Whole genome analysis of Rwandan G9P [8] rotavirus strains pre- and post-RotaTeq® vaccine introduction

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DECLARATION

I, Ms. Robyn-Lee Potgieter, declare that the master's degree research dissertation, publishable manuscripts/published articles, that I herewith submit for the Master's Degree qualification in Medical Sciences with specialisation in Virology, in the Next Generation Sequencing Unit, at the University of the Free State is my own original work, and that I have not previously submitted it for a qualification at another institution of higher education.

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Robyn-Lee Potgieter

06 December 2023

DEDICATION

This dissertation is dedicated to my late close family friend Duncan Grenfell, who was always so supportive and encouraging of my passion for science.

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RESEARCH DISSEMINATION

Publication emanating from this study.

1. **Potgieter R**, Mwangi P, Mogotsi M, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Seheri M, Steele D, Mwenda J, Nyaga MM. Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort. Viruses 2023, 15(12), 2321; https://doi.org/10.3390/v15122321
2. **Potgieter R,** Mwangi P, Mogotsi M, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Seheri M, Steele D, Mwenda J, Nyaga MM. Whole Genome Analysis of Rwandan G9P[8] Rotavirus Strains Reveals Positive Selection in Amino Acid Site 5 and 587 of Viral Protein 4 (under development for publication).

Other publications during the duration of the study.

1. Mwangi PN, **Potgieter RL**, Simwaka J, Mpabalwani EM, Mwenda JM, Mogotsi MT, Magagula N, Esona MD, Steele AD, Seheri ML, Nyaga MM. Genomic Analysis of G2P[4] Group A Rotaviruses in Zambia Reveals Positive Selection in Amino Acid Site 7 of Viral Protein 3. Viruses. 2023 Feb 11;15(2):501.

2. Mwangi PN, **Potgieter RL**, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Mwenda JM, Mogotsi MT, Rakau K, Esona MD. The Evolution of Post- Vaccine G8P [4] Group a Rotavirus Strains in Rwanda; Notable Variance at the Neutralisation Epitope Sites. Pathogens. 2023 Apr 28;12(5):658.

International conference output dissemination

1. Insights into Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine: reveals significant distinct sub-clustering in the postvaccination cohort. **Potgieter R**, Mwangi PN, Mogotsi MT, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Seheri ML, Steele AD, Mwenda JM, Nyaga MM. The 14th African Rotavirus Symposium, 08-10 November 2023, Abuja, Nigeria (poster presentation).

2. Vigilance in the Vaccine Era: Emerging Threats of Potentially Dangerous Rotavirus Strains. SACEV Satellite Meeting, 02-07 November 2023, Accra, Ghana (oral presentation).

3. Whole genome analysis of Rwandan G9P[8] strains pre- and postvaccine introduction. **Potgieter R**, Mwangi PN, Mogotsi MT, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Seheri ML, Steele AD, Mwenda JM, Nyaga MM. 14th international rotavirus symposium, 14-16 March 2023, Bali, Indonesia (poster presentation).

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1. Vigilance in the Vaccine Era: Emerging Threats of Potentially Dangerous Rotavirus Strains. **Potgieter R**. University of the Free State Health Science Three Minute Thesis (3MT) competition, 18 September 2023, University of the Free State, Bloemfontein, South Africa (oral presentation).

2. Insights into Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort. **Potgieter R**, Mwangi P, Mogotsi M, Uwimana J, Mutesa L, Muganga N, Murenzi D, Tusiyenge L, Seheri M, Steele D, Mwenda J, Nyaga MM. University of the Free State (UFS) Health Science Faculty Forum, 24 August 2023, University of the Free State, Bloemfontein, South Africa (oral presentation).

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1. Oxford Nanopore Technologies Day, 18 October 2022, Cape Town, South Africa.

2. University of the Free State Next Generation Sequencing Unit big data and Bioinformatics Workshop, 03-07 July 2023, Bloemfontein, South Africa. Part of the organisation committee and one of the facilitators for this workshop.

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ABBREVIATIONS

3D Three-Dimensional

cDNA	Complementary Deoxyribonucleic acid
DLP	Double-layered particle
DPRU	Diarrhoeal Pathogens Research Unit
DRC	Democratic Republic of Congo
dsRNA	Double Stranded Ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassays
ELISA	Enzyme-linked immunosorbent assays
EPI	Expanded programme of immunization
ER	Endoplasmic reticulum
FEL	Fixed-Effects Likelihood
FUBAR	Fast Unconstrained Bayesian AppRoximation for inferring selection
GSK	GlaxoSmithKline
HSREC	Health Sciences Research Ethics Committee
ICTV	International Committee on Taxonomy of Viruses
IgA	Immunoglobulin G
IgG	Immunoglobulin G
MEGA	Molecular Evolutionary Genetics Analysis
MEME	Mixed-Effects Model of Evolution
mRNA	messenger RNA
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
UFS-NGS	University of the Free State Next Generation Sequencing
NSPs	Non-structural proteins
nt	Nucleotide
ORF	Open reading frame
ORS	Oral Rehydration Solutions
ORT	Oral Rehydration Therapy

PAGE	polyacrylamide gel electrophoresis
PBS	Phosphate buffered solution
PCR	Polymerase chain reaction
PRRs	Pattern Recognition Receptors
RdRp	RNA-dependent RNA polymerase
RRL	Regional Reference Laboratory
RV	Rotavirus
RVA	Rotavirus Group A
RVE	Rotavirus Group E
SBS	Sequencing by synthesis
UV	Ultraviolet
VPs	Viral Proteins
WHO	World Health Organization

ABSTRACT

Children under the age of five who live in developing countries, are consistently exposed to the threat posed by Group A rotaviruses (RVA), despite the introduction of vaccines. Prior to the introduction of the rotavirus vaccine, over 3500 children in Rwanda died per year due to RVA associated disease. Therefore, the RotaTeq® vaccine was implemented in May 2012 to alleviate the disease burden. This led to a significant decline in rotavirus infections and fatalities in this region. Considering the extensive diversity of rotavirus strains found in Africa, additional data is necessary to assess the impact of vaccine introduction on RVA strains that are currently circulating in Rwanda. This study aimed to perform whole genome analysis of Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine introduction.

This was a descriptive observational experimental study which was executed at the University of the Free State - Next Generating Sequencing (UFS-NGS) Unit at the Faculty of Health Sciences, Bloemfontein, South Africa. This study formed part of a larger project aimed at addressing the Terms of Reference (ToRs) for Technical Service Agreement (TSA) between the World Health Organisation (WHO) and the UFS-NGS Unit. This study involved 30/158 faecal samples from Rwandan children that had already been conventionally genotyped as G9P[8] (based on the outer capsid proteins), as part of routine surveillance by World Health Organization African Regional Office (WHO/AFRO). A total of 23 samples were retrieved from the period prior to vaccination, while seven samples were used from the period following vaccination. To conduct whole genome sequencing on the Illumina MiSeq® platform, double-stranded RNA (dsRNA) was isolated from the faecal samples and complementary DNA (cDNA) was synthesised from the extracted material using the Maxima Kit. Subsequently, library preparation was carried out utilising the NextEra XT Kit. Following this, a variety of bioinformatics tools were employed to analyse the study data.

Whole genome analysis revealed that all 30 of the Rwandan G9P[8] study strains displayed the characteristic Wa-like constellation. Phylogenetic analysis in the form of maximum likelihood phylogenetic trees were constructed and revealed that the Rwandan study strains were clustering closely together. It was observed that three study strains from the post- vaccination study era were clustering distinctly from the rest of the G9P[8] study strains. The ranges of high similarity between nucleotides in all 11 genome segments agreed with the phylogenetic relationships that were observed. Amino acid differences were observed in the neutralisation epitope regions of the VP4 and VP7 genome segments compared to the Rwandan G9P[8] study strains and the RotaTeq® vaccine strain. These changes were mainly seen in the post- vaccine study strains and may lead to the escape of vaccine mutants, which could potentially affect the efficacy of the vaccine. The protein surfaces displayed these amino acid variations, as observed in three-dimensional (3D) protein structures. Analysis of selection pressures revealed that ten of the genome segments were subject to purifying selection, except for the VP4 genome segment, where two sites were subject to positive selection, which could influence the protein's evolutionary dynamics.

To unpack the unknown information surrounding rotavirus strain diversity, evolution, and the epidemiology of circulating RVA strains in Rwanda during the pre- and post- vaccine introduction periods, it remains important to continue performing whole genome studies in this region.

Keywords: rotavirus; Rwanda; G9P[8]; whole genome analysis, vaccine, phylogenetic analysis, antigenic epitope, protein modelling

CHAPTER ONE: INTRODUCTION

1.1. Preamble:

Chapter one provides a general overview of this study which includes a general introduction to rotavirus, a problem statement, the study significance, the aim and objectives of the study.

1.2. Background:

Diarrhoeal diseases are the second leading cause of morbidity and mortality among children aged five and below, following pneumonia (Walker *et al.,* 2013). Group A rotavirus is particularly notable among these illnesses due to its status as the principal viral agent that causes acute and severe gastroenteritis in neonates and young children (Parashar *et al.,* 2006). It is noteworthy that an alarming 95% of children have contracted rotavirus by the age of five, according to comprehensive epidemiological data (Troeger *et al.,* 2018). Group A rotavirus is a cause of considerable mortality on a global scale, accounting for around 128,500 deaths annually (Troeger *et al.,* 2018). Developing countries, specifically those in southeast Asia and sub-Saharan Africa, are the primary regions where 81% of these fatalities transpire (Troeger *et al.,* 2018). In the context of sub-Saharan Africa, the impact is even more severe, with an estimation that 37% of children hospitalised in the region due to rotavirus in 2013 ultimately succumbed to the infection (Tate *et al.,* 2016). Rwanda bears a heavy burden of morbidity and mortality associated with RVA-related diseases (Ngabo *et al.,* 2016). With approximately 3500 deaths recorded annually, the disease accounts for a significant proportion of child mortality in this region (GAVI, 2012). Addressing this

public health challenge is critical to reducing the devastating impact of rotavirus on young children in Rwanda and other low-income settings.

Rotavirus is transmitted via faecal-oral route and can also spread via aerosol droplets (Esona *et al.,* 2021). Since infection rates occur in children from both high-income and low-income countries, rotavirus is considered a "democratic virus" (Glass *et al.*, 2005). However, rotavirus remains a significantly prevalent and devastating disease among children living in low- and middle-income countries, where access to adequate sanitation facilities and effective medical care is relatively scarce (CDC, 2003). The World Health Organization (WHO) has undertaken extensive efficacy and safety evaluations to prequalify four rotavirus vaccines for global distribution with the aim of alleviating the burden of rotavirus disease (https://extranet.who.int/gavi/PQ_Web/). These are: Rotavac®, Rotarix®, RotaTeq®, and Rotasiil® (Serum Institute of India, Pune, India; GlaxoSmithKline Biologicals, Rixensart, Belgium; Merck & Co. Inc., Kenilworth, NJ, USA; Serum Institute of India, Pune, India, respectively). The implementation of vaccines as part of the expanded programme of immunisation (EPI) and sanitation improvements have contributed significantly to the decline in the rotavirus disease burden. The number of cases decreased from approximately 500,000 in the pre-vaccination period to 128,500 in the postvaccination period (Parashar *et al.,* 2006; Troeger *et al.,* 2018).

The RotaShield® vaccine was initially marketed in the United States in 1998, making it the inaugural rotavirus vaccination. Nevertheless, it was subsequently removed from the market because of detrimental consequences, such as the occurrence of bloody stool and intussusception (Glass *et al.,* 2005). Following that, two main rotavirus vaccines were created: RotaTeq® and Rotarix®. RotaTeq® is a vaccine that contains five different weakened strains of the virus, including G1-G4 and P1A[8]. On the other hand, Rotarix® is based on a single strain of the virus, specifically the G1P[8] human strain (Vesikari *et al.,* 2006). In addition, two more vaccines, Rotasiil® and

Rotavac®, were created. Rotasiil® is a pentavalent vaccine that is a combination of bovine and human strains, while Rotavac® is a monovalent vaccination specifically designed for neonates and is based on the G9P[11] strain (Bharat Biotech, 2019).

The RotaTeq® vaccine was introduced to several African countries, including Morocco, Gambia, Rwanda, Burkina Faso, and Libya (Seheri *et al.,* 2018). Rwanda was among the pioneering low-income countries to introduce RotaTeq® in May 2012, and it achieved an impressive 99% coverage within the first year of implementation (Gatera *et al.,* 2016). Significantly, within the initial three years following the implementation of RotaTeq® in Rwanda, there was a notable decline of 25–44% in hospitalisations attributed to diarrhoeal illnesses among children (Sibomana *et al.,* 2018). Moreover, a substantial decline of between 61% to 70% was reported in the incidence of RV-specific diagnoses in the region (Vesikari *et al.,* 2016). Rwanda, for economic reasons, opted to transition to the Rotarix® vaccine in April 2017 (Mandomando *et al.,* 2021). The implications of this change on potential novel circulating strains of rotavirus in the foreseeable future remain uncertain and warrant further investigation.

While introduction of the vaccine suggested that a positive impact was being made on the population, concerns emerged that administration of the vaccine might be selecting emergence of putative vaccine-escape mutants (Leshem *et al.,* 2014; Linhares *et al.,* 2014; Zeller *et al.,* 2017; Jere *et al.,* 2018). Rotavirus vaccines are designed to confer protective immunity against rotavirus strains (Wang *et al.,* 2021). However, the complex evolutionary mechanisms such as genome reassortment, zoonotic transmission, and rearrangement, have resulted in the emergence of different novel RVA strains capable of spreading throughout the community (Kirkwood *et al.,* 2011; Velasquez and Jiang, 2019). The presence of these newly identified strains in circulation could potentially diminish the efficacy of vaccines in regions characterised by a high diversity of strains, such as low-income countries in sub-Saharan Africa and

southeast Asia (Carvalho and Gill, 2019; Burnett *et al.,* 2020). It is worth noting that developed countries have documented greater rates of vaccine efficacy in comparison to developing and low-income countries, emphasises the significance of undertaking whole genome analysis (Narang *et al.,* 2009; Cunliffe *et al.,* 2012). Such analysis allows for a deeper understanding of the dynamic changes in RVA strains in Africa, particularly in low-income countries such as Rwanda (Seheri *et al.,* 2018; Mwangi *et al.,* 2020; Rasebotsa *et al.,* 2021).

1.3. Problem statement

Rotavirus vaccines offer cross-protection against various rotavirus strains. However, the potential exists for the emergence of novel or unusual genomic constellations that may circulate and spread throughout the population. Since the introduction of the rotavirus vaccines, surveillance of the epidemiology has become increasingly important, to assess the impact of vaccine introduction on circulating rotavirus strains (Jere *et al.,* 2018; Rasebotsa *et al.,* 2020 Maringa *et al.,* 2021; Mwangi *et al.,* 2021).

African countries have shown a greater disease burden and major strain diversity to rotavirus than other countries (Seheri *et al.,* 2014). The G9P[8] strain along with five other predominant strains, namely G1P[8], G2P[4], G3P[8], G4P[8], and G12P[8], account for approximately 74.7% of all circulating rotavirus strains in African countries (Mwenda *et al.,* 2010; Seheri *et al.,* 2014; Nyaga *et al.,* 2020). Several African countries, including Kenya, Ghana, Uganda, and Zambia, experienced the emergence of G9P[8] after the implementation of the rotavirus vaccine. However, this phenomenon was also noted in countries where the vaccine had not yet been administered to the population (Mwenda *et al.,* 2010; Seheri *et al.,* 2018; Damanka *et al.,* 2019; Kawata *et al.,* 2021; Omatola *et al.,* 2021). Therefore, circulating patterns of various rotavirus strains are not entirely understood.

There has been limited whole genome analysis studies on the G9P[8] genotype in Africa, more specifically Rwanda. In addition to the ability to fully evaluate the impact of RVA vaccinations on the genetic and antigenic composition of circulating RVA, whole genome analysis offers more insightful information, including the origin of strains, the detection of unusual reassortants and zoonotic events, and the genetic linkage of RVA gene segments (Nyaga *et al.,* 2020; Donato *et al.,* 2021).

1.4. Significance of study

The results of this research will provide valuable insights into the genomic epidemiology of G9P[8] strains, particularly in the era following rotavirus vaccination. This knowledge may ultimately aid in comprehending the impact of the introduction of the RVA vaccine in Rwanda. Furthermore, the submitted sequenced data to the National Centre for Biotechnology Information (NCBI), a public database, will provide supplementary reference sequence data that can be utilised in subsequent genomic investigations relating to strains originating from Rwanda.

1.5. Aim of the study

To analyse the whole genome of Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine introduction.

1.6 Specific objectives of the study

- **Objective 1:** To generate whole genome sequences of pre- and post- vaccine Rwandan rotavirus G9P[8] strains

- **Objective 2:** To perform phylogenetic analysis and correlate pre- and post-vaccine Rwandan rotavirus G9P[8] strains through pair-wise sequence analysis

- **Objective 3:** To perform selective pressure analysis and computational protein modelling on Rwandan G9P[8] rotavirus strains

1.7. Dissertation organisation

Each of the five chapters comprising this dissertation is introduced with a concise context section that offers an overview of the subject matter addressed in that chapter. The first chapter presents an overview of rotavirus, a problem statement, the significance of the research, as well as the aims and objectives.

An extensive literature review on rotavirus relevant to this dissertation is presented in chapter two.

Chapter three is designed to address the first two objectives of this study as an original manuscript published online entitled "Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort". In addition to introductory literature, general materials, and methodologies, this chapter will detail the particulars of the data analysis that was conducted.

Chapter four of this study addresses the third objective by presenting results of a publishable article titled "Whole Genome Analysis of Rwandan G9P[8] Rotavirus Strains Reveals Positive Selection in Amino Acid Site 587 of Viral Protein 4" This chapter will comprise an overview of the relevant literature and a methodology section that will exclusively detail elements of the analysis that are distinct from those explained in chapter three.

Chapter five serves as a comprehensive summary of the entire dissertation.

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CHAPTER TWO: LITERATURE REVIEW

2.1. Preamble:

This chapter primarily discussed the rotavirus disease burden, epidemiology of rotavirus, the characteristics of rotaviruses, the mechanisms of genetic diversity in rotaviruses, rotavirus vaccines and the burden of disease, followed by rotavirus in the Rwandan context.

2.2. Rotavirus discovery:

The discovery of rotavirus marked a significant milestone in the field of virology and paediatric medicine (Bishop *et al.,* 2009). Rotavirus (RV) was first identified in 1973 by an Australian paediatrician, Ruth Bishop, and her colleagues (Bishop *et al.,* 1973). Their work involved isolating the virus from stool samples of infected children that were admitted with gastroenteritis, where they looked in depth at the cytoplasm of duodenal biopsies (Bishop *et al.,* 1973). Bishop's pioneering research transformed the understanding of viral gastroenteritis and laid the foundation for subsequent studies and public health interventions (Flewett *et al.,* 1974). The term 'Rotavirus' descended from the Latin word 'rota' meaning wheel, which is attributed to the wheel-like structure of this virus (Flewett *et al.,* 1974). Rotavirus was similar to viruses previously causing diarrhoea in vervet monkeys, calves, and mice (Edwards and Sier, 1960, Adams and Kraft, 1963, Mebus *et al.,* 1969). Today, rotaviruses are documented to be the cause of diarrhoea in many avian species and young mammals (Ramig *et al*, 2004).

2.3. Rotavirus disease burden:

Rotavirus is the predominant viral agent responsible for inducing acute and severe gastroenteritis in neonates and young children (Parashar *et al.,* 2006). By five years of age, 95% of all children have been infected by rotavirus (Troeger *et al.,* 2018). Previously, rotavirus global mortality rate was approximately 500,000 deaths annually, but with the rollout of RVA vaccines, the global mortality rate reduced to 128,500, with 90% of these deaths having occurred in developing countries in southeast Asia and sub-Saharan Africa (Parashar *et al.,* 2006; Troeger *et al.,* 2018). India, the Democratic Republic of Congo (DRC), Angola, Nigeria, and Pakistan, account for more than half of rotavirus mortalities globally. Looking more closely at Africa, eight countries namely, Chad, Burkina Faso, DRC, Niger, Ethiopia, Nigeria, Cote d'Ivoire, and Uganda, account for over 80% of Africa's rotavirus associated moralities (Troeger *et al.,* 2018).

Rotavirus is transmitted via faecal-oral route and can also spread via aerosol droplets (Esona *et al.,* 2021). Rotavirus is regarded as a "democratic virus" as infection incidences occur in children in both high-income and low-income settings (Glass *et al.,* 2005). However, the incidence and fatalities of rotavirus are significantly greater among children residing in countries with low- or middle- incomes, where access to effective medical care is limited and sanitation facilities are notably inadequate, which can be seen in figure 2.1 (CDC, 2003). Despite the considerable reduction in RVA mortality rate attributed to the efficacy of RVA vaccines, rotavirus-induced diarrhoea remains the primary cause of diarrhoeal mortality among children aged below five years (Troeger *et al.,* 2018).

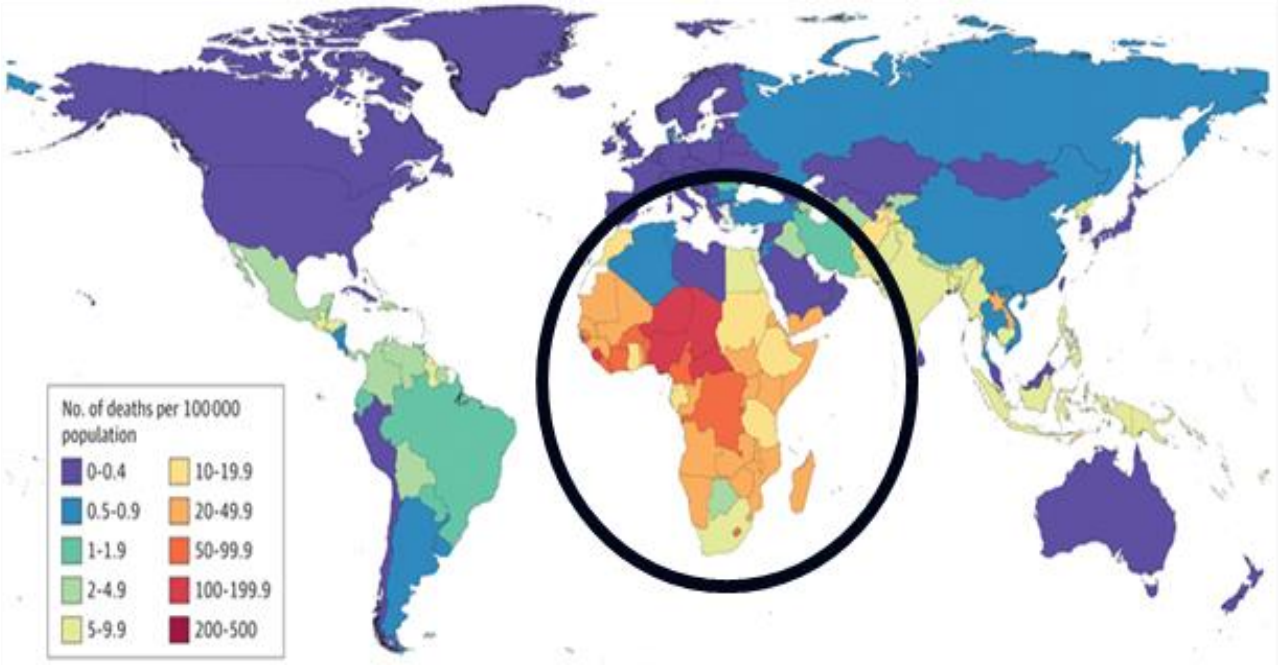

Figure 2.1: The global rotavirus disease burden. The colours represent the severity, with countries coloured in red, having a high burden of rotavirus disease, showing a high disease burden in sub-Saharan Africa and Asia (Troeger et al.,2018).

2.4. Rotavirus structure:

Rotavirus is part of the *Sedoreoviridae* family and has a genome size of 18,552 bp (https://ictv.global/report/chapter/reovirales accessed on 26 February 2023). Eleven segments of the RVA genome are composed of dsRNA encased in icosahedral capsids (Patton *et al.,* 2006). These RV segments all vary in size from 668 to 3302 base pairs (Matthijnssens *et al.,* 2008). RVA gene translation produces 11 or 12 viral proteins, five or six of which are non-structural proteins (NSPs) and six of which are structural viral proteins (VPs) (Figure 2.2.) (Patton *et al.,* 2006). The 11 segments are monocistronic as they each encode for one protein, except for segment 11 which is sometimes distronic (NSP5 and sometimes NSP6) (Figure 2.3) (Patton *et al.,* 2006). The virion particles' six

structural capsid proteins are encoded by VP1-4 and 6-7, as well as the five NSPs which encode NSP1-5/6 (Desselberger *et al.*, 2014).

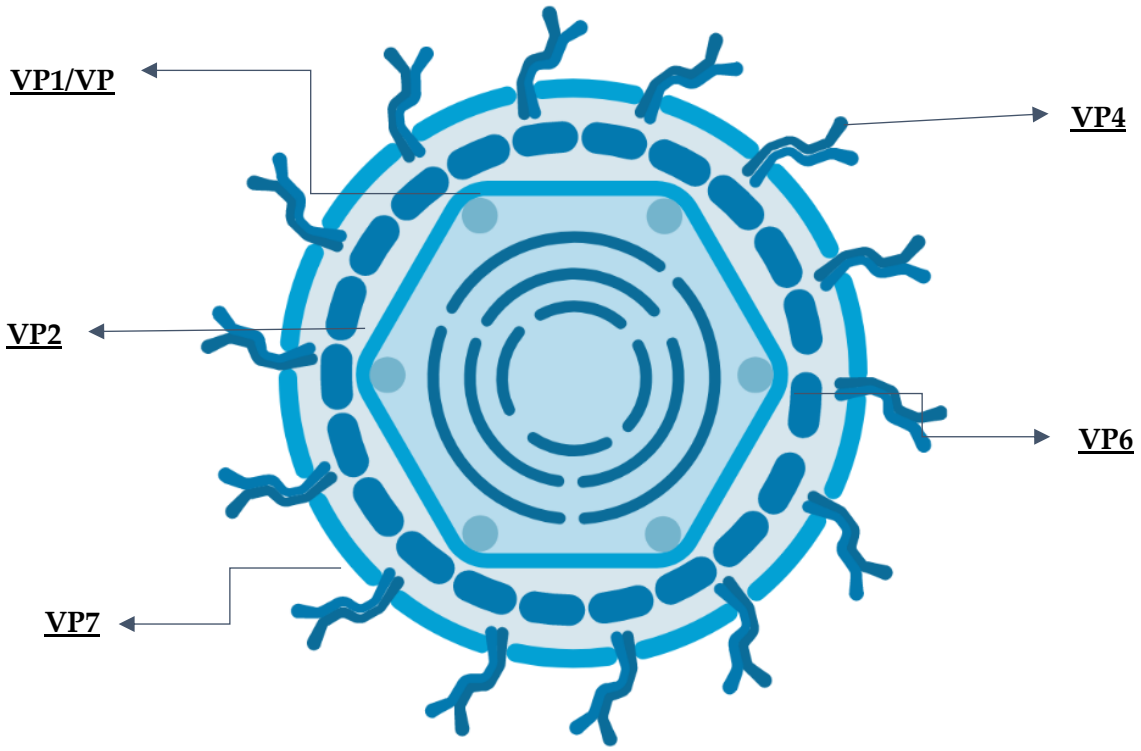

Figure 2.2: The figure shows the structural viral proteins of rotavirus in a wheel-like manner, with the VP1-3 representing the inner capsid layer, the VP6 making up the middle layer, and the VP4 and VP7 making the outer capsid proteins.

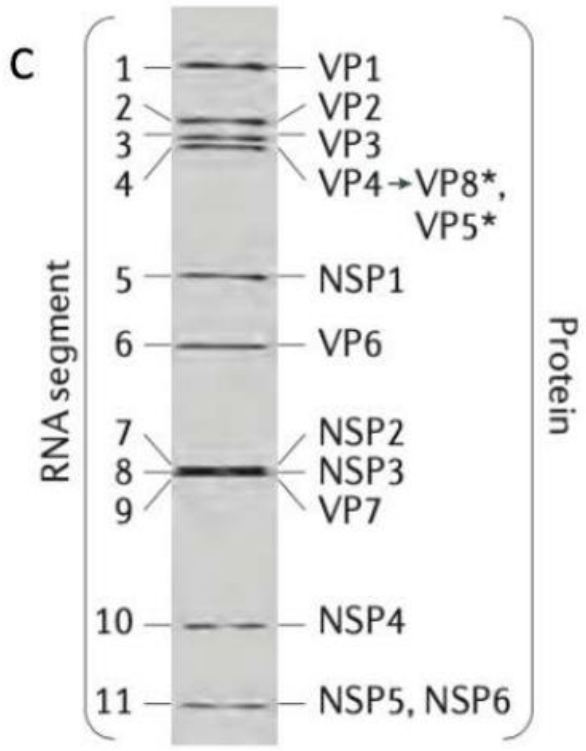

Figure 2.3: The gel electrophoresis separation of the 11 genome segments beside their gene-protein assignments, showing the six structural proteins and the five or six non-structural proteins (Crawford *et al.,* 2017).

Rotaviruses possess a distinctive triple-layered structural organisation crucial for their infectivity and survival (Estes and Greenberg, 2013). These structural proteins play a crucial role in attachment to host cells, entry into the host, and protection of the viral genome (Estes and Greenberg, 2013).

2.4.1. Structural Viral Proteins

At the core of this structure are the inner layer proteins (VP1 and VP2). The VP1, RNA-dependent RNA polymerase (RdRp), is responsible for replicating the viral RNA genome inside the host cell (Tao *et al.,* 2002). The VP1 is involved in the transcription of viral proteins, and it interacts with capping enzymes (Steger *et al.,* 2019). The VP2 is the core protein which associates with viral RNA (Matthijnssens *et al.,* 2008). It aids in the stability and packaging of the viral genome during assembly (McClain *et al.,*

2010). The VP3 is essential for capping the messenger RNA (mRNA), which enables it to mimic the hosts capped RNA which results in the escape of immune response (Kumar *et al.,* 2020). The VP6 forms part of the middle capsid layer and plays a crucial role in viral assembly (Corthesy *et al.,* 2006). It stabilises the core structure and is highly immunogenic, making it a common target for vaccines and diagnostic tests (Mathieu *et al.,* 2001). The VP7, also known as the "glycoprotein-sensitive protein", is a key outer capsid protein (Matthijnssens *et al.,* 2008). This protein is also responsible for the virus's serotype classification and genotype based on genomics (Kapikian *et al.,* 2007; Heiman *et al.,* 2008). The other key outer capsid protein is the VP4 (protease sensitive), which gives the virus its spike-like appearance (Matthijnssens *et al.,* 2008). This protein is essential for receptor binding and is a target for host immune responses (Estes and Cohen, 1989). It plays a major role in cell attachment and penetration and is also involved in serotype classification (Svensson *et al.,* 1987).

2.4.2. Non-structural Viral Proteins

NSP1 degrades additional host proteins and inhibits the innate immune response (Barro and Patton, 2005; Arnold *et al.,* 2013; Morelli *et al.,* 2015). Non-structural protein 2 functions as a molecular motor that aids in the production of pre-capsid intermediates from template mRNAs (Borodavka *et al.,* 2017). The NSP3 functions as a translation enhancer by producing a dimer that contains binding domains for translation initiation factors and a conserved region seen in viral mRNAs (Piron *et al.,* 1998; Arnold *et al.,* 2013). As a result, the circularisation of polysomes is stimulated, leading to the enhancement of viral mRNA translation (Kuhn and Wahle, 2004). Non-structural protein 4 is an intracellular receptor for double-layered particle (DLPs) and interacts with capsid proteins (Zhang *et al.,* 2014). Essentially, the NSP4 serves as an enterotoxin which causes diarrhoea, suggesting the NSP4s role in pathogenesis (Matthijnssens *et al.,* 2008; Zhang *et al.,* 2014). The NSP5 localises in the viroplasms of rotavirus infected cells (Eichwald *et al.,* 2004). Due to the interaction between the NSP5 and NSP2, a posttranslational hyperphosphorylation occurs which results in groups

with a reduced polyacrylamide gel electrophoresis (PAGE) mobility (Jiang *et al.,* 2006). Occasionally, a distinct open reading frame (ORF) of genome segment 11 produces non-structural protein 6, which modifies and interacts with the 3D structure of NSP5 to augment its activity (Torres-Vega *et al.,* 2000). The NSP6 is a crucial factor in facilitating cellular death and functions as a filter (Halasz *et al.,* 2010; Tait and Green *et al.,* 2013; Komoto *et al.,* 2017).

2.5. Rotavirus diversity and classification:

The rotavirus genus is divided into ten definite groups (A-J), which is based on the antigenic properties of VP6 (Matthijnssens *et al.,* 2011; Bányai *et al.,* 2017). However, according to International Committee on Taxonomy of Viruses (ICTV), there is currently no sequence data available on the Group E rotavirus (RVE) (ICTV, 2015). Group A Rotavirus are primarily human pathogens and are known to be important enteric pathogens (Tate *et al.,* 2016). Groups B, C, H, I, and J rotaviruses primarily infect animals, with sporadic transmission to humans (Mulherin *et al.,* 2008; Martella *et al.,* 2010; Bányai *et al.,* 2017). Group B and C rotaviruses are commonly found in bovines and swine, while Groups H, I, and J have been identified in birds (Johne *et al.,* 2011).

Traditionally, categorisation of rotaviruses into G types (due to the glycoprotein nature of VP7) and P types (due to the protease sensitivity of the VP4) was achieved using a binary classification system based on the immunological properties of the two outer capsid proteins (VP4 and VP7), which function as neutralisation antigens (Estes and Cohen, 1989; Matthijnssens *et al.,* 2008). The G and P genotypes are essential for serotyping and epidemiological surveillance, with numerous combinations representing distinct serotypes (Estes and Greenberg, 2013). However, to comprehensively describe the diversity of rotavirus strains, a classification scheme

based on all the 11 genome segments is currently preferred (Estes and Greenberg, 2013). All the segments of the genome are sequenced and analysed, where the genetic relatedness among the strains may be identified (Matthijnssens *et al.,* 2008). The classification method for RV being used is largely based on the percentage identification cut-off values per genotype for each segment, which allows the identification of different genotypes (Matthijnssens *et al.,* 2012). The nomenclature used for the comparison among the RVA genome is classified as follows, Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx which are used for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 encoding genes, respectively, whereby x denotes the number of the genotypes (Matthijnssens *et al.,* 2012). This classification system is largely based on nucleotide sequences of the complete ORF, thus the whole ORF of all genes included in a new strain must be sequenced to assign it to an established genotype or a new genotype must be established (Matthijnssens *et al.,* 2012). Based on the whole-genome classification scheme, RVA are further classified into three genogroups, the Wa-like and the DS-1-like (major) and the AU-1-like (minor), which is illustrated in figure 2.4 below (Matthijnssens *et al.,* 2008).

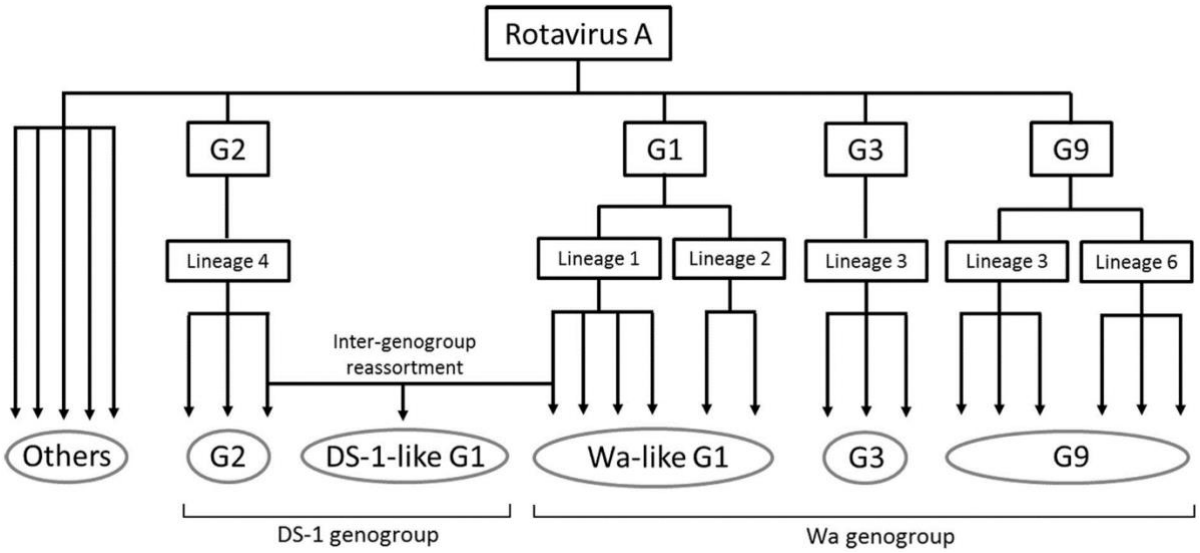

Figure 2.4: Rotaviruses three genogroups classification, the Wa-like and the DS-1-like (major) and the AU-1-like (minor) (Matthijnssens *et al.,* 2008).

2.6. Rotavirus epidemiology:

The RVA genotypes that are most identified on a global scale are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Dóró *et al.,* 2014). They collectively account for up to 75% of rotavirus infections in humans across the globe (Dóró *et al.,* 2014). Conversely, the rotavirus genotype combinations that were most prevalent in Africa were G1P[8], G2P[4], G3P[8], G9P[8], and G12P[6] (Mwenda *et al.,* 2010). The G1P[8] genotype is predominant in human RVA infections in Africa, comprising around 29% of the total RVA strains that are circulating in the area (Doro *et al.,* 2014). The G9 genotype was discovered in the 1980s, being the last to emerge in Africa (Waggie *et al.,* 2010). The G9 genotype is now considered the fifth most widespread genotype among the rotavirus strains (Page *et al.,* 2010; Doro *et al.,* 2014). During the mid-1990s, the G9 genotype became common in Africa and was then further characterised at whole genome level (Nyaga *et al.,* 2013). Globally, only a few of the G9 rotaviruses have been fully characterised (Nyaga *et al.,* 2013; Jere *et al.,* 2018). The G9 rotavirus strains have been commonly associated with six different genotypes globally namely, G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Moure *et al.,* 2018).

The G9P[8] genotype is becoming increasingly prevalent in many countries (Wu *et al.,* 2017). The G9P[8] strain along with five other predominant strains, namely G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8], account for approximately 74.7% of all circulating rotavirus strains in African countries (Mwenda *et al.,* 2010; Seheri *et al.,* 2014; Nyaga *et al.,* 2020). The G9P[8] emerged in several African nations, including Kenya, Ghana, Uganda, and Zambia, after the implementation of the rotavirus vaccine [8]. However, this phenomenon was also noted in countries where the vaccine had not yet been administered to the populace (Mwenda *et al.,* 2010; Seheri *et al.,* 2018; Damanka *et al.,* 2019; Kawata *et al.,* 2021; Omatola *et al.,* 2021). In a study conducted in China, G9P[8] was the predominant genotype present throughout the duration of the study (2012-2019), with an increasingly prevalence of 3.4% in 2009 to 60.9 % in 2015

(Zhou *et al.,* 2020). In 2012 in Hungary, the G9P[8] genotype emerged as a prevalent strain in the country, where clonal spread was suggested due to the re-emergence of conserved genomic constellations of the G9P[8] genotype (Doro *et al.,* 2014).

In Africa, whole genome studies on genotype G9 have been conducted in Cameroon and South Africa which identified the genomic constellations of the G9, on the Wa-like backbone with sporadic reassortment gene segments in some G9P[6] combinations (Esona *et al.,* 2009; Nyaga *et al* 2013). South African G9 strains were associated with VP4 genes and showed increased homology with G9P[8] strains (Page *et al.,* 2010). It has been noted that the VP7 genes of G9 strains sequenced in Ghana were associated with G9P[8] strains from other parts of Africa and other parts of the world such as Europe and South America (Damanka *et al.,* 2019).

2.7. Rotavirus clinical features and diagnosis:

One hundred viral particles are sufficient to induce an infection of rotavirus, owing to its exceptionally transmissible characteristics (Graham *et al.,* 1987). In addition to faecal-oral transmission and shedding in the stool of infected children, aerosol particulates may also be used to transmit this virus (Fragoso *et al.,* 1986; CDC, 2019). The main clinical features of rotavirus include watery diarrhoea and vomiting for several days, accompanied by additional symptoms such as fever, dehydration, abdominal pain, loss of appetite, fussiness, or sleepiness (Omatola *et al.,* 2022).

Diagnosing rotavirus infection is a critical process, especially in cases of severe gastroenteritis, as early identification can provide the appropriate treatment and infection control measures (Omatola *et al.,* 2022). The diagnosis of rotavirus infection involves various laboratory methods that aim to detect the presence of the virus or its antigens in clinical specimens, such as stool samples from patients with gastroenteritis

symptoms (Gentsch *et al.,* 1992). Enzyme immunoassays (EIAs) and polymerase chain reaction (PCR) are the most common and reliable techniques. Certain EIAs, such as enzyme-linked immunosorbent assays (ELISAs), are efficient and widely utilised due to their rapid results and high sensitivity (Wilde *et al.,* 1991). In contrast, PCR is a molecular technique that detects the virus's genetic material, making it highly specific and sensitive (Watzinger *et al.,* 2006). Other methods, like electron microscopy, allow for direct visualisation of the virus particles but are less commonly used in clinical settings due to their complexity (Goldsmith *et al.,* 2009). Rapid antigen tests offer quick results and are suitable for bedside diagnosis, although they might not be as sensitive as other methods (Wang *et al.,* 2021). Advanced diagnostic technologies, such as multiplex molecular assays, enable the simultaneous detection of multiple enteric pathogens, enhancing diagnostic efficiency (Anderson and Weber, 2004). Accurate diagnosis is essential for epidemiological surveillance (Anderson and Weber, 2004).

2.8. Replication cycle of rotavirus:

The replication cycle of rotavirus is a complex and highly orchestrated process (demonstrated in figure 2.5) that involves several key steps as follows:

1. **Attachment and entry**:

The replication cycle begins with the attachment of rotavirus to host cells (Amimo *et al.,* 2021). The virus primarily targets mature enterocytes lining the small intestine (Ramig *et al.,* 2004). As mentioned before, rotavirus attachment is mediated by the outer capsid proteins. Prior to entering the host cells, the VP4 protein is separated into VP8* and VP5* by trypsin-like proteases. The VP8* protein then attaches to sialic acid residues on the surface of the host cell, while VP7 assists in the initial attachment at the lipid raft by interacting with co-receptors (Lopez and Arias, 2006; Baker and Prasad, 2010). Following attachment, rotavirus enters the host cell through receptor-

mediated endocytosis (Gutierrez *et al.,* 2010). The viral particles are internalised into endosomes (Gutierrez *et al.,* 2010).

2. Uncoating:

Once inside the endosome, the low pH environment due to the low Ca^{2+} triggers the uncoating process (Crawford *et al.,* 2017). This involves the removal of the outer capsid proteins exposing the inner capsid, which releases the DLPs into the cell cytoplasm, where transcription and translation occurs (Yoder *et al.,* 2009).

3. Transcription and translation:

The released viral RNA serves as a template for transcription and replication within the host cells cytoplasm. Using mRNA, the viroplasm is produced by the interaction between the NSP2 and NSP5 (Crawford *et al.,* 2017). RNA synthesis and translation occurs in the viroplasm (Ramani *et al.,* 2016; Saxena *et al.,* 2016). The newly synthesised mRNAs are translated into viral proteins (Mitzel., 2004).

4. Assembly:

Newly synthesised RNA is packaged into DLPs which binds to the NSP4, which is then buds out of the endoplasmic reticulum (ER) and Golgi apparatus (Crawford *et al.,* 2017). For virus replication to occur, the level of calcium in the cytoplasm must be increased, which is carried out by the NSP4 (Trask *et al.,* 2012).

5. Maturation and Release:

Following assembly, the viral particles obtain the VP4 and VP7 proteins, which then transform into infectious virions in the endoplasmic reticulum (Estes and Cohen, 1989; Settembre *et al.,* 2011; Desselberger *et al.,* 2014). By means of cell lysis or non-classical vesicular transport, these progenies are liberated from the infected host cell, enabling them to invade enterocytes within the lumen (Gardet *et al.,* 2006). The progression of this replication cycle results in the emergence of diarrhoea (Gardet *et al.,* 2006).

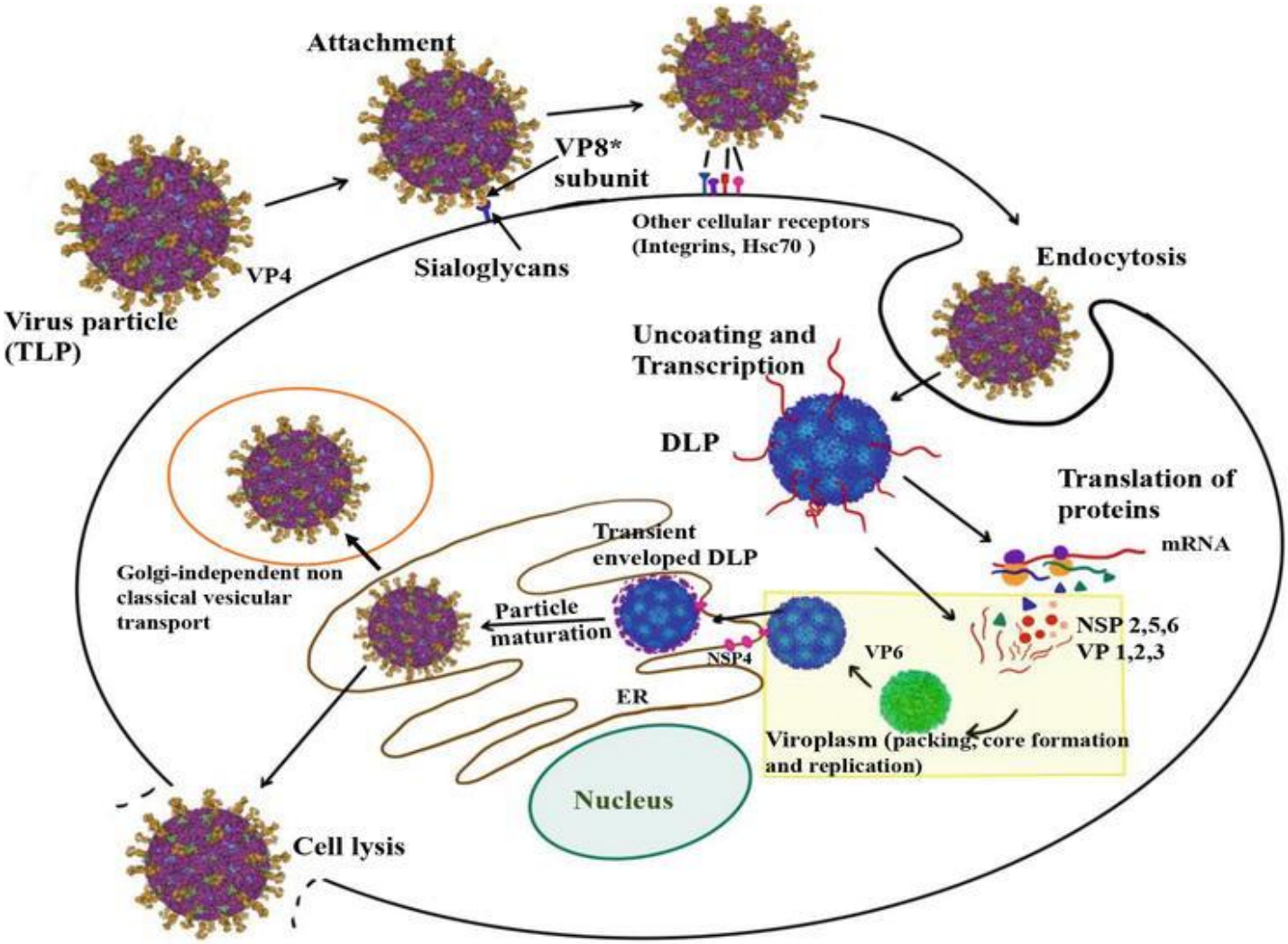

Figure 2.5: This figure represents the key features of the rotavirus replication cycle, first demonstrating the attachment and entry, the uncoating process, transcription followed by translation, thereafter assembly which reaches the final stage of maturation and release (Nirmal and Gangar, 2023).

2.9. Rotavirus Immune Response:

The immune response to rotavirus infection is an intricate and diverse process that includes both cellular and humoral responses, aiming to establish immunity against the virus (Offit *et al.*, 1996; Franco *et al.*, 2006; Desselberger and Huppertz, 2011). Upon infection, RVA is detected by pattern recognition receptors present in enterocytes,

dendritic cells, adaptive B and T cells, as well as macrophages, all of which are components of the immune system (Offit, 1996; Broquet *et al.,* 2011; Crawford *et al.,* 2017). Specific B lymphocytes targeting the VP6 protein hinder the transcription process (Aiyegbo *et al.,* 2013). Rotavirus infections are detected by pattern recognition receptors (PRRs) that activate type I and type III interferon responses, which aid in the innate immune system's elimination of the infection (Aiyegbo *et al.,* 2013; Holloway *et al.,* 2014; Lin *et al.,* 2016). CD8+ cytotoxic T lymphocytes are the main agents responsible for eradicating an RVA infection (Jaimes *et al.,* 2002). The CD4+ T helper cells regulate the immune response by assisting B cells in producing antibodies and activating cytotoxic CD8+ T cells (Jaimes *et al.,* 2002; Kim *et al.,* 2008; Mesa *et al.,* 2010). CD8+ T cells possess the ability to selectively attack and eliminate infected cells, hence halting the creation of further viruses. However, in certain instances, their immune response may not be substantial enough to effectively combat the infection (Franco and Greenberg, 1995). Successful resolution of a rotavirus infection leads to the development of immune memory (Gomez-Rial *et al.,* 2019). Memory T cells and B cells can retain information regarding the infection which allows a quicker and more effective immune response in the case of reinfection (Gomez-Rial *et al.,* 2019). Newborn babies receive maternal antibodies, including rotavirus-specific immunoglobulin G (IgG), through the placental transfer (Caddy *et al.,* 2020). This provides some level of protection during the first few months of life (Caddy *et al.,* 2020). Maternal immunoglobulin A (IgA) antibodies in breast milk can further protect infants against this infection (Chan *et al.,* 2011). The production of antibodies, specifically IgA and IgG, and the development of immune memory are key elements in protection against rotavirus infections (Chan *et al.,* 2011).

2.10. Mechanisms of genetic diversity of rotaviruses:

Numerous types of mechanisms contribute to the continuous evolution of rotavirus strains. These mechanisms include point mutations, gene reassortment, gene rearrangement, genetic recombination, and zoonotic transmission (Kirkwood *et al.,* 2011; Hoxie and Dennehy, 2020).

2.10.1. Point mutations:

Point mutations one of the most important mechanisms associated with rotavirus disease (Desselberger *et al.,* 2014). Point mutations occur continuously due to the elevated error rate of RdRp present in the viral genome (Blackhall *et al.,* 1996). These mutations involve frameshift and base substitutions, where there will be a single base pair change at a specific location within the RNA sequence (Bányai and Pitzer, 2016). A point mutation may sequentially accumulate or can occur sporadically (Martínez-Laso *et al.,* 2009). The emergence of new genetic lineages and sub-lineages can be accredited to the accumulation of point mutations (Iturriza-Gomara *et al.,* 2000). The occurrence of amino acid substitution at neutralisation epitopic regions of the VP4 and VP7 result in neutralising antibody escape mutants (Guo *et al.,* 2020; Rasebotsa *et al.,* 2020).

2.10.2. Genome reassortment:

Genome reassortment is another important mechanism of rotavirus which is often associated with zoonotic transmission (Todd *et al.,* 2010; Cowley *et al.,* 2013). This mechanism involves exchanges of genome segments originating from the same rotavirus species, which results in progeny with a mixture of genome segments from the parental strains (Bányai and Pitzer, 2016). This evolution mechanism is a major contributing factor in the total diversity of all RVA strains and may produce predominant variants with advantageous characteristics against strains already circulating in the population (Gentsch *et al.,* 2005; Strydom *et al.,* 2019; Mwangi *et al.,* 2020).

2.10.3. Gene arrangement:

This type of mechanism is commonly seen in chronically infected children who are immunocompromised, and is also found in animals (Pedley *et al.,* 1984). Gene arrangements involve insertions, partial duplications and deletions of nucleotides present in individual sequences, with gene duplications being the most abundant form of rearrangements (Pedley *et al.,* 1984; Bányai and Pitzer, 2016). These genes are typically found in non-structural segments of the genome and are positioned downstream from the open reading frame (Patton *et al.,* 2006).

2.10.4. Genetic recombination:

Genetic recombination is an evolutionary mechanism which is considered rare in rotavirus evolution, due to the segmented genome and the transcription and replication mechanisms (Desselberger *et al.,* 1996; McDonald *et al.,* 2016). For genome recombination to occur, a single host cell must be infected by different strains of rotavirus (co-infection) (Parra *et al.,* 2004; Jere *et al.,* 2011). Intergenotype, inter-lineage and sub-lineage, intragenic, and intersegmental recombination events have been reported in structural and non-structural genome segments (Woods *et al.,* 2015; Esona *et al.,* 2017).

2.10.5. Zoonotic transmission:

Zoonotic transmission is the natural reassortment event between animals and humans. There is evidence to suggest that zoonotic transmission is more prevalent in low-income and developing countries, where the close proximity of humans and animals may facilitate interspecies transmission (Cook *et al.,* 2004; Bányai *et al.,* 2009; Maringa *et al.,* 2020). It is suggested that animals may serve as reservoirs for certain strains of rotavirus, which creates greater diversity among the strains (Dóró *et al.,* 2014). These zoonotic genes may result in an asymptotic infection but can also lead to mild to serve diarrhoeal symptoms in humans (Palombo *et al.,* 2002; Martella *et al.,* 2010).

2.11. Prevention and treatment of rotavirus:

Children with primary rotavirus infections, typically present with more severe symptoms, especially those who show signs of severe diarrhoea (Bishop *et al.,* 1983; Crawford *et al.,* 2017). Hydration and timely treatment are crucial in managing rotavirus infections (Mokomane *et al.,* 2018). Dehydration can be a severe complication of this disease, it is essential to provide sufficient fluids to individuals, especially children (Mokomane *et al.,* 2018). Oral rehydration solutions (ORS) can be used to replenish lost fluids and electrolytes (Marlin *et al.,* 1998). In healthcare settings, proper infection control measures, such as isolation of people presenting with symptoms of gastroenteritis can reduce the likelihood of transmission, as well as the use of appropriate personal protective equipment by healthcare workers, are important to prevent the nosocomial spread of the virus (Mokomane *et al.,* 2018). Frequent handwashing with soap and water is a simple yet effective measure to prevent contamination, especially after diaper changes, using the toilet, and before preparing or consuming food (Mokomane *et al.,* 2018). Zinc is beneficial for the rehydration process (Liberato *et al.,* 2015). Zinc plays a crucial role in reducing the severity and duration of diarrhoea, it boosts the immune system and supports intestinal health (Liberato *et al.,* 2015). Zinc is not a direct replacement for fluids but rather complements rehydration efforts (Liberato *et al.,* 2015). Health education is also important to raise awareness about rotavirus, its symptoms, and preventive measures (Parashar *et al.,* 2013). This helps in promoting vaccination and implementing good hygiene practices (Parashar *et al.,* 2013).

It is important to note that while these preventive measures are effective, vaccination is considered the most significant intervention for controlling rotavirus infections (Patel *et al.,* 2011). These vaccines are administered in multiple doses and have demonstrated a high degree of efficacy in reducing the severity and incidence of rotavirus gastroenteritis (Vesikari *et al.,* 2012). Vaccination is typically recommended

for infants, often starting at around six weeks of age (Patel *et al.,* 2010). Several vaccines are available and included in many national immunisation programs (Vesikari *et al.,* 2012).

2.12. WHO prequalified vaccines:

Four rotavirus vaccines have been prequalified by the WHO for global use after undergoing thorough studies of their effectiveness and safety. The objective is to further alleviate the impact of rotavirus sickness. Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium), Rotavac® (Bharat Biotech International Limited, Hyderabad, India), RotaTeq® (Merck & Co. Inc, Kenilworth, NJ, USA), and Rotasiil® (Serum Institute of India, Pune, India) are the WHO prequalified vaccines.

In 1998, the RotaShield® vaccine was introduced in the United States as the first rotavirus vaccine. However, it was later withdrawn from the market due to adverse effects, including bloody stool and intussusception (Glass *et al.,* 2005). Subsequently, two primary rotavirus vaccines were developed: RotaTeq® and Rotarix®. RotaTeq® is a pentavalent live attenuated vaccine, containing reassortant strains G1-G4 and P1A[8], while Rotarix® is a monovalent vaccine based on the G1P[8] human strain (Vesikari *et al.,* 2006). Additionally, two other vaccines, Rotasiil® and Rotavac®, were developed – Rotasiil® is a bovine-human reassortant pentavalent vaccine, and Rotavac® is a monovalent neonatal human attenuated vaccine based on the G9P[11] strain (Bharat Biotech, 2019).

2.12.1. RotaTeq® vaccine:

RotaTeq® is a pentavalent human-bovine reassortant rotavirus vaccine and is composed of live attenuated rotaviruses derived from both human and bovine strains (Vesikari *et al.,* 2006). The vaccine contains five reassortant rotaviruses representing the G1, G2, G3, G4, and P1A[8] serotypes, bovine backbone of the parental strain WC3

(G6P7[5]), which are responsible for most severe rotavirus infections (Heaton *et al.,* 2005). RotaTeq® is typically administered to infants in a three-dose series, starting at six weeks of age (Heaton *et al.,* 2005). The subsequent doses are given at 10 and 32 weeks (Matthijnssens *et al.,* 2010). The vaccine is often co-administered with other routine childhood vaccinations (Tanaka *et al.,* 2017).

2.12.2. Rotarix® vaccine:

Rotarix® is a monovalent rotavirus vaccine, which was developed by GlaxoSmithKline (GSK) (Bernstein *et al.,* 1999). Rotarix® is a live attenuated rotavirus vaccine derived from a licenced human G1P[8] strain (Dennehy *et al.,* 2008). The vaccine undergoes a series of genetic reassortment processes to reduce its virulence while maintaining its ability to stimulate a protective immune response (Dennehy *et al.,* 2008). The recommended schedule for Rotarix® vaccination involves two doses (WHO, 2013). The first dose is typically administered at 6 weeks of age, and the second dose is given at least four weeks apart but not after 24 weeks of age (Dennehy *et al.,* 2008; WHO, 2013). The vaccine is administered orally, either directly into the infant's mouth or mixed with a small amount of water.

2.12.3. RotaVac® vaccine:

Rotavac® is an oral, live attenuated rotavirus vaccine that was developed in India by Bharat Biotech in collaboration with various national and international partners, and was licensed in 2014, and prequalified by the WHO in 2018 (Kirkwood *et al.,* 2019). Rotavac® is a monovalent vaccine based on a naturally occurring strain of human rotavirus (G9P[11]) (Das *et al.,* 1993; Bhandari *et al.,* 2014). The vaccination schedule for Rotavac® typically involves three doses (Ella *et al.,* 2018). The first dose is usually given at 6 weeks of age, followed by two additional doses at 10 weeks and 14 weeks of age (Bharat Biotech, 2019).

2.12.4. Rotasiil® vaccine:

Rotasiil® is an oral vaccine produced by the Serum Institute in India, and was licensed in 2014 (WHO, 2014; Zade *et al.,* 2014). Rotasiil® is a pentavalent bovine-human reassortant live attenuated vaccine, which includes strains representing the G1, G2,

G3, G4, and P[8] serotypes (Kapikian *et al.,* 2005). The recommended vaccination schedule for Rotasiil® involves three doses (Zade *et al.,* 2014). The first dose is typically administered at around 6-8 weeks of age, with the second and third doses given at 10 and 14 weeks respectively (Kapikian *et al.,* 2005; Zade *et al.,* 2014).

2.13. Introduction of the rotavirus vaccine in African countries:

The introduction of vaccines in the EPIs of the countries and in part, improvements in sanitation have greatly reduced the rotavirus disease burden which was around 500,000 during the prevaccine period to 128,500 during the postvaccination period (Parashar *et al.,* 2006; Troeger *et al.,* 2018). Hospital admissions associated with rotavirus disease decreased significantly following the introduction of the vaccine (Burnett *et al.,* 2020). Hospitalisations of children under the age of five in African countries due to rotavirus decreased by a median of 59% after the introduction of the vaccine (Aliabadi *et al.,* 2019; Burnett *et al.,* 2020).

Several countries that introduced the rotavirus vaccine in its early stages, including Togo, Malawi, Burkina Faso, Ghana, and Zimbabwe, observed significant reductions in rotavirus-associated hospitalisations (35-80%) (Bar-Zeev *et al.,* 2016; Tsolenyanu *et al.,* 2016; Mujuru *et al.,* 2017; Bonkoungou *et al.,* 2018). Additionally, research has revealed that rotavirus vaccines possess the capability to confer herd immunity, a protective effect that extends to unvaccinated members of the population due to a portion of the population receiving the vaccine (John and Samuel, 2000). This was reported by Bennett and colleagues in Malawi, where the positivity rate of rotavirus decreased in unvaccinated babies, which proves the indirect effects of the vaccine (Bennett *et al.,* 2018). While there has been a significant positive impact post- vaccine introduction, a lower efficacy of the vaccine has been reported in developing countries in sub- Saharan Africa and Asia (Bányai *et al.,* 2011; Tate *et al.,* 2016; Velasquez *et al.,*

2017). Probable explanations for this observation in these regions, may be the constant evolutionary mechanisms, strain diversity, seasonality, maternal antibodies, malnutrition, co-infections with other enteric pathogens, and the limited access to health care facilities and proper sanitation (Glass *et al.,* 2006; Bányai *et al.,* 2011; Moon *et al.,* 2016; Soares-Weiser *et al.,* 2019).

Africa has introduced the rotavirus vaccine to over 70% of their countries, and 35 countries have introduced the rotavirus vaccine to their nation immunisation programs, yet the disease burden remains extremely high compared to other higher-income countries (Tate *et al.,* 2016). South Africa was the initial African country to administer the rotavirus vaccine, utilising the Rotarix® vaccine, in 2009. In May 2012, Rwanda became the first low-income country to implement the Rotateq® vaccine (Madhi *et al.,* 2012; Seheri *et al.,* 2018). The RotaTeq® vaccine was introduced to several African countries, including Morocco, Gambia, Rwanda, Burkina Faso, and Libya (Seheri *et al.,* 2018). From 2012 to 2015, the Rotarix® vaccine was introduced in countries such as Kenya, Tanzania, Ethiopia, Zimbabwe, Zambia, and Uganda (Weldegebriel *et al.,* 2018).

2.14. The Rwandan context:

Rwanda is a small low-income country located in East Africa, with a high burden of rotavirus disease. Rwanda bears a heavy burden of morbidity and mortality associated with RVA-related diseases (Ngabo *et al.,* 2016). With approximately 3500 deaths recorded annually, the disease accounts for a significant proportion of child mortality in this region (GAVI, 2012). Rwanda was one of the early countries with low incomes to implement RotaTeq®, and the coverage rate in the first year of use was a remarkable 99% (Gatera *et al.,* 2016). It was reported that 98% of the population received the RotaTeq® vaccine in 2019 (Sibomana *et al.,* 2018; WHO 2020).

Significantly, within the initial three years following the implementation of RotaTeq® in Rwanda, there was a notable decline of 25–44% in hospitalisations attributed to diarrhoeal illnesses among children (Sibomana *et al.,* 2018). Furthermore, a significant reduction of 61% to 70% in the occurrence of diagnoses specific to RV was documented in the area (Vesikari *et al.,* 2016). On the contrary, Rwanda implemented the Rotarix® vaccine in April 2017 because of financial constraints (Mandomando *et al.,* 2021). The implications of this change on potential novel circulating strains of rotavirus in the foreseeable future remain uncertain and warrant further investigation. Rwanda also shows a correlation between the dry season (July to September) and increased rotavirus cases, as during this time there is less rain, which results in low water supply leading to improper sanitation (Uwimana *et al.,* 2015).

According to Seheri and colleagues, the G1P[8] was the prevailing strain that was in circulation in 2011 (prevaccination era) (Seheri., et al 2018). The G8P[4] emerged as the prevailing strain in 2013 (regardless of vaccination status), comprising 56% of reported cases (Doro *et al.,* 2014). The G4P[8] and G12P[8] genotypes became the dominant strains in Rwanda circulating in 2014, with G8P[4] re-emerging in 2015 (Seheri *et al.,* 2015). An in-depth understanding of various aspects, including the genetic linkage of RVA gene segments, the origin of strains, the identification of atypical reassortants and zoonotic events, and the potential consequences of RVA vaccines on the genetic and antigenic composition of circulating RVA in the Rwandan region, can be obtained through whole genome analysis (Nyaga *et al.,* 2020; Donato *et al.,* 2021).

2.15. Next generation sequencing (NGS) technologies:

Next Generation sequencing techniques enables full genome analysis of bacteria, viruses, fungi, plants, animals, and other microorganisms, which is now an essential

part of modern-day science (Vincent *et al.,* 2017). Next generation sequencing technologies have revolutionised the study of viruses, offering unprecedented insights into their genomic diversity, evolution, and pathogenicity (Vincent *et al.,* 2017). This technology involves a suite of high-throughput sequencing techniques that allow rapid and simultaneous sequencing of millions of DNA or RNA fragments (Ansorge *et al.,* 2009). In the context of viruses, Next Generation Sequencing (NGS) has become a vital tool for comprehensive genome analysis (Ansorge *et al.,* 2009).

One significant application is metagenomic sequencing, which enables the identification and characterisation of viral populations within complex biological samples, including clinical specimens and environmental samples (Bibby *et al.,* 2013). This unbiased approach has been instrumental in the discovery of novel viruses and the monitoring of viral diversity (Bibby *et al.,* 2013). Additionally, NGS facilitates the tracking of transmission and the spread of viral outbreaks by providing detailed genomic information on viral strains, aiding in epidemiological investigations (Quer *et al.,* 2022). The technology's ability to generate large datasets promptly, has also enhanced the understanding of viral evolution, transmission dynamics, and the emergence of drug-resistant variants (Schloss *et al.,* 2008). As NGS technologies evolve, they will continue to advance the ability to diagnose, monitor, and combat viral infections, ultimately contributing to more effective public health strategies (Schloss *et al.,* 2008).

Sanger sequencing was the first sequencing method developed, thereafter more cost effective and faster NGS techniques were established (Ansorge *et al.,* 2009). These parallel sequencing techniques offers high throughput and speed (Quer *et al.,* 2022). The Illumina MiSeq® sequencing platform, which was utilised in this project, is one of the current market leaders and is a widely used NGS system that has played a pivotal role in advancing genomics research across various disciplines (Van Dijk *et al.,* 2014; Hernandez *et al.,* 2018). Known for its versatility, scalability, and accuracy, the

MiSeq® platform enables the sequencing of targeted regions, small genomes, and entire microbial genomes with high throughput (Bentley *et al.,* 2008). The technology is based on sequencing by synthesis (SBS) technology, allowing for the simultaneous determination of nucleotide sequences in millions of clusters on a flow cell (Mardis, 2008). One of the key features of the MiSeq® is its flexibility in read length, accommodating various applications, including whole-genome sequencing, amplicon sequencing, and metagenomics (Mardis, 2008). This Illumina MiSeq® platform can retrieve data within hours, depending on the application, and can generate up to 25 million reads and 15 Gigabases of data per run (Illumina, 2021) and is able to sequence short fragments with exceptional accuracy (Illumina, 2021). The platforms SBS technology allows for highly accurate base calling, contributing to the generation of high-quality sequencing data (Kumar *et al.,* 2019). The MiSeq® has found widespread use in diverse research areas, including microbiology, oncology, and agricultural genomics (Kumar *et al.,* 2019). Its ability to deliver reliable and reproducible results has made it an essential tool for projects demanding both precision and efficiency (Liu *et al.,* 2012). As sequencing technologies continue to advance, the Illumina MiSeq® remains a fundamental principle in the genomics toolkit, contributing to the ongoing exploration of genetic information and its applications in various scientific endeavours (Rockett *et al.,* 2020).

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CHAPTER THREE: Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort

3.1. Preamble

Chapter three was structured to address the first two objectives of this study as an original article published online in the special issue "Viral Gastroenteritis 2022'' of the *Viruses* Journal. This manuscript is entitled "**Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort**". A copy of the first page of the published manuscript (abstract) can be found in Appendix B. This chapter will include introductory literature, an in-depth look into the general materials and methods, as well as the specific aspects of the data analysis which was performed.

Author contributions to this manuscript are as follows: Conceptualisation (Robyn-Lee Potgieter, Peter Mwangi and Martin Nyaga); methodology (Robyn-Lee Potgieter, Peter Mwangi, Milton Mogotsi, and Martin Nyaga); software (Robyn-Lee Potgieter, Peter Mwangi and Martin Nyaga); formal analysis and data curation (Robyn-Lee Potgieter, Peter Mwangi and Martin Nyaga); writing—original draft preparation (Robyn-Lee Potgieter, Peter Mwangi and Martin Nyaga); writing—review and editing

(all co-authors); supervision (Martin Nyaga); co-supervision (Peter Mwangi); funding acquisition (Martin Nyaga).

Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort

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ABSTRACT:

Although the introduction of rotavirus vaccines has substantially contributed to the reduction in rotavirus morbidity and mortality, concerns persist about the re-emergence of variant strains that might alter vaccine effectiveness in the long term. The G9 strains re-emerged in Africa during the mid-1990s and have more recently become predominant in some countries, such as Ghana and Zambia. In Rwanda, during the 2011 to 2015 routine surveillance period, G9P[8] persisted during both the pre- and postvaccine periods. The prevaccination cohort was based on the surveillance

period of 2011 to 2012, and the postvaccination cohort was based on the period of 2013 to 2015, excluding 2014 as no from this period was identified as the G9P[8] genotype. The RotaTeq® vaccine that was first introduced in Rwanda in 2012 is genotypically heterologous to VP7 G9. This study elucidated the whole genome of Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine introduction. Fecal samples from Rwandan children under the age of five years (prevaccine n = 23; postvaccine n = 7), conventionally genotyped and identified as G9P[8], were included. Whole-genome sequencing was then performed using the Illumina® MiSeq platform. Phylogenetic analysis and pair-wise sequence analysis were performed using MEGA6 software. Distinct clustering of three postvaccination study strains was observed in all 11 gene segments, compared to the other Rwandan G9P[8] study strains. Specific amino acid differences were identified across the gene segments of these three 2015 postvaccine strains. Important amino acid differences were identified at position N242S in the VP7 genome segment of the three postvaccine G9 strains compared to the other G9 strains. This substitution occurs at a neutralisation epitope site and may slightly affect protein interaction at that position. These findings indicate that the Rwandan G9P[8] strains revealed a distinct sub-clustering pattern among postvaccination study strains circulating in Rwanda, with changes at neutralisation epitopes, which may play a role in neutralisation escape from vaccine candidates. This emphasises the need for continuous whole-genome surveillance to better understand the evolution and epidemiology of the G9P[8] strains postvaccination.

Keywords: rotavirus; Rwanda; G9P[8]; vaccination; whole genomic analysis

3.2. Introduction

Group A rotaviruses are a major viral etiological agent of acute and severe gastroenteritis among young children under the age of five years (Walker *et al.*, 2013).

Extensive epidemiological data have demonstrated that by the age of five, an overwhelming 95% of children get infected by the virus, irrespective of their socioeconomic status (Parashar *et al.,* 2006). Before the introduction of rotavirus vaccines, the average annual global mortality cases of rotavirus were estimated at 500,000 (Parashar *et al.,* 2006). After the introduction of rotavirus vaccines, the mortality cases have significantly reduced to roughly 128,500 deaths with 81% of these deaths having occurred in developing countries in southeast Asia as well as sub-Saharan Africa (Troeger *et al.,* 2018). This high rate of morbidity and mortality among RVA-related disease among children from low- and middle-income countries is due to challenges in accessing efficient medical care and living in settings with relatively poor facilities that cannot offer optimal rehydration services (CDC, 2004; Troeger *et al.,* 2018). Other factors that may affect vaccine effectiveness include, among others, maternal antibodies, and high strain diversity of circulating strains in these regions compared to the developed world (Madhi *et al.,* 2010).

To combat the rotavirus disease burden, the WHO has prequalified four rotavirus vaccines for global use after extensive efficacy and safety studies (Kirkwood *et al.,* 2018). In 2006, RotaTeq® (Merck & Co. Inc., Kenilworth, NJ, USA) and Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) were the first two vaccines to be prequalified by the WHO (Vesikari *et al.,* 2016). Later in 2018, the WHO prequalified two additional vaccines: Rotavac® (Bharat Biotech, Hyderabad, India) and Rotasiil® (Serum Institute of India, Pune, India) (https://extranet.who.int/gavi/PQ_Web/ accessed on 17 March 2023). Rwanda introduced the RotaTeq® vaccine in May 2012 and achieved 99% vaccination coverage within the first year of introduction (Gatera *et al.,* 2016). This relatively high vaccination coverage resulted in a sharp decrease in diarrhea-associated hospitalisation (25–44%) and hospitalisation rates among Rwandan children in the first three years post-RotaTeq® introduction (Sibomana *et al.,* 2018). However, in April 2017, Rwanda made a switch to the Rotarix® vaccine due to economic considerations (Sibomana *et al.,* 2018).

Rotavirus is classified in the *Sedoreoviridae* family and has a dsRNA genome of 18,552 bp (https://ictv.global/report/chapter/reovirales accessed on 26 February 2023). The RVA genome has 11 segments contained within icosahedral capsids (Matthijnssens *et al.,* 2008). The RVA gene translation produces 11 and sometimes 12 viral proteins, six structural VPs identified as VP1-VP4, VP6 and VP7, and five or six NSPs denoted as NSP1-5/6 (Patton *et al.,* 2006; Matthijnssens *et al.,* 2008). Conventionally, a binary classification system derived from the immunological properties of the two outer capsid proteins (VP4 and VP7) was used and is still globally accepted to classify rotaviruses into G types and P types, respectively (Patton *et al.,* 2006). These proteins act as neutralisation antigens, providing specificity to RVA strains (Estes and Cohen *et al.,* 1989). With the increase of the use of next generation sequencing, the whole-genome classification scheme is rapidly becoming the more preferred way to classify rotaviruses. Based on this scheme, human RVAs were classified into three genogroups, the Wa-like and the DS-1-like, which are the two major genogroups, and the AU-1-like, which is a minor genogroup (Matthijnssens *et al.,* 2008).

Globally, the most prevalent human RVA genotypes detected are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Moure *et al.,* 2018). Together, they account for up to three quarters of human rotavirus infections worldwide (Zhou *et al.,* 2020). However, in Africa, the most prevalent rotavirus genotype combinations detected between 2006 and 2008 were G1P[8], G2P[4], G9P[8], G12P[8], and G12P[6] (Mwenda *et al.,* 2010). The G9 strains re-emerged in Africa during the mid-1990s and have more recently become predominant in many countries (Nyaga *et al.,* 2013). The G9P[8] genotype was first identified in 1983 in the United States of America and quickly became prevalent globally (Doro *et al.,* 2014). The origin of the VP4 and VP7 genome segments for the vaccine strains are as follows: RotaTeq®—G1, G2, G3, G4, P[8], and G6P[5]; Rotarix®—G1P[8]; RotaVac®—G9P[11]; and Rotasiil®—G1, G2, G3, G4, G9, and G6P[5] (Doro *et al.,* 2014; Sadiq *et al.,* 2022).

The P[8] component is not present in some vaccine candidates such as the RotaVac® and Rotasiil® vaccine (Sadiq *et al.,* 2022). In Rwanda, the G1P[8] was the predominant strain circulating in 2011 (prevaccination era) (Seheri *et al.,* 2018). In 2013 (postvaccination era), G8P[4] become the dominant strain, accounting for 56% of cases (Seheri *et al.,* 2018). The G2P[4] and G12P[6] genotypes became the predominant strains circulating in 2009 to 2010, with G1P[8] and G9P[8] circulating in 2011 to 2012 and the G9P[8] genotype dominating 2013, and in 2014 to 2015, the G12P[8] genotype was circulating in this region, showing how G9P[8] persisted during both the pre- and postvaccine periods (Seheri *et al.,* 2018; Kabayiza *et al.,* 2023).

Although the rotavirus vaccines appear to be having a beneficial impact on the disease burden, there are concerns that their administration may be selecting for the emergence of potential vaccine-escape mutants (Burnett *et al.,* 2020; Kabayiza *et al.,* 2023). Evolutionary mechanisms such as genome reassortment, and rearrangement, have resulted in the emergence of different novel RVA strains that may have the ability to circulate throughout the community (Burnett *et al.,* 2020). These novel circulating strains may contribute to suboptimal effectiveness of the vaccines in regions such as the low-income countries in sub-Saharan Africa and southeast Asia, where strains are highly diverse (Burnett *et al.,* 2020). Vaccines have shown higher effectiveness rates in high-income countries compared to low-income countries (Velasquez *et al.,* 2018; Steele *et al.,* 2019). This emphasises the importance of performing whole-genome analysis, which allows for a greater understanding of the changing aspects of RVA strains pre- and postvaccination in Africa, specifically in low-income countries such as Rwanda (Seheri *et al.,* 2018; Mwangi *et al.,* 2020; Rasebotsa *et al.,* 2021). Therefore, the purpose of this study was to assess any changes in the evolution of the genomic makeup of the G9P[8] strains that were circulating in Rwanda during the pre- and post-RotaTeq® vaccination periods.

3.3. Materials and Methods

3.3.1. Ethical Statement

Ethical approval for this study was sought from the University of the Free State Health Sciences Research Ethics Committee (HSREC), where the study was granted approval under the reference UFS-HSD2022/0983/2709 (Appendix A). The archived patient samples had all their personal information delinked and anonymised.

3.3.2. Sample Description

Fecal samples (n = 158) from Rwandan children under the age of five years were sequenced at the University of the Free State Next Generation Sequencing Unit (UFS-NGS), Bloemfontein, South Africa. Of these samples, 46 were sequenced from the prevaccination era, and 112 from the postvaccine era. Samples (n = 30) that had been previously conventionally genotyped for their VP7 and VP4 genes as G9P[8] were included in this study. From these successfully sequenced samples, 23 were from the prevaccination period and seven from postvaccination period. The focus on the G9P[8] strains was due to their recognised epidemiological significance as well as being not only important in Rwanda, but also well recognised as a globally prevalent genotype.

3.3.3. Rotavirus dsRNA Extraction and Purification

The total viral nucleic acid material was extracted as described previously (Nyaga *et al.*, 2018). Briefly, approximately 100 mg of stool sample was added to 200 μL of phosphate-buffered solution (PBS) (Sigma-Aldrich®, Saint Louis, MO, USA) to create a stool suspension. A total of 300 μL of the stool suspension was added to 900 μL of TRI Reagent® (Sigma-Aldrich®, Saint Louis, MO, USA) after the stool suspension stood for 10 min at room temperature. Thereafter, the mixture was centrifuged (Eppendorf centrifuge 5427R, Hamburg, Germany) at 18,000 RPM for 20 min at 4 °C. A volume of

700 µL of ice-cold isopropanol (Sigma-Aldrich®, Saint Louis, MO, USA) was added, and the mixture was left to dry for 10 min to precipitate the supernatants. The extracted nucleic material was incubated in 8M LiCl2 (Sigma-Aldrich®, St Louis, MO, USA) at 4 °C for 16 h to enrich the dsRNA viruses and remove impurities. The extracted nucleic acid material was thereafter purified using a MinElute gel extraction kit (Qiagen, Hilden, Germany), and the integrity and enrichment of the dsRNA were verified via 1% agarose gel electrophoresis and visualised using an ultraviolet (UV) transilluminator (Sigma-Aldrich®, Saint Louis, MO, USA).

3.3.4. Double-Stranded cDNA Synthesis

Complementary DNA (cDNA) was synthesised on the purified enriched dsRNA by utilising the optimised Maxima H Minus Double Stranded Synthesis Kit protocol (ThermoFisher Scientific, Waltham, MA, USA). This modified protocol entails a first- and second-strand cDNA synthesis step. Briefly, a 13 µL volume dsRNA was denatured for 5 min at 95 °C, allowing the dsRNA to unwind. The denatured RNA was then spun down for 10 s. A 1 µL volume of the random hexamer primer (ThermoFisher Scientific, Waltham, MA, USA) was added to 13 µL of the denatured dsRNA. The tubes were once again spun down and were then placed in a thermocycler (Labnet, Edison, NJ, USA) for 5 min to allow for annealing at 65 °C of the random hexamer primer to the template RNA. Thereafter, 5 µL of the 4 X first-strand reaction mix and 1 µL of the first-strand enzyme mix was added and they were incubated at 50 °C for 30 min. Second-strand synthesis proceeded immediately using the kit's master mix, where 55 µL of nuclease-free water was added to the reaction mixture. This was followed by the addition of a 20 µL volume of 5 X second-strand reaction mix, followed by the addition of 5 µL of second-strand enzyme mix (Thermo Fischer Scientific, Waltham, MA, USA). This mixture was then placed in the thermocycler (Labnet, Edison, NJ, USA) for 60 min at 16 °C to incubate. Thereafter, a 6 µL volume of 0.5 M EDTA (ThermoFisher Scientific, Waltham, MA, USA) with a pH

of 8.0 was added to bring the reaction to a stop. A 10 µL volume RNase 1 (ThermoFisher Scientific, Waltham, MA, USA) was added to remove residual RNA, and this was further incubated for 5 min at room temperature. An MSB® Spin PCRapace purification kit (Stratec Molecular, Berlin, Germany) was used to purify the synthesised cDNA. Purified cDNA was quantified using a Qubit™ 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

3.3.5. NextEra® XT DNA Library Preparation and Whole-Genome Sequencing

DNA libraries were prepared using the NextEra® XT DNA Library Kit (Illumina, San Diego, CA, USA). Briefly, the template DNA was tagmented, followed by indexing, amplification, and clean-up of the genomic DNA using Ampure XP beads (Beckman Coulter, Pasadena, CA, USA). The quality of the libraries as well as the fragment size distribution were thereafter assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). The libraries were normalised to 4 nM and pooled together for MiSeq® sequencing. This was accomplished by combining 5 µL of the individually barcoded libraries into a single Eppendorf® tube (Eppendorf AG, Hamburg, Germany). Thereafter, sodium hydroxide (NaOH) (Sigma-Aldrich®, Saint Louis, MO, USA) was used to denature the pooled libraries. The pooled libraries together with the NaOH resulted in a total volume of 10 µL, which was incubated to allow the DNA to denature into single strands. After denaturation occurred, 990 µL of pre-chilled hybridisation buffer HT1 (Illumina, San Diego, CA, USA) was added, resulting in 20 pM denatured libraries. A further dilution of the 20 pM library was carried out to a sequencing concentration of 8 pM, and a denaturation step of the PhiX positive control (Illumina, San Diego, CA, USA) was added for optimal cluster density. A final volume of 600 µL of diluted library with a 2% PhiX control was loaded into a V3 (600 cycle) reagent kit (Illumina, San Diego, CA, USA), and whole-genome sequencing was performed at 301 × 2 paired-end reads using a MiSeq® benchtop

sequencer (Illumina, San Diego, CA, USA) at the UFS-NGS Unit, Bloemfontein, South Africa.

3.3.6. Data Analysis

Quality control of the raw data was performed using FASTQC v. 0.11.9 to proceed with a Phred data quality score of Q30 (Andrews *et al.,* 2010). The Illumina sequence read ends were analysed using Geneious Prime® software v2022.0.1 (https://www.geneious.com; Kearse *et al.,* 2012), which comprised reference mapping to obtain full-length genomes. The study strains achieved 100% coverage of the ORF in the whole-genome sequencing, as well as 100% coverage of the genome, including the entire coding and noncoding regions. For phylogenetic analysis, the MUSCLE algorithm which is implemented in MEGA X (https://www.megasoftware.net/) was used to align the ORF of each gene segment. After alignment, the DNA Model Test program in MEGA X was utilised for identification of the optimal evolutionary models for phylogenetic analysis. Thereafter, maximum likelihood trees were constructed together with approximately 30 random globally selected RVA reference strains for each gene segment with 1000-replicate bootstrap support, and the p-distance algorithm was used to calculate the genetic distance matrixes between amino acid and nucleotide sequences. The ORF sequences for all 11 genes of these Rwandan G9P[8] strains were sequenced and deposited in the NCBI GenBank under the references OR401005-OR401334 (Appendix C1).

3.4. Results

3.4.1. Whole-Genome Constellation Analysis

All 30 Rwandan G9P[8] strains from both the pre- and postvaccination periods during the 2011 to 2015 surveillance season exhibited the typical Wa-like genotype constellation (G9-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1).

3.4.2. Comparative Analysis of the Neutralising Epitope Regions in the VP7 and VP4 Genome Segments

Amino acid differences were observed when comparing the neutralisation epitopes in the VP7 genome segment between the Rwandan G9P[8] study strains and the four WHO-prequalified vaccine strains. Within the antigenic epitope region of the VP7 genome segment (Aoki *et al.,* 2009), 26 of the 29 amino acids residues were conserved among the Rwandan G9 strains. The Rwandan study strains differed from some of the vaccine strains at three amino acid sites (96, 100, and 242) in the 7-1a and 7-1b antigenic epitope regions. The amino acid difference T96A, observed in one postvaccination study strain, involved a polarity change from a polar to a nonpolar amino acid. This amino acid difference did not correlate with any of the vaccine candidates. When comparing the study strains to the vaccine strains, 20 of the Rwandan study strains (19 from the prevaccination era, and one from the postvaccination era), including the RotaVac® G9 vaccine strain, experienced the amino acid difference D100G, which involved a polarity change from a nonpolar to a polar amino acid. Three study strains from the postvaccination era, with the inclusion of the RotaTeq® G2 vaccine strain, demonstrated the amino acid difference N242S, which involved an amino acid substitution (Table 3.1). No amino acid differences were detected outside the VP7 epitopes.

Table 3.1: Amino acid differences in the neutralising epitope regions of the VP7 gene segment between Rwandan G9P[8] study strains and rotavirus vaccine strains. The coloured shading is used to indicate amino acid differences that were observed. The array of coloured shading of the amino acid residues for the vaccine strains represents changes that were observed when comparing the G9P[8] study strains to these vaccine strains.

		Neutralisation epitopes																																		
		7-1a														7-1b						7-2														
		87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Vaccine strains	RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
	RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G2	A	N	S	D	E	W	E	N	Q	D	T	M	N	K	Q	D	V	S	N	S	R	D	N	T	S	D	I	S	G
	RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G3	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	A	N	K	D	K	D	A	T	L	S	E	A	G
	RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G4	S	T	S	T	E	W	K	D	Q	N	L	I	D	K	Q	D	T	A	D	T	R	A	S	G	E	S	T	S	G
	RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
	RVA/Vaccine/IND/Rotavac-116E/AG/G9P[11]	I	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	N	S	T	L	S	E	N	G
	RVA/Vaccine/IND/Rotasiil-Au32/2016/G9	A	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	T	K	D	S	T	L	S	E	S	G
Pre-vaccination strains	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1618/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1616/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1589/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1586/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1584/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1577/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1573/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1567/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1550/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU1534/2011/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU541/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU471/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU464/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU425/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU413/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU240/2012/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU238/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU226/2012/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU222/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU214/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU213/2012/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU210/2012/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/NGS-UFS-MRC-DFRU229/2012/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
Post-vaccination strains	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU615/2013/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU543/2013/G9P[8]	T	T	G	T	E	W	K	G	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU433/2013/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU8058/2015/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	S	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU8039/2015/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	S	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU10026/2015/G9P[8]	T	T	G	T	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	S	K	D	S	T	L	S	E	S	G
	RVA/Human-wt/RWA/UFS-NGS-MRC-DFRU8031/2015/G9P[8]	T	T	G	A	E	W	K	D	Q	D	A	I	D	K	Q	N	T	A	D	N	K	D	S	T	L	S	E	S	G

Antigenic analysis of the Rwandan G9P[8] study strains compared to the RotaTeq®, Rotarix®, Rotasiil®, and RotaVac® vaccines was performed based on the three VP7 antigenic residues. The amino acids highlighted in light green signify amino acid differences in the Rwandan study strains and the vaccine strains. The postvaccine and prevaccine G9P[8] study strains are in bold and coloured in red and black, respectively.

The neutralising epitope regions of the VP4 genome segment comprise 37 amino acid residues (Dormitzer *et al.,* 2002). Amino acid differences were observed when comparing the VP4 genome segment of the Rwandan G9P[8] study strains and the Rotarix® and RotaTeq® vaccines at three amino acid sites (195, 196, and 113) in the VP8* 8-1 and 8-3 antigenic epitope regions. The amino acid difference G195S, observed in one postvaccination study strain, involved a change from polar to nonpolar. One

Rwandan G9P[8] study strain from the prevaccine era, compared to the vaccine strains, demonstrated the amino acid difference I196L. Seven study strains, including three G9 study strains from the postvaccination era, experienced the amino acid N113D (Table 3.2). When comparing the study strains to the RotaTeq® vaccine, it is noted that the prevaccine study strains already differed from the vaccine strains at multiple epitopes, including one prevaccine strain (I96L) and four prevaccine strains (N113D). No amino acid differences were detected outside the VP4 epitopes.

Table 3.2: Amino acid differences in the neutralising epitope regions of the VP4 gene segment between Rwandan G9P[8] study strains and two of the rotavirus vaccine strains. The coloured shading is used to indicate amino acid differences that were observed. The array of coloured shading of the amino acid residues for the vaccine strains represents changes that were observed when comparing the G9P[8] study strains to these vaccine strains.

		Neutralisation epitopes																																			
		8-1											8-2		8-3								8-4			5-1								5-2	5-3	5-4	5-5
		100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	125	131	132	133	135	87	88	89	384	386	388	393	394	398	440	441	434	459	429	306
Vaccine strains	RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8	D	S	Q	E	S	T	N	L	N	N	I	T	A	N	P	V	S	S	N	D	N	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A8	D	S	Q	E	S	T	N	L	N	D	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	L	W	P	G	R	T	P	E	L	R
Pre-vaccination strains	**RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]**	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	L	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
Post-vaccination strains	**RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]**	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	D	S	Q	D	S	T	N	L	N	S	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	D	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	D	S	Q	D	S	T	N	L	N	G	I	T	A	N	P	V	N	R	N	D	D	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R

Antigenic analysis of the 30 Rwandan G9P[8] study strains compared to the RotaTeq® and Rotarix® vaccines was carried out based on the two structural proteins of the VP4 genome. The amino acids highlighted in light green and light yellow signify amino acid differences when comparing the Rwandan G9P[8] study strains and the vaccine strains. The postvaccine and prevaccine G9P[8] study strains are in bold and coloured in red and black, respectively.

3.4.3. Sequence and Phylogenetic Analysis of the VP7 and VP4 Genome Segments

To determine the genetic relationship between the Rwandan G9P[8] study strains and other global RVA strains (Appendix C2), a phylogenetic analysis was performed. To construct the phylogenetic tree for the VP7 genome segment, lineage designation as per Gupta et al., 2021 was used, which defines six lineages for this genome segment (Figure 3.1). The Rwandan G9 study strains and global RVA reference G9 sequences for the VP7 genome segment were segregated into six lineages. All 30 of the Rwandan G9P[8] study strains clustered into lineage III, which comprised sequences predominantly from the Eastern African region. The Rwandan study strains within lineage III were closely related amongst each other, with nucleotide similarities ranging from 92.5% to 100%. Three postvaccination study strains, which were identified to be noticeably sub-clustered together, demonstrated a nucleotide similarity range of 95,1% to 100%. Within lineage III, several sub-lineages were observed, where a 70% threshold node value was used to define a robust sub-lineage. Therefore, using this criterion for this study, we identified that Rwandan G9 sequences clustered into sub-lineage IIId.

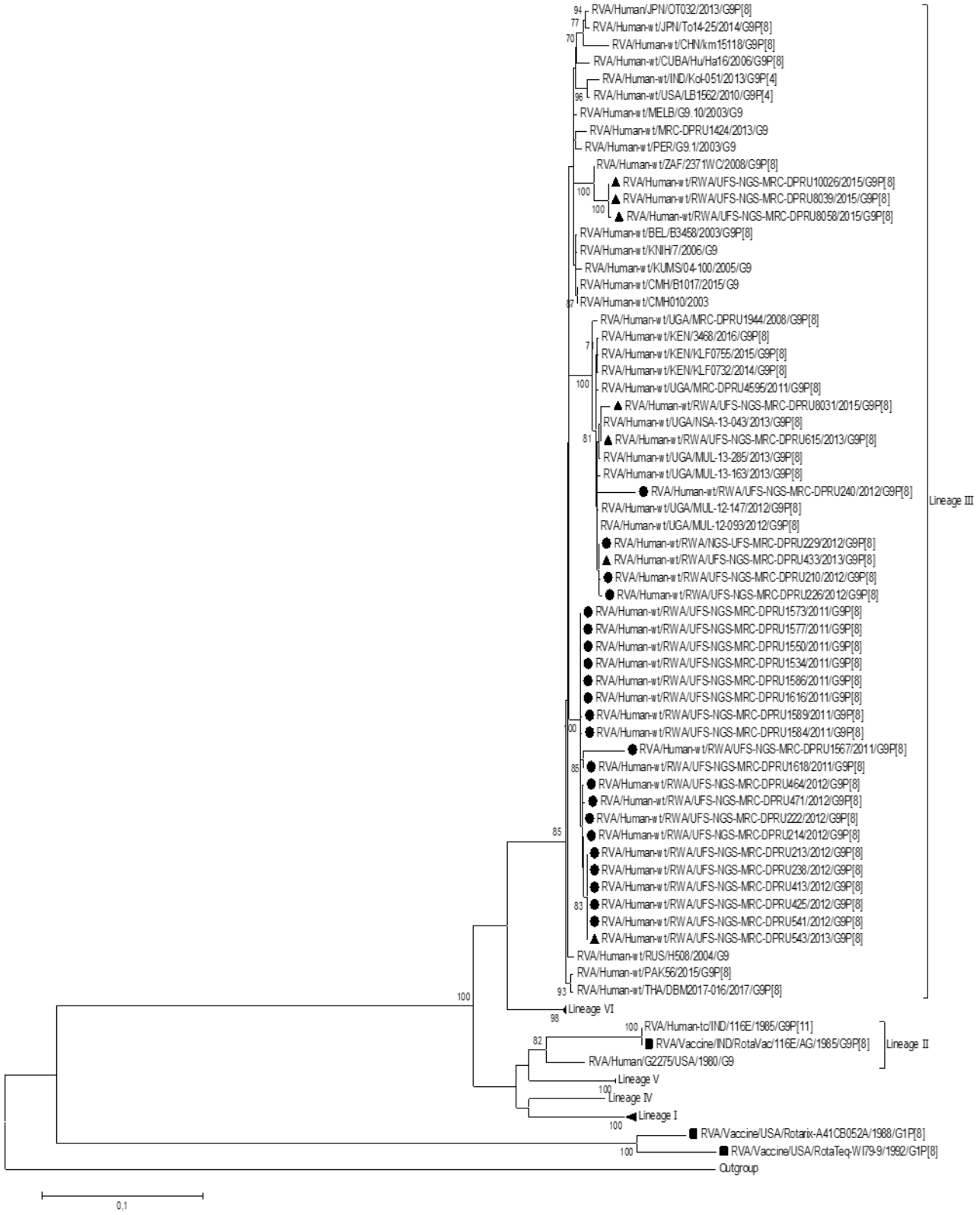

Figure 3.1: Maximum likelihood phylogenetic trees constructed from the nucleotide sequences of the Rwandan G9 strains for the VP7 genome. Using 1000 bootstrap replicates, the branch support was thoroughly evaluated. All G9P[8] study strains clustered into lineage

III. The prevaccination study strains are indicated by a black circular shape, the postvaccination study strains are indicated by a triangular shape, and the vaccine strains are indicated by a square shape.

The VP4 genome segment of the G9 Rwandan study strains was phylogenetically compared to four established lineages (I-IV) (Yan *et al.*, 2019; Gupta *et al.*, 2021) (Figure 3.2). The P[8] sequences of the Rwandan G9P[8] study strains, together with global reference P[8] sequences, were segregated into four lineages. The P[8] study strains all clustered into lineage III, and this lineage comprised sequences primarily from East Africa, more specifically, Kenya and Uganda. Rwandan strains within lineage III were discovered to be highly linked, with nucleotide similarity ranging from 96% to 100%. The VP4 genome segment showed 96,6% to 100% nucleotide similarity within the different sub-clustering pattern of the three 2015 postvaccination research strains. Several robust sub-lineages were defined within lineage III using a 70% threshold node. Thus, we identified that the Rwandan G9P[8] sequences clustered into sub-lineage IIIa and IIIb, respectively, within lineage III. Sub-lineage IIIa comprised 23 Rwandan G9 sequences, including three G9 sequences from 2015, and sub-lineage IIIb comprised seven Rwandan G9P[8] study strains.

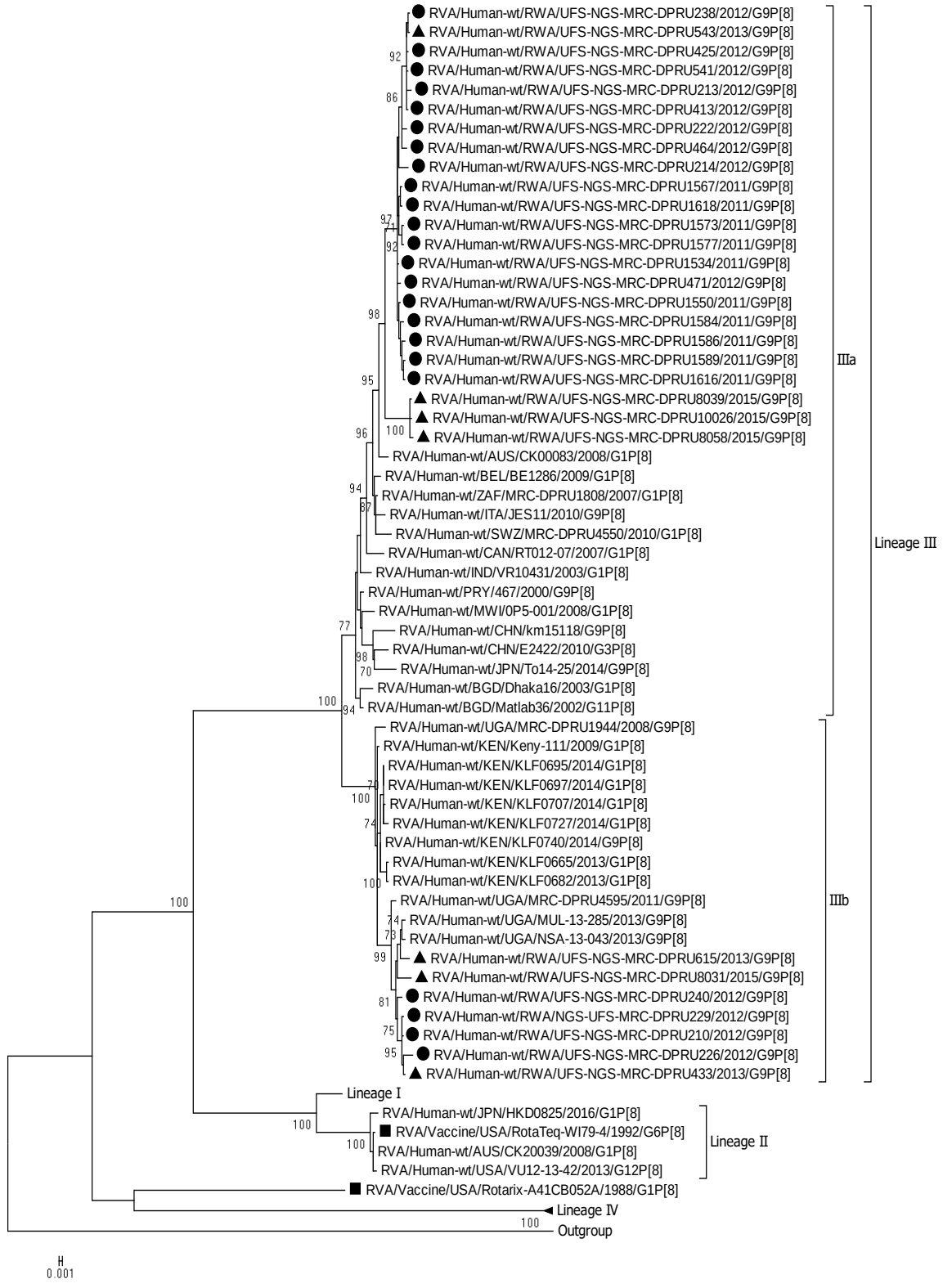

Figure 3.2: Maximum likelihood phylogenetic trees constructed from the nucleotide sequences of the Rwandan P[8] strains for the VP4 genome. Using 1000 bootstrap replicates, the branch support was thoroughly evaluated. All G9P[8] study strains clustered into Lineage III. The prevaccination study strains are indicated by a black circular shape, the

postvaccination study strains are indicated by a triangular shape, and the vaccine strains are indicated by a square shape.

3.4.4. Sequence Analysis and Phylogenetic Analysis of the VP1-3, VP6, and NSP1-5 Genomes

The remaining nine genome segments, (VP1-3, VP6, and NSP1-5) (Supplementary Materials) together with the Rwandan G9 pre- and postvaccination study strains, phylogenetically clustered closely with other selected reference global RVA strains. For these genome segments, the Rwandan G9P[8] study strains also clustered closely with reference strains originating from Eastern African regions, with nucleotide similarity ranges of 95.4% to 100%.

The VP1 sequences were found to be closely related amongst each other, with a moderate nucleotide similarity range of 93.7% to 100%, and amongst the three sub-clustering postvaccine strains, a 93.7% to 100% similarity range was reported. Within the VP2 gene sequences, the Rwandan study strains shared high nucleotide identities in a range of 95.8% to 100%. The three distinct postvaccine strains, which sub-clustered closely together, demonstrated a nucleotide similarity range of 97.9% to 100% for the VP2 genome segment. A nucleotide similarity range of 94.2% to 100% was observed amongst the VP3 genome segment of the G9 study strains. A range of 96.9% to 100% was observed amongst the three postvaccine strains, which were noticeably sub-clustered together. For the VP6 genome segment, the 30 Rwandan study strains, when compared to the RVA reference strains, were found to be closely related amongst each other, with a moderate nucleotide similarity range of 94.7% to 100%, and amongst the sub-clustering postvaccine strains, a 94.7% to 100% similarity range was reported.

The five non-structural proteins presented a diverse range of nucleotide similarities, firstly, with a nucleotide similarity range of 95.7% to 100% amongst the Rwandan G9 study strains for the NSP1 genome segment. The three distinct postvaccine strains, which were sub-clustered closely together, demonstrated a nucleotide similarity range of 97.7% to 99.9% for this genome segment. The NSP2 genome of the 30 G9P[8]

study strains presented a moderate nucleotide similarity range of 90.4% to 100%. A range of 97.5% to 100% was observed amongst the postvaccine strains, which were noticeably sub-clustered together. For the NSP3 gene sequences, the pre- and postvaccination study strains clustered closely together and revealed a shared nucleotide identity in the range of 92% to 100%, and amongst the three sub-clustering postvaccine strains, a 98.9% to 100% similarity range was reported. Phylogenetically, the global RVA reference strains were compared to the NSP4 genome of 30 Rwandan G9P[8] study strains and found to have a distinct nucleotide similarity range of 81.5% to 100% amongst each other. The postvaccine strains, which were sub-clustered distinctly together demonstrated a nucleotide similarity range of 97.1% to 100%. The NSP5 gene sequences of the 30 Rwandan study strains clustered closely together and demonstrated the highest level of similarity, with a range of 97.4% to 100%. A high range of 99.7% to 100% was observed amongst the three postvaccine strains, which were strikingly sub-clustering together.

3.5. Discussion

This study analysed 30 Rwandan G9P[8] study strains at the whole-genome level, revealing that all the G9 sequences from both the pre- and postvaccination periods exhibited the pure Wa-like genotype constellation. Pure genome constellations originating from the same genotype are suggested to be a result of the co-evolution of optimal functioning proteins and epidemiological fitness (McDonald *et al.,* 2009; Nyaga *et al.,* 2015). This may also suggest that the pure nature of the constellation of these strains has contributed to them persisting in the population during the pre- and postvaccination periods (McDonald *et al.,* 2009; Koukou *et al.,* 2022).

Phylogenetically, all 11 genome segments of the Rwandan G9P[8] study strains were highly similar and clustered closely together with the global reference RVA G9P[8]

strains and were further segregated into lineages that either consisted solely of pre- or postvaccination strains, or that consisted of a mixture of the two vaccination periods. The emergence of defined lineages or sub-lineages is credited to diverse evolutionary mechanisms such as recombination, mutations, and reassortments (Kirkwood *et al.,* 2010; Agbemabiese *et al.,* 2019; Rasebotsa *et al.,* 2021).

A unique sub-clustering pattern was observed in three of the 2015 Rwandan G9P[8] postvaccination study strains, which created a significant difference between the pre- and postvaccination strains that are circulating in Rwanda. This may be due to the vaccine candidate being used, which failed to provide protection against the ever-changing virus and may not be as affective against these postvaccination strains (Jiang *et al.,* 2010; Steele *et al.,* 2012; Bányai *et al.,* 2018). All 30 of the Rwandan G9 study strain sequences clustered into lineage III for the VP7 and VP4 genome segments, and furthermore, clustered into further defined sub-lineages (Gupta *et al.,* 2021). The same has been reported in other studies in Africa and Asia, where both pre- and post-G9P[8] vaccination strains have clustered into lineage III, highlighting its predominance (Hoshino *et al.,* 2005; Santos *et al.,* 2005; Page *et al.,* 2010; Jere *et al.,* 2011; Mullick *et al.,* 2014). The G9 genotype typically clusters into lineage III, as all the Rwandan G9 study strains clustered into lineage III; this may demonstrate pre-existing G9 immunity in the population due to G9 strains being present previously.

Three postvaccination Rwandan strains from 2015 distinctly sub-clustered together for all 11 genome segments. A close phylogenetic relationship was observed between the G9 study strains and reference sequences from the Eastern African regions, more specifically, Kenya and Uganda, suggesting co-circulation amongst these neighbouring countries (Nakagomi *et al.,* 2013; Burnett *et al.,* 2022). Due to the high nucleotide similarities between the Rwandan G9P[8] study strains and those strains from neighbouring countries, this suggests local evolution. This underscores the importance of sharing epidemiological data amongst neighbouring countries to

monitor the circulation and emergence of strains, as rotavirus can readily disseminate throughout the population (Burnett *et al.,* 2013).

Sequence analysis of each gene segment revealed high nucleotide similarities (92.4–100%) for all gene segments except for the NSP2 and NSP4 genome segments, which presented a moderate nucleotide similarity range of 90.4% to 100%, and a diverse nucleotide similarity of 81.5% to 100%, respectively, when compared with the globally selected RVA G9P[8] reference strains. This may suggest that the G9 study sequences are related through evolutionary changes from a common ancestral sequence due to the high degree of nucleotide similarity amongst themselves (Martinez-Laso *et al.,* 2009).

This study identified amino acid differences in the neutralisation epitope regions between the VP7 and VP4 study strains and the Rotarix®, RotaTeq®, Rotavac®, and Rotasiil® vaccine strains. The Rotasiil® and RotaVac® vaccine strains were not included in the analysis of the VP4 neutralisation epitope, as VP4 in these vaccine strains is of non-human origin (Sadiq *et al.,* 2022; Vetter *et al.,* 2022). In addition, the RotaTeq® VP4 P[8] clustered in lineage II, and the VP4 P[8] of the Rwandan study strains clustered into lineage III, which translates to VP4 amino acid differences prior to vaccination. At position 113, 4/23 prevaccine strains contained aspartic acid. The 19 remaining prevaccine strains and the RotaTeq® vaccine strain contained asparagine at that position. Postvaccination, 3/7 study strains contained the N113D substitution, which could suggest vaccine pressure at this epitope, but additional data would be required to support this conclusion. The RotaTeq® vaccine introduced in Rwanda is genotypically heterologous to VP7 G9, and this may be a factor in the dominance of the G9P[8] genotype before vaccine introduction, as it was circulating in 2012 and 2013 (Ansaldi *et al.,* 2007; Tatte *et al.,* 2011).

The observed amino acid differences included amino acid substitutions, as well as radical changes, meaning a change was observed in polarity or charge. These changes

may play a role in the neuralisation escape of the vaccine candidate for these strains and may contribute to the escape of host immunity, as these findings were specifically found postvaccination introduction (Trinh *et al.,* 2007). These changes may also contribute to the decreased antibody binding at those regions of the epitopes, which results in the epitope becoming inaccessible (Zeller *et al.,* 2015).

Due to the asymmetric nature and limited sample size of the presented dataset, a limitation to our study was observed, as a greater number of samples were sequenced prevaccination compared to samples that were sequenced from the postvaccine era. Despite this limitation, we believe that this study provides significant insight into the evolution of G9P[8] strains circulating in Rwanda, which can be valuable in potentially predicating the vaccine's impact in this region.

In conclusion, our findings indicate that the Rwandan G9P[8] strains revealed a distinct sub-clustering pattern among postvaccination 2015 study strains circulating in Rwanda, with changes at neutralisation epitopes, which may play a role in neutralisation escape from vaccine candidates for these strains. This emphasises the need for continuous whole-genome surveillance to better understand the evolution and epidemiology of the G9P[8] strains postvaccination, and to further assess the vaccine's impact on circulating rotavirus strains in Rwanda.

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CHAPTER FOUR:

Whole Genome Analysis of Rwandan G9P[8] Rotavirus Strains Reveals Positive Selection in Amino Acid Site 5 and 587 of Viral Protein 4

4.1. Preamble

This chapter was presented in a publishable manuscript format (Appendix B) entitled "**Whole Genome Analysis of Rwandan G9P[8] Rotavirus Strains Reveals Positive Selection in Amino Acid Site 5 and 587 of Viral Protein 4**", addressing the third objective of this study in an overlapping manner. This manuscript is under development to be published. This chapter will include introductory literature, as well as a methodology section which only includes aspects of analysis that are different to what was described in chapter three above.

Robyn-Lee Potgieter, Martin Nyaga, and Peter Mwangi conceptualised this project. Robyn-Lee Potgieter, Peter Mwangi, and Martin Nyaga performed the laboratory work and bioinformatics analysis. Robyn-Lee Potgieter prepared the original manuscript draft, with reviews from Peter Mwangi, Martin Nyaga, and all the other co-authors. This project was supervised and funded by Martin Nyaga, with co-supervision from Peter Mwangi.

Whole Genome Analysis of Rwandan G9P[8] Rotavirus Strains Reveals Positive Selection in Amino Acid Site 5 and 587 of Viral Protein 4

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ABSTRACT:

Young children and infants below the age of five years in low- and middle- income countries such as Rwanda, have a higher burden of rotavirus disease and diarrhoeal-associated disease compared to high-income countries. The G9 strains re-emerged in Africa during the 1990s and has become predominant in some countries, with the G9P[8] genotypes persisting both in the pre- and the post- vaccination eras. A comprehensive whole-genome analysis study was performed on 30 Rwandan Group A rotavirus (RVA) samples from the pre- and post- RotaTeq® vaccination era. Selection pressure analysis was performed using a suite of tools from the DataMonkey Webserver. Protein modelling was performed using the Swiss-Model protein structure homology-modelling server. The amino acid sites across all the 11 genome segments

were under purifying selection, although positively selected sites (5, 587) were identified on the VP4 genome segment, which may affect cell attachment on this genome segment. Amino acid differences in the neutralisation epitope regions are observed on the VP7 and VP4 Rwandan study strains. The observed amino acid differences included radical changes meaning a change was observed in polarity or charge, as well as amino acid substitutions. These changes may play a role in neutralisation escape of the vaccine candidate for these strains and may contribute to the escape of host immunity. Protein modelling analysis indicated high structural homology between pre- and postvaccine structures because of originating from a common ancestor. This highlights the need for constant whole-genome surveillance to better recognise the evolution and epidemiology of G9P[8] rotavirus strains, and to further assess the vaccine impact on circulating rotavirus strains in Rwanda.

Key words: rotavirus; Rwanda; G9P[8]; vaccination; epitope; whole-genomic analysis, amino acid, selection pressure, protein modelling

4.2. Introduction

Diarrhoeal diseases are the second major cause of morbidity and mortality in children under the age of five, second only to pneumonia (Walker *et al.,* 2013). Among these diseases, RVA stands out as the major viral etiological agent responsible for acute and severe gastroenteritis in young children and infants (Parashar *et al.,* 2006). To alleviate the rotavirus disease burden, the WHO has prequalified four rotavirus vaccines for global use (Vesikari *et al.,* 2016). The introduction of vaccines in the EPI of countries and in part, improvements in sanitation have greatly reduced the rotavirus mortality rate (Parashar *et al.,* 2006; Troeger *et al.,* 2018).

Rwanda bears a heavy burden of morbidity and mortality associated with RVA-related diseases (Ngabo *et al.,* 2016). Notably, the introduction of RotaTeq® in Rwanda in 2012 resulted in a significant reduction in diarrhoeal-associated hospitalisations among children (25-44%) within the first three years (Sibomana *et al.,* 2018). Additionally, a substantial decline of between 61% to 70% was reported in the incidence of RV-specific diagnoses in the region (Vesikari *et al.,* 2016). However, in April 2017, Rwanda made a switch to the Rotarix® vaccine due to economic factors (Mandomando *et al.,* 2021). The implications of this change on potential novel circulating strains of rotavirus in the foreseeable future remain uncertain and justify further investigation.

Rotavirus is part of the *Sedoreoviridae* family and has a genome size of ~18 kp (Matthijnssens *et al.,* 2022). The genome of rotavirus is segmented, which allows for events of reassortment which may result in the emergence of reassortant RVA strains (Rasebotsa *et al.,* 2020; Maringa *et al.,* 2021). The RdRp can cause an accumulation of repeated point mutations, due to its error-prone nature (Estes and Greenberg *et al.,* 2013). Due to the variation in mutation rates among the different RVA genome segments, this may exert different selective pressures on these genome segments (Jere *et al.,* 2018; Nyaga *et al.,* 2018).

While introduction of the vaccine suggested that a positive impact was being made on the population, concerns emerged that administration of the vaccine might be selecting emergence of potential vaccine-escape mutants (Leshem *et al.,* 2014; Linhares *et al.,* 2014; Zeller *et al.,* 2017; Jere *et al.,* 2018). Due to the complex evolutionary mechanisms such as genome reassortment, zoonotic transmission, and rearrangement, have resulted in the emergence of different novel RVA strains capable of spreading throughout the community (Kirkwood *et al.,* 2011; Velasquez and Jiang, 2019). These novel circulating strains may contribute to low effectiveness of the vaccines in low-income countries where strains are highly diverse (Carvalho and Gill,

2018; Burnett *et al.,* 2020). Notably, developed countries have reported higher vaccine efficacy rates compared to developing and low-income countries (Narang *et al.,* 2009; Cunliffe *et al.,* 2012).

Using powerful bioinformatics tools such as selection pressure analysis and protein modelling, we were able to significantly contribute to the understanding of biological processes, evolution, and the structure-function relationships of proteins (Caddy *et al.,* 2021; Wu *et al.,* 2022).

Selection pressure analysis is performed to gain insights into the evolutionary forces acting on RVA strains, which can explain advantageous and disadvantageous mutations (Domingo *et al.,* 2015). For RVA, this type of analysis is particularly valuable for understanding the dynamics of host-pathogen interactions (Raque *et al.,* 2022). Looking at the selective pressures which are acting on the genomes, specific regions can be identified to be under positive, negative, or neutral selection (Biswas *et al.,* 2006). Positive selection refers to the evolutionary process that favours the retention of mutations that enhance the ability to survive and replicate within its host (Aguileta *et al.,* 2009). Positive selection acts on the VP4 and VP7 genome segments, as they are crucial for host cell attachment and immune evasion (Amimo *et al.,* 2021). These regions undergo continuous adaptive changes to evade host immune responses, allowing the virus to persist and spread effectively (Amimo *et al.,* 2021). Positive selection primarily driven by the host immune systems' selective pressure, leading to the emergence of new strains with altered antigenic properties (Doro *et al.,* 2015). Understanding positive selection is essential for vaccine development, as it helps identify conserved regions that can be targeted to create effective vaccines (Takala *et al.,* 2009). Additionally, positive selection studies contribute to the understanding of the evolutionary dynamics of RVA, aiding in the development of strategies to control and prevent infections in young children (Omatola *et al.,* 2022). Identification of regions under negative selection highlights the conservation of genetic elements

essential for viruses functionality or stability (Song *et al.,* 2009). This information is helpful in designing effective therapeutic interventions. Therefore, selection pressure analysis contributes significantly to predicting potential changes in circulating strains, which assist in surveillance and preparedness efforts to counter emerging threats (Pitzer *et al.,* 2011).

Protein modelling is a crucial approach that involves the computational construction of 3D structures for viral proteins (Almeida *et al.,* 2017). The structures that are usually visualised are those that have key interacts with host cells, therefore for RVA, we look at the VP4 and VP7 (Mendoza *et al.,* 2023). Protein modelling aids in predicting the impact of mutations on the structure and function of proteins (Kuhlman *et al.,* 2019). As RVAs are undergoing continuous evolution, studying these variations through protein modelling allows us to distinguish potential changes in antigenic properties, which play an essential role in vaccine development (Omatola *et al.,* 2022). Therefore, making computational protein modelling a vital tool which contributes to our understanding of RVA and strategies to combat these infections (Usman *et al.,* 2023).

This illustrates the value of performing whole-genome analysis, as it enables an expanded understanding of the ever-evolving variations in RVA strains across Africa, specifically in economically disadvantaged countries like Rwanda (Seheri *et al.,* 2018; Rasebotsa *et al.,* 2021). Therefore, the aim of this study was to assess changes in the evolutionary makeup of the G9P[8] strains circulating in Rwanda during the pre- and post-RotaTeq® vaccine eras.

4.3. Materials and Methods

4.3.1. Ethical Statement

The Health Science Research Ethics Committee of the University of the Free State granted ethical authorisation for this research under the reference number UFS-HSD2022/0983/2709. Each piece of personally identifiable information on the archived patient samples was anonymised and delinked.

4.3.2. Rotavirus dsRNA extraction to whole genome sequencing

Rotavirus dsRNA extraction, purification, cDNA synthesis, library preparation, and whole genome sequencing was performed on 30 Rwandan G9P[8] samples as described in chapter three.

4.3.3. Data analysis

Quality control of the raw data was performed using FASTQC v. 0.11.9 to proceed with data quality of Q30 Phred score (Andrews *et al.,* 2010). The Illumina sequence read ends were analysed using the Geneious Prime® software v2022.0.1 (https://www.geneious.com; Kearse *et al.,* 2012), which comprised trimming, reference-based mapping to obtain full length genomes. The ORF sequences for all 11 genes of these Rwandan G9P[8] strains were deposited in the NCBI GenBank under the reference OR401005-OR401334.

In order to increase the approximation of sites in the genome of the G9P[8] Rwandan RVA strains undergoing a collection of selection pressures (positive, negative and purifying), the genome segments were analysed using a suite of analysis tools from the Webserver DataMonkey (https://www.datamonkey.org/), such as Fast Unconstrained Bayesian AppRoximation for inferring selection (FUBAR), Mixed-Effects Model of Evolution (MEME), as well as Fixed-Effects Likelihood (FEL) (Murrell *et al.,* 2013; Weaver *et al.,* 2018). The FUBAR analysis employs a Bayesian approach and

was performed with a 0.9 posterior probability, while the MEME and FEL methods use a maximum likelihood approach and utilise a 0.1 p-value threshold (Weaver *et al.*, 2018). The Swiss-Model protein structure homology-modelling server (https://swissmodel.expasy.org) was used to construct the VP4 and VP7 proteins, where the validity of these protein structures was assessed. Thereafter, the full image was visualised using PyMol (https://pymol.org; Krieger *et al.*, 2002; Pettersen *et al.*, 2004).

4.4. Results

4.4.1. Whole genome constellation analysis

The whole genome sequences of all 11 genome segments of the 30 Rwandan G9P[8] study strains were determined (Table 1). The study identified that all the G9P[8] Rwandan study strains, both before and after vaccination, belonged to the Wa-like constellation, as shown in Table 2.

Table 4.1: The complete nucleotide and amino acid lengths of all 11 genome segments of the Rwandan G9P[8] study strains

Genome segment	VP1	VP2	VP3	VP4	VP6	VP7	NSP1	NSP2	NSP3	NSP4	NSP5
Nucleotide length	3264	2637	2505	2325	1191	978	1458	951	930	525	600
Amino acid length	1088	879	835	775	397	326	486	317	310	175	200

Table 4.2: Whole genome constellations of the 30 G9P[8] Wa-like genotype constellation

	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Prevaccination (n=23)	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
Post-vaccination (n=7)	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1

4.4.2. Selection pressure analysis

The amino acid sites of all 11 genome segments of the 30 Rwandan G9P[8] study strains were undergoing purifying selection apart from the amino acid site 5 and 587 in the VP4 genome segment, which were subjected to positive/diversifying (Figure 4.1; Table 4.3).

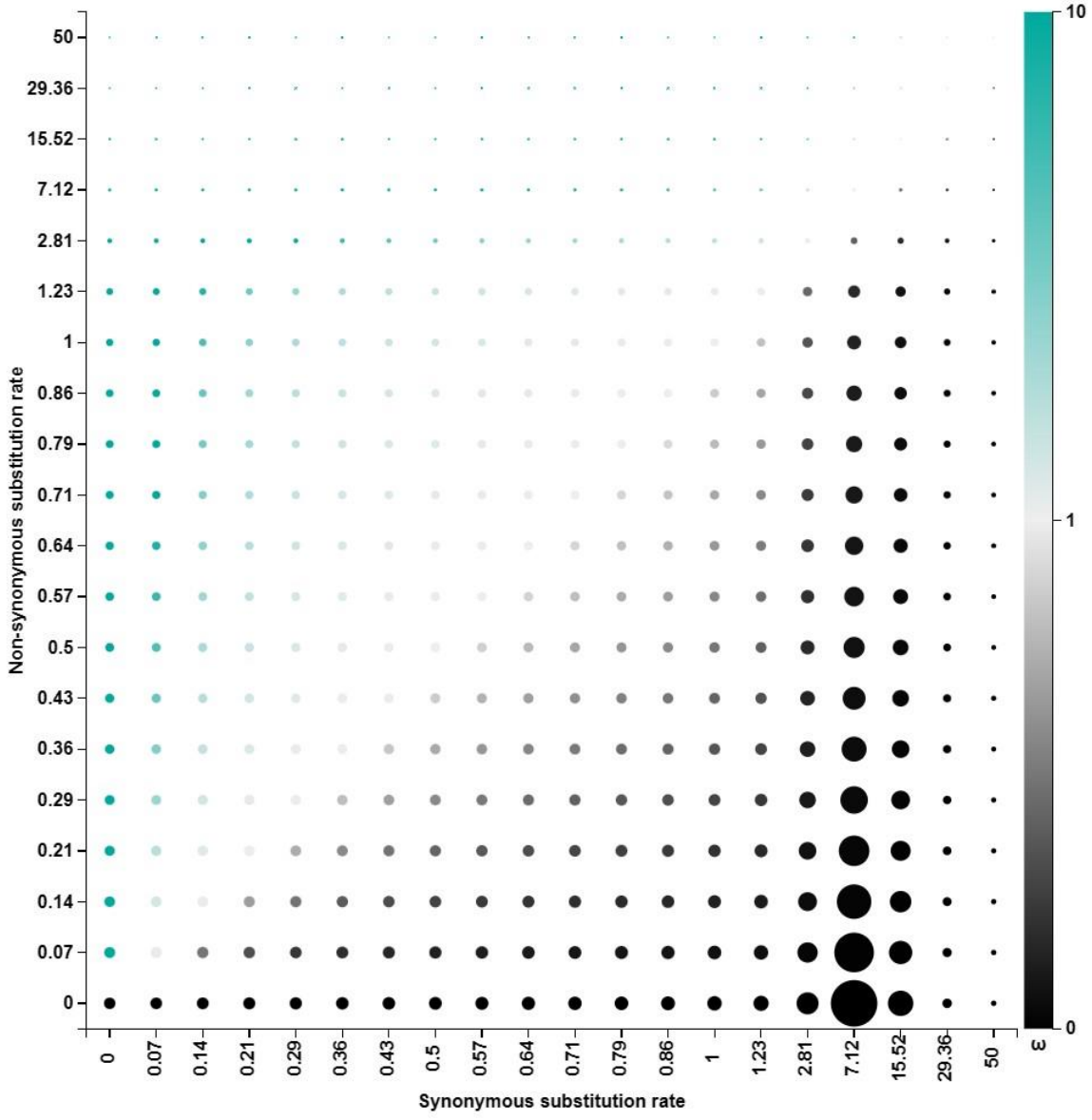

Figure 4.1: This figure shows the posterior distribution over the discretised rate grid for the VP4 genome selection which had two sites under positive selection. The size of a dot is

proportional to the posterior weight allocated to that grid point, and the colour shows the power of selection.

Table 4.3: Positively selected amino acid sites in the 30 Rwandan G9P[8] rotavirus sequences as identified by MEME, FUBAR and FEL analysis

Gene segment	**Positively selected sites**		
	MEME	**FUBAR**	**FEL**
NSP1	-	-	-
NSP2	-	-	-
NSP3	-	-	-
NSP4	-	-	-
NSP5	-	-	-
VP1	-	-	-
VP2	-	-	-
VP3	-	-	-
VP4	**5,587**	**5,587**	**5**
VP6	-	-	-
VP7	-	-	-

Positively selected amino acid sites are indicated by being bolded, with the VP4 genome segment being shaded with a light green. The dash (-) sign indicates that no positive selection site was identified.

The first site (site 5) was found to have two amino acids: Phenylalanine occurring in ~77% (23/30) of the Rwandan G9P[8] study strains and Isoleucine occurring in ~33% (7/30) of the study sequences (Table 4.4). In the second site (site 587) it was found to have three amino acid residues: Tyrosine being predominant and occurring in ~70% (21/30) of the G9P[8] sequences, the second amino acid residue was Asparagine which

only occurred in ~7% (2/30) of the Rwandan study strains, and lastly, Valine occurring in ~23% (7/30) of the strains (Table 4.4).

Table 4.4: Amino acid residues identified at VP4 site 5 and 587 of Rwandan G9P[8] study sequences

Rwandan G9P[8] VP4 study sequences	Amino acid site **5**	Amino acid site **587**
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	F	N
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	F	N
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	F	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	I	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	I	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	I	V
RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	F	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	F	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	I	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	F	Y

RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	I	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU533/2013/G9P[8]	I	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	I	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	F	V
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	F	Y
RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	F	Y

Amino acid residues which were identified at the VP4 genome segment for all 30 Rwandan G9P[8] strains are displayed, with the light blue shading representing the Rwandan study strains from the prevaccination era, and the G9 study sequences from the post- vaccination era shaded in a light orange.

4.4.3. Sequence analysis and protein modelling

The VP7 genome segment is comprised of three established neutralising epitopes namely, 7-1a, 7-1b, and 7-2 (Aoki *et al.*, 2009; Zeller *et al.*, 2015). Within these antigenic regions, 29 amino acids residues have been defined, with 14 residues found within the 7-1a antigenic epitope region, six residues found within the 7-1b region, and nine amino acid residues found within the 7-2 antigenic epitope region (Zeller *et al.*, 2012). When examining the VP7 genome segment, distinctions in amino acids were detected between the RotaTeq® vaccine strain and the Rwandan G9P[8] study strains containing neutralisation epitopes. A polarity shift was detected in the 7-1a antigenic epitope region, which contained the amino acid difference T96A that was identified in one postvaccination study strain. The amino acid difference D100G was detected in 19 strains from the prevaccination era and one strain from the postvaccination era, all of which belonged to the 7-1a antigenic epitope region. This difference also exhibited a polarity change. Three study isolates from the postvaccination era exhibited the amino acid difference N242S in the 7-1b epitope, signifying an amino acid substitution.

Table 4.5: The amino acid properties of observed amino acid differences and the antigenic epitope regions in the VP7 genome segment

Amino acid difference	Antigenic epitope regions	Amino acid property
T96A	7-1a	A change in polarity from a polar to a nonpolar amino acid
D100G	7-1a	A change in polarity from nonpolar to polar amino acid
N242S	7-1b	An amino acid substitution

This table summarises the amino acid differences observed between the G9P[8] study strains, the properties of the amino acid residues, as well as their antigenic epitopic region in the VP7 genome segment.

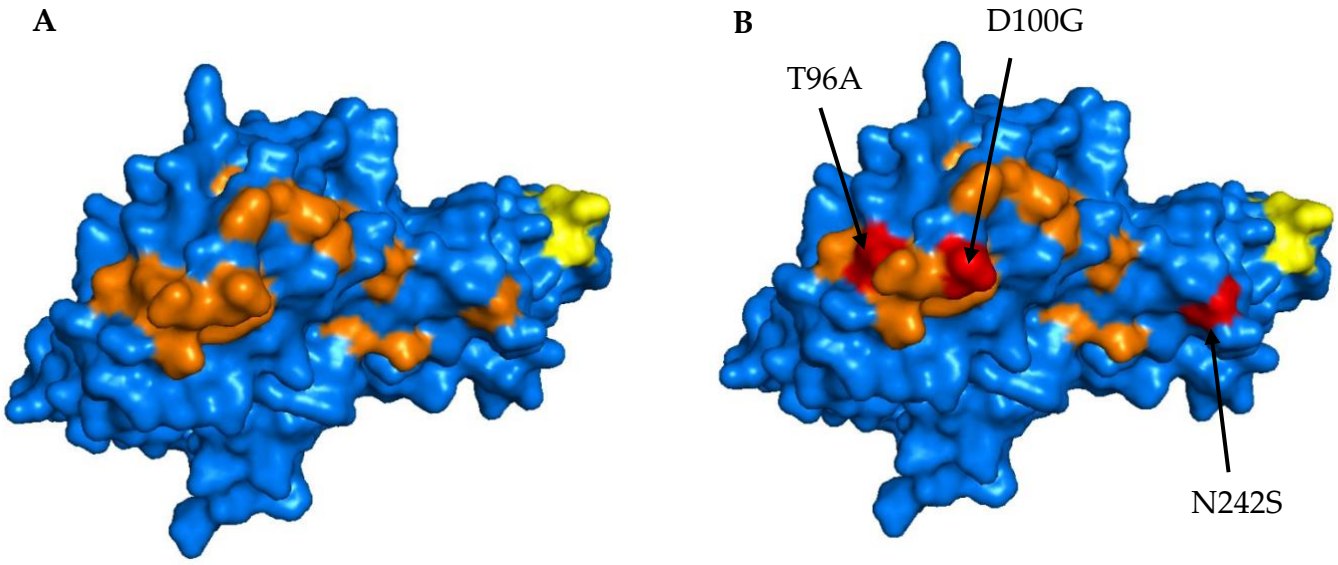

Figure 4.2 (A and B): Three-dimensional protein model representing the location of exposed amino acid difference between the VP7 protein of the G9 component of the RotaTeq® vaccine (A) compared to G9P[8] merged study strain from Rwanda (B). The antigenic epitopic regions 7-1a and 7-1b are coloured in orange and yellow, respectively, with the red colour

representing the amino acid changes observed on the Rwandan study strain compared to the vaccine strains.

The VP4 genome segment is cleaved into two distinct structural proteins, VP8* and VP5* by trypsin during entry into the host cell (Crawford *et al.,* 2001). The VP8* region is comprised of four neutralising antigenic epitopes namely, 8-1, 8-2, 8-3, and 8-4, whereas the VP5* region contains five namely, 5-1, 5-2, 5-3, 5-4, and 5-5 (Dormitzer *et al.,* 2002). Within the VP8* and VP5* antigenic epitope region, 37 amino acid residues have been defined, 25 of which are found within the VP8* region and 12 of which are present in the VP5* region (Dormitzer *et al.,* 2002). Distinction in amino acids was detected between the RotaTeq® and the VP4 genome segment of the Rwandan G9P[8] study strains. A polarity change was observed in the VP8* epitope 8-1 of one postvaccination study strain, where the amino acid difference G195S was identified. One prevaccine Rwandan G9P[8] study strain exhibited the amino acid difference I196L, which corresponded to an amino acid substitution, within the VP8* epitope 8-1. The amino acid difference N113D occurred in the VP8* region at epitope 8-3 in seven study strains (four from the prevaccination era and three from the postvaccination era), resulting in a comparable amino acid substitution.

Table 4.6: The amino acid properties of observed amino acid differences and the antigenic epitope regions in the VP4 genome segment.

Amino acid difference	**Antigenic epitope regions**	**Amino acid property**
G195S	8-1	A polarity change from a polar to a nonpolar amino acid
I196L	8-1	An amino acid substitution
N113D	8-3	An amino acid substitution

This table summarises the amino acid differences observed between the Rwandan G9 study strains, the properties of the amino acid residues, as well as their antigenic epitopic region in the VP4 genome segment.

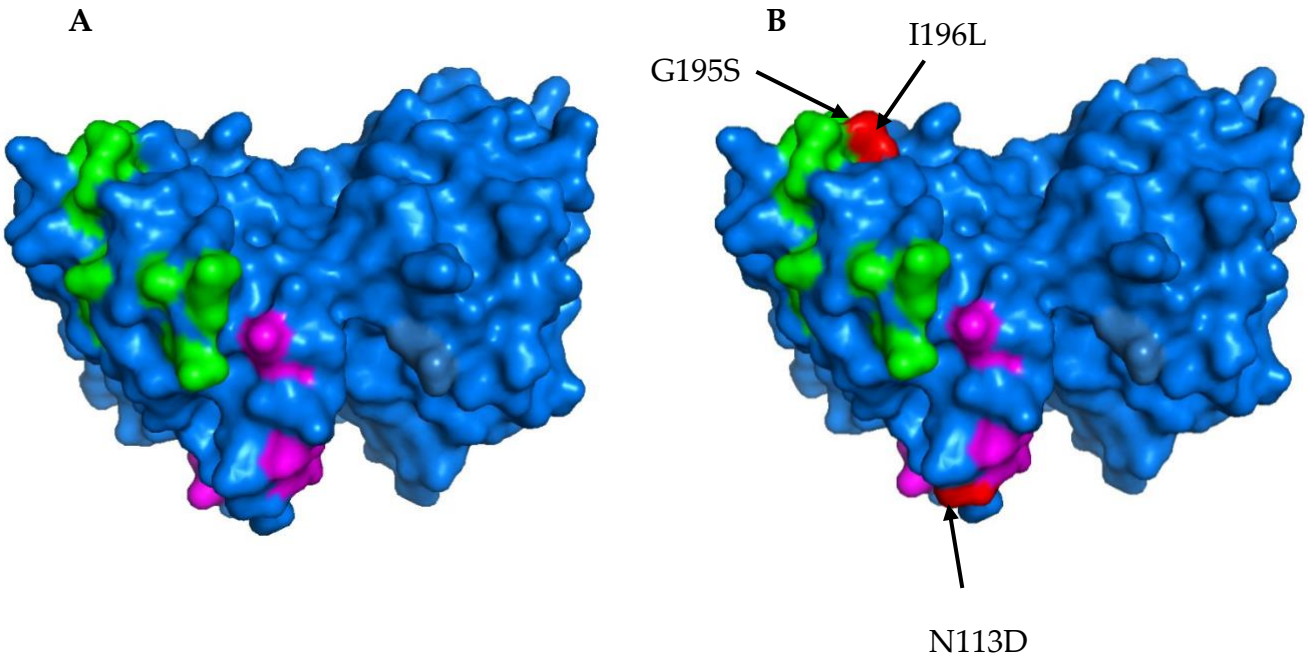

Figure 4.3 (A and B): Three-dimensional protein model representing the location of exposed amino acid difference between the VP4 protein of the P[8] component of the RotaTeq® vaccine (A) compared to G9P[8] merged study strain from Rwanda (B). The antigenic epitopic regions 8-1 and 8-3 are coloured in green and pink, respectively, with the red colour representing the amino acid changes observed on the Rwandan study strain compared to the vaccine strain.

4.5. Discussion

Rotavirus disease has been alleviated on a global scale since the introduction of rotavirus vaccine (Steele *et al.*, 2019). Therefore, it is critical to implement continuous surveillance in all regions in order to identify circulating strains both before and after vaccination, possible emergence of vaccine-escape mutants' post- vaccination, and the

potential impact rotavirus vaccination has on a population (Zeller *et al.,* 2015; Donato and Bines, 2021). Therefore, with this study a whole genome analysis of all the genome segments of 30 Rwandan G9P[8] study strains collected from 2011 to 2015 is provided. This analysis revealed that all the G9 sequences from both vaccination eras (pre- and post- vaccination) was confirmed to exhibit the pure Wa-like genotype constellation. These findings are typical of the G9 genotype according to various whole genome studies, as the G9 genotypes are usually associated with the typical Wa-like constellation (Dóró *et al.,* 2014). Pure genome constellations, which arise from proteins with optimal functionality and epidemiological fitness, are the consequence of co-evolution (Kirkwood *et al.,* 2010; McDonald *et al.,* 2012; Koukou *et al.,* 2022). The possibility exists that the pure nature constellation of these G9 strains is responsible for persistence both before and after vaccination (McDonald *et al.,* 2012). These Rwandan G9P[8] are suggested to be of human origin, as they emerged independent of interspecies-transmission events (Dennis *et al.,* 2014).

Non-synonymous–synonymous substitution ratio is an important measure for calculating selective pressure based on the protein-coding sequences (Li *et al.,* 2001). Non-synonymous mutations that offer fitness advantages or amino acids which are under positive selection, have a ω value which is significantly greater than one (Boyko *et al.,* 2008). Yet, most amino acids that are present in proteins are under functional or structural limitations, with evolution only happening at a minimal time point which results in only a few amino acids being affected (Anisimove *et al.,* 2001). When an average ω value is observed, this may lack the ability to accurately determine sites under positive selection, which may be a limitation to this model (Anisimove *et al.,* 2001).

The amino acid sites across all the 11 genome segments were undergoing purifying selection, which may act as a strategy to eliminate deleterious polymorphisms that may arise due to RNA polymerase enzymes error-prone nature (Donker *et al.,* 2012).

Although, positively selected sites (5, 587) were identified in the VP4 genome segment in this study. Phenylalanine and isoleucine that were observed at site 5 have similar physiochemical properties, as they are both considered non-polar amino acids, meaning they are neutrally charged residues (Damodaran *et al.,* 2017). Valine, Asparagine, and Tyrosine that were identified at site 587 have physiochemical differences, as they are non-polar, polar, and non-polar amino acids, respectively. These residues are positively charged, neutrally charged, and neutrally charged, respectively (Damodaran *et al.,* 2017). The amino acids that are under selective pressure are proposed to have crucial catalytic functions (Heiman *et al.,* 2008; Ogden *et al.,* 2014). Due to this, these amino acid sites are proposed to enhance cell attachment and penetration of the VP4 genome segment (Dormitzer *et al.,* 2004).

Upon comparison with the RotaTeq® vaccine strain, it was found that none of the strains studied in Rwanda displayed identical features to the G9 RotaTeq® vaccine strain (Rasebotsa *et al.,* 2021; Mwangi *et al.,* 2023). During this study, we observed differences in the amino acid composition inside the neutralisation epitope areas of the VP7 and VP4 strains when compared to the RotaTeq® vaccination strain. Differences in amino acid composition were observed at positions 196, 113, and 100 for the VP4 genome segment, and at sites 96, 100, and 242 for the VP7 genome segment. The amino acid difference at position 195 on the VP4 epitope region and at position 96 and 100 on the VP7 genome segment suggest a radical change in polarity. Amino acid substitutions were reported at position 242 for the VP7 genome segment and at position 196 and 113 for the VP4 genome segment. Changes in polarity that were observed may be a result of the natural fluctuation of RVA, as these changes were seen during the prevaccination period, as well as after vaccine introduction (Jere *et al.,* 2018). These changes may also contribute to the decreased antibody binding (Jere *et al.,* 2018). Additionally, these modifications might contribute to the reduced affinity for antibodies towards those specific areas of the epitopes, which could impact their fitness via selection pressure and ultimately render the epitope unattainable

(McDonald *et al.,* 2009). A notable disparity was identified at particular epitope regions between the strains used in the prevaccination and postvaccination studies. This finding could potentially indicate the influence of vaccine pressure at those epitope sites; however, further data are necessary to validate this conclusion. Antigenic variations between the Rwandan study strains and the vaccine strain are associated with decreased effectiveness in low- income countries (Steele *et al.,* 2019).

Protein modelling analysis indicated high structural homology between pre- and postvaccine structures (Mwangi *et al.,* 2022). The genotypic heterology between the RotaTeq® vaccine and the VP7 G9 may have contributed to the G9P[8] genotype's prevalence in Rwanda prior to the introduction of the vaccine, as it was circulating in 2012 and 2013 (Kabayiza *et al.,* 2013). At this time, there is insufficient evidence to suggest that the amino acid variations observed in the VP4 and VP7 epitope regions are due to vaccination; rather, these variations could have arisen naturally during evolution.

In conclusion, our results suggest that purifying selection was applied to the amino acid sites throughout all eleven genome segments of the Rwandan G9P[8] strains. However, we did identify positively selected sites (5, 587) in the VP4 genome segment. Alterations at neutralisation epitopes were additionally documented, potentially contributing to the evasion of neutralisation by vaccine candidates targeting these strains. This highlights the significance of ongoing monitoring of the entire genetic makeup of organisms to better understand the spread and development of G9P[8] strains in Rwanda. It also allows for the assessment of how effective vaccinations are against the specific rotavirus strains present in this region.

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CHAPTER FIVE:

GENERAL DISCUSSION, CONCLUSIOIN AND RECOMMENDATION

5.1. Preamble

This chapter provides a concise overview and final thoughts on the primary discoveries of the study, while also explaining the methods used to accomplish the objectives.

5.2. General discussion and conclusions:

Countries in low-income countries especially in Africa are known to have the greatest rotavirus strain diversity in the world (Ghosh and Kobayashi, 2011). Due to the segmented nature of this rotavirus, it is susceptible to recombination, reassortments, zoonotic transmissions and genomic agreements, which can all be confirmed through whole genome analysis (Santos and Hoshino, 2005). Next generation sequencing has provided a platform for researchers to gain insight into the whole genome of globally circulating rotavirus strains, which has made RVA whole genome studies more widespread (Jere *et al.*, 2018). By leveraging the highly impactful Illumina® MiSeq platform, we conducted an analysis of the complete genomic composition and genetic diversity of circulating RVA strains in Rwanda both before and after the introduction of the RotaTeq® vaccine.

This study primarily processed and analysed RVA samples at whole-genome level (beyond the conventional binary (G/P) surveillance performed in most WHO sentinel surveillance sites) and aimed to analyse the whole genome of Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine introduction. A total of 158 faecal samples were gathered from children aged five and below who were admitted to hospitals in Rwanda as a result of RVA associated with diarrhea. Out of the samples examined, 112 were identified from the post-immunisation period (2013-2015) and 46 were classified from the pre-immunisation era (2011 and 2012). The DPRU in Pretoria is where the WHO rotavirus Regional Reference Laboratory (RRL) conventionally genotyped these samples. From the pool of 158 samples, only 30 were conventionally genotyped as G9P[8] and included in this research. The remaining 128 samples that was not disseminated in this research, was distributed elsewhere. The G9P[8] genotype was the focus of this study as there is currently limited information on this genotype at whole genome level, not only in Rwanda, but in the rest of Africa, as well as these genotypes recognised epidemiological significance. This genotype, only with five other strains namely, G1P[8], G2P[4], G3P[8], G4P[8], and G12P[8], are globally dominant strains (Moure *et al.*, 2018), and contributes to the high morbidity and mortality rates.

The three study objectives were addressed in an overlapping manuscript format in chapter three and chapter four.

The first objective where whole genome sequences of the G9P[8] Rwandan study strains were generated, together with the second objective, where phylogenetic and pairwise sequence analysis was performed, was presented in chapter three as a published article entitled "Genomic analysis of Rwandan G9P[8] rotavirus strains pre- and post- RotaTeq® vaccine reveals significant distinct sub-clustering in the postvaccination cohort".

This chapter provides a concise analysis of the Rwandan G9P[8] study strains, examining their phylogenetic characteristics both before and after vaccination. It was observed that each of the 30 study strains, belonged to the customary Wa-like constellation. The examination of the data unveiled clear characteristics separating the strains prior to and after vaccination. Across all 11 genome segments three strains from the post vaccination era clustered differently from the others. Interestingly, looking closely at the VP4 and the VP7 as they are the outer capsid proteins and play a role in vaccine effectiveness, all 30 of the study strains were closely related with high nucleotide similarity ranges and clustered into lineage III, which is consistent with other studies. Additionally, co-circulation was suggested by the close clustering of the study strains with global reference strains originating from neighbouring countries, specifically Uganda and Kenya (Nakagomi *et al.,* 2013; Burnett *et al.,* 2022). The antigenic regions of the VP4 and VP7 genome segments were utilised to compare the vaccine strains with the study strains (Sadiq *et al.,* 2018). The three postvaccine study strains exhibited the identical amino acid substitution as the vaccine strains, which could potentially impact protein functionality at those specific sites and lead to vaccine candidates evading neutralisation.

The last study objective to perform selection pressure analysis and computational protein modelling were addressed in an overlapping manner with the antigenic region analysis from chapter three in a publishable manuscript format in chapter four.

It was found that across all the 11 genome segments of the virus, the amino acids were undergoing purifying selection, apart from the VP4 genome segment. Within this genome segment, two amino acid sites (5, 587) were found to be under positive selection. Furthermore, comparative analysis between our Rwandan G9P[8] study strains and RotaTeq® vaccine strain revealed the amino acid differences which was visualised on the 3D protein structures of the VP4 and VP7 protein. Two changes were observed in the 7-1a antigenic epitope region of the VP7 protein, and one change was

observed in the 7-2 region, whereas the VP4 protein contained two changes in the 8-1 region and one change in the 8-3 region. These changes are of particular interest as they may play a role in vaccine effectiveness in Rwanda.

This research, which utilises whole genome surveillance to examine circulating RVA strains before and after vaccination, has the potential to significantly benefit the Rwandan region by supplementing the existing knowledge on RVA strains in the area. In the postvaccination study strains, a sub-clustering pattern was identified, and the VP4 genome segment revealed two positive selection sites. Disparities in amino acids were identified at antigenic epitope regions on the VP4 and VP7 genome segments. These differences could potentially aid in neutralisation evasion by the vaccine candidates, given that the VP4 and VP7 are attachment-related outer capsid proteins. In addition, the continuous application of the comprehensive genomic data acquired from this study will contribute to the examination of the evolutionary patterns exhibited by G9P[8] strains in Rwanda.

5.3. Limitations and recommendations:

The present investigation was constrained by the asymmetric dataset, which comprised a greater number of sequenced samples (n=23) from the prevaccination era than from the postvaccination era (n=7). Despite this limitation, we maintain that the study provides significant contributions to our understanding of the evolution of G9P[8] strains that are widespread in Rwanda. Acquiring such knowledge could potentially be crucial in predicting the impacts of vaccines in this domain. In future studies we would recommend having a more balanced dataset, so that a more comprehensive comparison can be made between the pre- and post- vaccination period.

The adoption of NGS technologies for comprehensive whole-genome sequencing of rotaviruses is highly recommended. This methodology offers a profound depth of insight into rotavirus diversity, with a particular emphasis on the African region, characterised by a notably diverse landscape of rotavirus strains. In 2017, Rwanda transitioned from RotaTeq® to Rotarix® vaccine. Subsequent comprehensive whole-genome studies are warranted to assess the potential impact of this vaccine switch on the prevailing strains within the Rwandan region.

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APPENDICES

Appendix A: Ethics approval letter

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HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

30-Aug-2022

Dear **Robyn Potgieter**

Ethics Clearance: **Whole genome analysis of Rwandan G9P[8] rotavirus strains pre and post-RotaTeq(R) vaccine introduction**

Principal Investigator: **Robyn Potgieter**

Department: **School of Medicine Department (Bloemfontein Campus)**

Submission Page

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-HSD2022/0983/2709**

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.

Research conducted in any Department of Health facility: Researchers are required to sign and return the HSREC approval letters to the provincial Department of Health where they applied. It is also a requirement for researchers to submit electronic copies of their final research findings, and/or make a presentation of their findings and recommendations at departmental research days when and where indicated.

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(2020); 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; International Council for Harmonisation (ICH) Harmonised Guideline, Integrated Addendum to ICH E6(R1), Guideline for Good Clinical Practice (GCP) E6(R2), 2016, SAHPRA Guidelines as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

Appendix B: Published manuscript

viruses

MDPI

Article

Genomic Analysis of Rwandan G9P[8] Rotavirus Strains Pre- and Post-RotaTeq® Vaccine Reveals Significant Distinct Sub-Clustering in a Post-Vaccination Cohort

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Abstract: Although the introduction of rotavirus vaccines has substantially contributed to the reduction in rotavirus morbidity and mortality, concerns persist about the re-emergence of variant strains that might alter vaccine effectiveness in the long term. The G9 strains re-emerged in Africa during the mid-1990s and have more recently become predominant in some countries, such as Ghana and Zambia. In Rwanda, during the 2011 to 2015 routine surveillance period, G9P[8] persisted during both the pre- and post-vaccine periods. The pre-vaccination cohort was based on the surveillance period of 2011 to 2012, and the post-vaccination cohort was based on the period of 2013 to 2015, excluding 2014. The RotaTeq® vaccine that was first introduced in Rwanda in 2012 is genotypically heterologous to Viral Protein 7 (VP7) G9. This study elucidated the whole genome of Rwandan G9P[8] rotavirus strains pre- and post-RotaTeq® vaccine introduction. Fecal samples from Rwandan children under the age of five years (pre-vaccine n = 23; post-vaccine n = 7), conventionally genotyped and identified as G9P[8], were included. Whole-genome sequencing was then performed using the Illumina® MiSeq platform. Phylogenetic analysis and pair-wise sequence analysis were performed using MEGA6 software. Distinct clustering of three post-vaccination study strains was observed in all 11 gene segments, compared to the other Rwandan G9P[8] study strains. Specific amino acid differences were identified across the gene segments of these three 2015 post-vaccine strains. Important amino acid differences were identified at position N242S in the VP7 genome segment of the three post-vaccine G9 strains compared to the other G9 strains. This substitution occurs at a neutralization epitope site and may slightly affect protein interaction at that position. These findings indicate that the Rwandan G9P[8] strains revealed a distinct sub-clustering pattern among post-vaccination study strains circulating in Rwanda, with changes at neutralization epitopes, which may play a role in neutralization escape from vaccine candidates. This emphasizes the need for continuous whole-genome surveillance to better understand the evolution and epidemiology of the G9P[8] strains post-vaccination.

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Appendix C.1: Accession numbers of the sequences generated in this study of Rwandan G9P[8] strains.

Submission ID	Strain Name	Accession Number
BankIt2731466 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401005
BankIt2731466 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401006
BankIt2731466 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401007
BankIt2731466 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401008
BankIt2731466 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401009
BankIt2731466 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401010
BankIt2731466 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401011
BankIt2731466 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401012
BankIt2731466 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401013
BankIt2731466 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401014
BankIt2731466 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401015
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BankIt2731476 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401140
BankIt2731476 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401141
BankIt2731476 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401142
BankIt2731476 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401143
BankIt2731476 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401144
BankIt2731476 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401145
BankIt2731476 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401146
BankIt2731476 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401147
BankIt2731476 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401148
BankIt2731476 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401149
BankIt2731476 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401150
BankIt2731476 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401151
BankIt2731476 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401152
BankIt2731476 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401153
BankIt2731476 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401154
BankIt2731477 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401155
BankIt2731477 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401156
BankIt2731477 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401157
BankIt2731477 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401158
BankIt2731477 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401159
BankIt2731477 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401160
BankIt2731477 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401161

BankIt2731477 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401162
BankIt2731477 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401163
BankIt2731477 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401164
BankIt2731477 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401165
BankIt2731477 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401166
BankIt2731477 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401167
BankIt2731477 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401168
BankIt2731477 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401169
BankIt2731477 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401170
BankIt2731477 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401171
BankIt2731477 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401172
BankIt2731477 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401173
BankIt2731477 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401174
BankIt2731477 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401175
BankIt2731477 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401176
BankIt2731477 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401177
BankIt2731477 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401178
BankIt2731477 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401179
BankIt2731477 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401180
BankIt2731477 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401181
BankIt2731477 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401182
BankIt2731477 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401183
BankIt2731477 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401184

BankIt2731479 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401185
BankIt2731479 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401186
BankIt2731479 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401187
BankIt2731479 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401188
BankIt2731479 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401189
BankIt2731479 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401190
BankIt2731479 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401191
BankIt2731479 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401192
BankIt2731479 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401193
BankIt2731479 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401194
BankIt2731479 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401195
BankIt2731479 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401196
BankIt2731479 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401197
BankIt2731479 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401198
BankIt2731479 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401199
BankIt2731479 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401200
BankIt2731479 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401201
BankIt2731479 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401202
BankIt2731479 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401203
BankIt2731479 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401204
BankIt2731479 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401205
BankIt2731479 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401206
BankIt2731479 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401207

BankIt2731479 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401208
BankIt2731479 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401209
BankIt2731479 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401210
BankIt2731479 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401211
BankIt2731479 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401212
BankIt2731479 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401213
BankIt2731479 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401214
BankIt2731480 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401215
BankIt2731480 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401216
BankIt2731480 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401217
BankIt2731480 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401218
BankIt2731480 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401219
BankIt2731480 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401220
BankIt2731480 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401221
BankIt2731480 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401222
BankIt2731480 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401223
BankIt2731480 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401224
BankIt2731480 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401225
BankIt2731480 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401226
BankIt2731480 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401227
BankIt2731480 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401228
BankIt2731480 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401229
BankIt2731480 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401230

BankIt2731480 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401231
BankIt2731480 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401232
BankIt2731480 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401233
BankIt2731480 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401234
BankIt2731480 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401235
BankIt2731480 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401236
BankIt2731480 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401237
BankIt2731480 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401238
BankIt2731480 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401239
BankIt2731480 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401240
BankIt2731480 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401241
BankIt2731480 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401242
BankIt2731480 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401243
BankIt2731480 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401244
BankIt2731481 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401245
BankIt2731481 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401246
BankIt2731481 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401247
BankIt2731481 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401248
BankIt2731481 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401249
BankIt2731481 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401250
BankIt2731481 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401251
BankIt2731481 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401252
BankIt2731481 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401253

BankIt2731481 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401254
BankIt2731481 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401255
BankIt2731481 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401256
BankIt2731481 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401257
BankIt2731481 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401258
BankIt2731481 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401259
BankIt2731481 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401260
BankIt2731481 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401261
BankIt2731481 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401262
BankIt2731481 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401263
BankIt2731481 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401264
BankIt2731481 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401265
BankIt2731481 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401266
BankIt2731481 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401267
BankIt2731481 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401268
BankIt2731481 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401269
BankIt2731481 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401270
BankIt2731481 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401271
BankIt2731481 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401272
BankIt2731481 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401273
BankIt2731481 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401274
BankIt2731482 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401275
BankIt2731482 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401276

BankIt2731482 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401277
BankIt2731482 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401278
BankIt2731482 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401279
BankIt2731482 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401280
BankIt2731482 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401281
BankIt2731482 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401282
BankIt2731482 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401283
BankIt2731482 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401284
BankIt2731482 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401285
BankIt2731482 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401286
BankIt2731482 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401287
BankIt2731482 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401288
BankIt2731482 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401289
BankIt2731482 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401290
BankIt2731482 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401291
BankIt2731482 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401292
BankIt2731482 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401293
BankIt2731482 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401294
BankIt2731482 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401295
BankIt2731482 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401296
BankIt2731482 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401297
BankIt2731482 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401298
BankIt2731482 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401299

BankIt2731482 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401300
BankIt2731482 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401301
BankIt2731482 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401302
BankIt2731482 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401303
BankIt2731482 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401304
BankIt2731485 Seq1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P[8]	OR401305
BankIt2731485 Seq2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P[8]	OR401306
BankIt2731485 Seq3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P[8]	OR401307
BankIt2731485 Seq4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P[8]	OR401308
BankIt2731485 Seq5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P[8]	OR401309
BankIt2731485 Seq6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P[8]	OR401310
BankIt2731485 Seq7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P[8]	OR401311
BankIt2731485 Seq8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P[8]	OR401312
BankIt2731485 Seq9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P[8]	OR401313
BankIt2731485 Seq10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P[8]	OR401314
BankIt2731485 Seq11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P[8]	OR401315
BankIt2731485 Seq12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P[8]	OR401316
BankIt2731485 Seq13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P[8]	OR401317
BankIt2731485 Seq14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P[8]	OR401318
BankIt2731485 Seq15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P[8]	OR401319
BankIt2731485 Seq16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P[8]	OR401320
BankIt2731485 Seq17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P[8]	OR401321
BankIt2731485 Seq18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P[8]	OR401322

BankIt2731485 Seq19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P[8]	OR401323
BankIt2731485 Seq20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P[8]	OR401324
BankIt2731485 Seq21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P[8]	OR401325
BankIt2731485 Seq22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P[8]	OR401326
BankIt2731485 Seq23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P[8]	OR401327
BankIt2731485 Seq24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P[8]	OR401328
BankIt2731485 Seq25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P[8]	OR401329
BankIt2731485 Seq26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P[8]	OR401330
BankIt2731485 Seq27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P[8]	OR401331
BankIt2731485 Seq28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P[8]	OR401332
BankIt2731485 Seq29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P[8]	OR401333
BankIt2731485 Seq30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P[8]	OR401334

Appendix C2: Accession numbers of the reference sequences used throughout the study

NSP1

Strain ID	**Accession number**
RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	KP752758
RVA/Human-wt/KEN/KLF0658/2013/G1P[8]	MZ094716
RVA/Human-wt/KEN/KLF0638/2013/G1P[8]	MZ094496
RVA/Human-wt/KEN/MRC-DPRU4150/XXXX/G1G10P[8]	KJ753644
RVA/Human-wt/ZAF/UFS-NGS-MRC-DPRU1980/2008/G1P[8]	MT854858
RVA/Human-wt/CAN/RT133-07/2008/G1P8	JQ069424
RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	KP753252
RVA/Human-wt/KEN/Keny-057/2009/G1P[8]	KP882698
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753418.1
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632249
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632326
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751752
RVA/Human-wt/BEL/B4633/2003/G12P[8]	DQ146644
RVA/Human-wt/Bel/BE00068/2000/G1P[8]	JN258821
RVA/Human-wt/BGD/Matlab36/2002/G11P[8]	GU199509
RVA/Human-wt/AUS/CK00053/2007/G1P[8]	JX027662
RVA/Human-wt/PRY/469/2000/G9P[8]	KJ626666
RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	MG181486
RVA/Human-wt/NPL/5N0031/2005/G12P[6]	LC372864
RVA/Human-wt/ZMB/MRC-DPRU3491/2009/G12P[6]	KF636141
RVA/Human-wt/COD/KisB521/2008/G12P[6]	KJ870917
RVA/Human-wt/ITA/AV28/2010/G9P8	JX195080
RVA/Human-wt/LBN/M21/2011/G12P[6]	MN746067
RVA/Human-wt/USA/VU12-13-113/2013/G12P[8]	KT918991
RVA/Human-wt/IND/CMC_00042/2012/G9P[8]	MN067005
Human-wt/JPN/Tokyo17-21/2017/G3P[8]	LC477543

NSP2

Strain ID	**Accession number**
RVA/Human-wt/ZMB/MRC-DPRU3491/2009/G12P[6]	KF636142
RVA/Human-wt/MWI/MW2-1274/2005/G1P[8]	MG181476
RVA/Human-wt/USA/2007719825/2007/G1P[8]	HM773751
RVA/Human-wt/SWZ/MRC-DPRU4506/2010/G2P[4]P[8]	KP752716
RVA/Human-wt/JPN/EnRo2339/2014/G1P[8]	LC464117

RVA/Human-wt/PAK56/2015/G9P8	MH182452
RVA/Human-wt/CZE/H186/2018/G9P[4]	MT005294
RVA/Human-wt/AUS/CK00088/2009/G1P[8]	JX027869
RVA/Human-wt/ITA/ME659/14/2014/G12P8	KU048685
RVA/Human-wt/MOZ/HCN1181/2016/G1P[8]	MT737629
RVA/Human-wt/IDN/STM453/2018/G1P[8]	LC469527
RVA/Human-wt/UGA/MUL-13-157/2013/G1P[8]	KX632349
RVA/Human-wt/RUS/Nov07-2799/2007/G9P[8]	KC155674
RVA/Human-wt/UGA/MUL-12-093/2012/G9P[8]	KX632316
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632327
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753419
RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	KP753253
RVA/Human-wt/KEN/Keny-058/2009/G1P[8]	KP882710
RVA/Human-wt/ZAF/MRC-DPRU135/2009/G1P[8]	KJ753078
RVA/Human-wt/KEN/KLF0642/2013/G1P[8]	MZ094541
RVA/Human-wt/KEN/KLF0696/2014/G1P[8]	MZ095033
RVA/Human-wt/KEN/KLF0647/2013/G1P[8]	MZ094596

NSP3

Strain ID	**Accession number**
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632251
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632306
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632328
RVA/Human-wt/UGA/MUL-13-163/2013/G9P[8]	KX632295
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753420
RVA/Human-wt/ZAF/MRC-DPRU135/2009/G1P[8]	KJ753079
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751754
RVA/Human-wt/COD/KisB521/2008/G12P[6]	KJ870919
RVA/Human-wt/PRY/371/1999/G1P[8]	KJ627109
RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	EF694227.1
RVA/Human-wt/CHN/Fuzhou19-36/2019/G9P[8]	MW384316
RVA/Human-wt/VNM/RVN16.0876/2016/G9P[8]	LC215270
RVA/Human-tc/AUS/McN13/1980/G3P2A[6]	JX416224
RVA/Human-wt/ESP/SS65130987/2015/G12P[8]	MH171437
RVA/Human-wt/ITA/RG176/13/2013/G12P8	KU048709
RVA/Human-wt/MWI/BID111/2012/G1P[8]	MG181510
RVA/Human-wt/MOZ/MAN0033/2012/G1P[8]	MT737672
RVA/Human-wt/KEN/KCH1187/2019/G3P[8]	LC600850
RVA/Human-wt/USA/VU12-13-189/2013/GXP[X]	MF168081
RVA/Human-wt/JPN/Tokyo17-21/2017/G3P[8]	LC477603

NSP4

Strain ID	Accession number
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632329
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751755
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753421
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632252
RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	KP753255
RVA/Human-wt/BRA/1A0755/2009/G12P6	KX274568
RVA/Human-wt/AUS/CK00091/2009/G1P[8]	JX027894
RVA/Human-wt/BGD/Matlab36/2002/G11P[8]	GU199512
RVA/Human-wt/USA/DC5685/1991/G1P[8]	KT695113
RVA/Human-wt/UGA/MUL-13-157/2013/G1P[8]	KX632351
RVA/Human-wt/ITA/ME659/14/2014/G12P8	KU048729
RVA/Human-wt/USA/VU12-13-188/2013/G1P[X]	MF168303
VA/Human-wt/GHA/DC949/2010/G9P[8]	LC439279
RVA/Human-wt/JPN/NS16-09/2016/G9P[8]	LC569403
RVA/Human-wt/ZAF/3133WC/2009/G12P[4]	HQ657147
RVA/Human-wt/LBN/M21/2011/G12P[6]	MN746070
RVA/Human-wt/COD/KisB504/2009/G1P[6]	KJ870931
RVA/Human-wt/UGA/MRC-DPRU4616/2011/G12P[6]	KJ753279

NSP5

Strain ID	Accession number
RVA/Human-wt/COD/KisB504/2009/G1P[6]	KJ870932
RVA/Human-wt/AUS/CK00083/2008/G1P[8]	JX027816
RVA/Human-wt/BEL/BE00034/2008/G1P[8]	HQ392292
RVA/Human-wt/IND/VR10040/2003/G1P[8]	KT920991
RVA/Human-wt/IND/VR11281/2003/G1P[8]	KT920892
RVA/Human-wt/JPN/Tokyo17-21/2017/G3P[8]	LC477663
RVA/Human-wt/IDN/STM457/2018/G1P[8]	LC469596
RVA/Human-wt/KEN/KCH1187/2019/G3P[8]	LC600852
RVA/Human-wt/ZAF/MRC-DPRU1191/2009/G12P[8]	KJ752338
RVA/Human-wt/ITA/ME659/14/2014/G12P8	KU048768
RVA/HuMAN-wt/RUS/Novosibirsk/Nov12-N4489/2012/G12P[8]	MN224028
RVA/Human-wt/MOZ/HJM1646/2017/G1P[8]	MT737751
RVA/Human-wt/MOZ/HGM0033/2014/G1P[8]	MT737772
RVA/Human-wt/MOZ/HGM0048/2014/G1P[8]	MT737759

RVA/Human-wt/MOZ/HGM0544/2015/G1P[8]	MT737771
RVA/Human-tc/USA/DC5685-40-AG/1991/G1P[8]	KT695147
RVA/Human-wt/BRA/QUI-59-F3/2010/G1P[8]	KU361040
RVA/Human-wt/ESP/SS96099331/2018/G12P[8]	MK417795
RVA/Human-wt/GHA/DC949/2010/G9P[8]	LC439281
RVA/Human-wt/CHN/SC18511031/2018/G9P[8]	ON993184
RVA/Human-wt/RUS/Novosibirsk/NS18-A1413/2018/G12P[8]	MN577190
RVA/Human/JPN/OT029/2013/G9P[8]	LC174262
RVA/Human/JPN/OT013/2013/G9P[8]	LC174249
RVA/Human/JPN/OT024/2013/G9P[8]	LC174257
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751756
RVA/Human-wt/UGA/MUL-13-163/2013/G9P[8]	KX632297
RVA/Human-wt/ZAF/MRC-DPRU7873/XXXX/G9P[8]	KJ753029
RVA/Human-wt/KEN/MRC-DPRU4150/XXXX/G1G10P[8]	KJ753637
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632330
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632253
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753422
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632308
RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	KP752752

VP1

Strain ID	**Accession number**
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632298
RVA/Human-wt/UGA/MUL-13-163/2013/G9P[8]	KX632287
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632320
RVA/Human-wt/KEN/KLF0642/2013/G1P[8]	MZ094545
RVA/Human-wt/KEN/KLF1044/2013/G1P[8]	MZ097058
RVA/Human-wt/KEN/KLF0658/2013/G1P[8]	MZ094721
RVA/Human-wt/KEN/KLF1046/2014/G1P[8]	MZ097080
RVA/Human-wt/KEN/KLF0944/2019/G3P[8]	MZ096717
RVA/Human-wt/KEN/KCH1184/2019/G3P[8]	LC600831
RVA/Human-wt/KEN/KLF0931/2019/G3P[8]	MZ096596
RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	KP752753
RVA/Human-wt/KEN/KLF0636/2013/G1P[8]	MZ094479
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751757
RVA/Human-wt/KEN/KLF0561/2012/G9P[8]	MZ093868
RVA/Human-wt/CHN/E2422/2010/G3P[8]	KF371840
RVA/Human/JPN/SP029/2013/G9P[8]	LC172977
RVA/Human-wt/USA/SSCRTV_00012/2013/GXP[8]	MF469230
RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	DQ146649

RVA/Human-wt/CMR/MRC-DPRU1417/2009/G9P[8]	KF636289
RVA/Human-wt/CAN/RT070-09/2009/G1P8	JQ069950
RVA/Human-wt/JPN/Tokyo17-21/2017/G3P[8]	LC477453
RVA/Human-wt/AUS/CK00102/2010/G1P[8]	KP645267
RVA/Human-wt/MOZ/0289/2012/G12P[6]	MG926714
RVA/Human-wt/UGA/MRC-DPRU4620/2011/G12P[8]	KJ753727
RVA/Human-wt/USA/VU12-13-79/2012/G12P[8]	KT918831
RVA/Human-wt/ESP/SS65130987/2015/G12P[8]	MH171309

VP2

Strain ID	**Accession number**
RVA/Human-wt/TGO/MRC-DPRU4578/2010/G12P[6]	KP752946
RVA/Human-wt/UGA/MRC-DPRU3713/2010/G12P[6]	KJ751868
RVA/Human-wt/UGA/MRC-DPRU4616/2011/G12P[6]	KJ753282
RVA/Human-wt/COD/KisB521/2008/G12P[6]	KJ870912
RVA/Human-wt/UGA/MUL-13-183/2013/G12P[6]	KX632266
RVA/Human-wt/UGA/KTV-13-023/2013/G12P[6]	KX655485
RVA/Human-wt/UGA/MUL-12-093/2012/G9P[8]	KX632310
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632321
RVA/Human-wt/NPL/5N0109/2005/G12P[6]	LC368100
RVA/Human-wt/NPL/06N0397/2006/G12P[6]	LC368122
RVA/Human-wt/BEL/BE00029/2008/G1P[8]	HQ392242
RVA/Human-wt/AUS/CK00083/2008/G1P[8]	JX027823
RVA/Human-wt/MOZ/HJM1646/2017/G1P[8]	MT737430
RVA/Human-wt/ITA/PA93/12/2012/G12P[8]	KU048549
RVA/Human-wt/ESP/SS257451/2012/G12P[8]	MH171322
RVA/Human-wt/JPN/Tokyo17-21/2017/G3P[8]	LC477483

VP3

Strain ID	**Accession number**
RVA/Human-wt/AUS/CK00088/2009/G1P[8]	JX027877
RVA/Human-wt/CAN/RT122-07/2008/G1P8	JQ069749
RVA/Human-wt/ITA/AV21/2010/G9P8	JX195065
RVA/Human-wt/BEL/BE00031/2008/G1P[8]	HQ392264
RVA/Human-wt/MOZ/HJM1646/2017/G1P[8]	MT737465
RVA/Human-wt/IND/CMC_00048/2013/G9P[8]	MN066756
RVA/Human-wt/USA2007719945/2007/G1P[8]	HM773834
RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	DQ146651

RVA/Human-tc/MWI/MW2-1274/2005/G1P[8]	AB751552
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632300
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632245
RVA/Human-wt/UGA/KTV-13-023/2013/G12P[6]	KX655486
RVA/Human-wt/COD/KisB521/2008/G12P[6]	KJ870913
RVA/Human-tc/KEN/KDH633/2010/G12P[6]	AB861947
RVA/Human-tc/KEN/KDH684/2010/G12P[6]	AB861969
RVA/Human-wt/UGA/MRC-DPRU3713/2010/G12P[6]	KJ751869
RVA/Human-wt/UGA/MRC-DPRU4616/2011/G12P[6]	KJ753283

VP4

Strain ID	**Accession number**
RVA/Human-wt/AUS/CK00083/2008/G1P[8]	JX027821
RVA/Human-wt/BEL/BE1286/2009/G1P[8]	JN849147
RVA/Human-wt/ZAF/MRC-DPRU1808/2007/G1P[8]	KP753020
RVA/Human-wt/ITA/JES11/2010/G9P8	JX195088
RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8]	KP752674
RVA/Human-wt/CAN/RT012-07/2007/G1P8	JQ069626
RVA/Human-wt/IND/VR10431/2003/G1P[8]	KT920643
RVA/Human-wt/PRY/467/2000/G9P[8]	KJ626908
RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	MG181483
RVA/Human-wt/CHN/km15118/G9P[8]	KX778583
RVA/Human-wt/CHN/E2422/2010/G3P[8]	KF371843
RVA/Human-wt/JPN/To14-18/2014/G9P[8]	LC105271
RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	DQ492672
RVA/Human-wt/BGD/Matlab36/2002/G11P[8]	GU199506
RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	KJ751760
RVA/Human-wt/KEN/Keny-111/2009/G1P[8]	KP882737
RVA/Human-wt/KEN/KLF0695/2014/G1P[8]	MZ095029
RVA/Human-wt/KEN/KLF0697/2014/G1P[8]	MZ095051
RVA/Human-wt/KEN/KLF0707/2014/G1P[8]	MZ095128
RVA/Human-wt/KEN/KLF0727/2014/G1P[8]	MZ095238
RVA/Human-wt/KEN/KLF0740/2014/G9P[8]	MZ095293
RVA/Human-wt/KEN/KLF0665/2013/G1P[8]	MZ094789
RVA/Human-wt/KEN/KLF0682/2013/G1P[8]	MZ094919
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753427
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632323
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632246
RVA/Human-wt/JPN/HKD0825/2016/G1P[8]	LC384333
RVA/Human-wt/AUS/CK20039/2008/G1P[8]	KC443601

RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G6P[8]	GU565055
RVA/Human-wt/USA/VU12-13-42/2013/G12P[8]	MF168111
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	JN849113

VP6

Strain ID	**Accession number**
RVA/Human-wt/COD/KisB521/2008/G12P[6]	KJ870915
RVA/Human-wt/COD/KisB504/2009/G1P[6]	KJ870926
RVA/Human-wt/KEN/MRC-DPRU4150/XXXX/G1G10P[8]	KJ753642
RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	KP753261
RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	KJ753428
RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	KX632247
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632302
RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	KX632324
RVA/Human-wt/BEL/BE00023/2007/G1P[8]	HQ392184
RVA/Human-wt/PRY/371/1999/G1P[8]	KJ627120
RVA/Human-wt/USA/SSCRTV_00006/2013/G12P[8]	MF469202
RVA/Human-wt/AUS/CK00064/2007/G1P[8]	KC769364
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RVA/Human-wt/VNM/12053_58	KX363098
RVA/Human-wt/ITA/ASTI23/2007/G9P8	JX185762
RVA/Human-wt/TGO/MRC-DPRU4578/2010/G12P[6]	KP752949

VP7

Strain ID	**Accession number**
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RVA/Human-wt/MELB/G9.10/2003	AY307090
RVA/Human-wt/MRC-DPRU1424/2013/G9	JN605412
RVA/Human-wt/PER/G9.1/2003/G9	AY184813
RVA/Human-wt/ZAF/2371WC/2008/G9P[8]	JN014001
RVA/Human-wt/BEL/B3458/2003/G9P[8]	EF990708

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RVA/Human-wt/CMH/B1017/2015/G9	KU991795
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RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	KX632303
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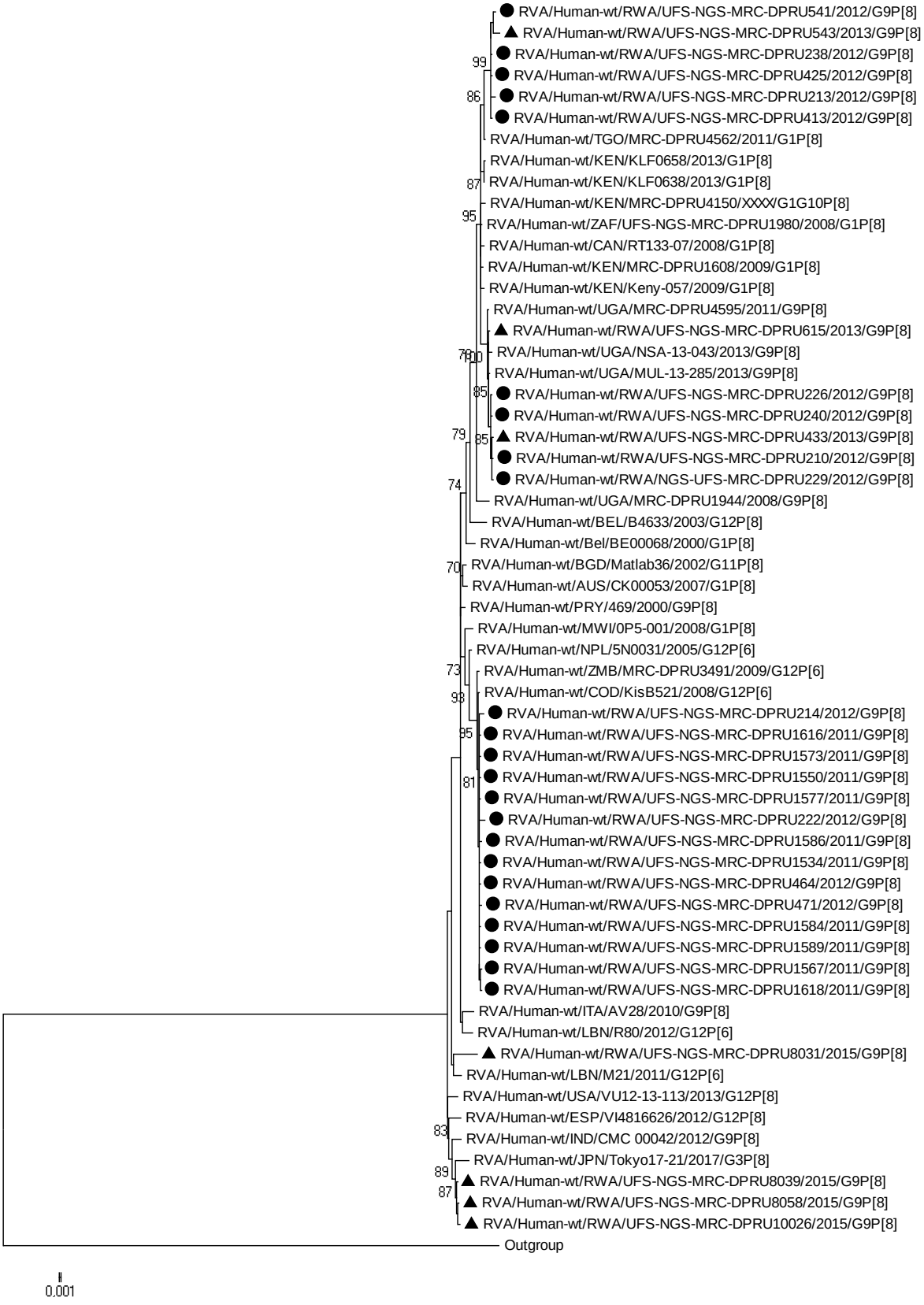
Appendix C.2: Maximum likelihood trees

NSP1



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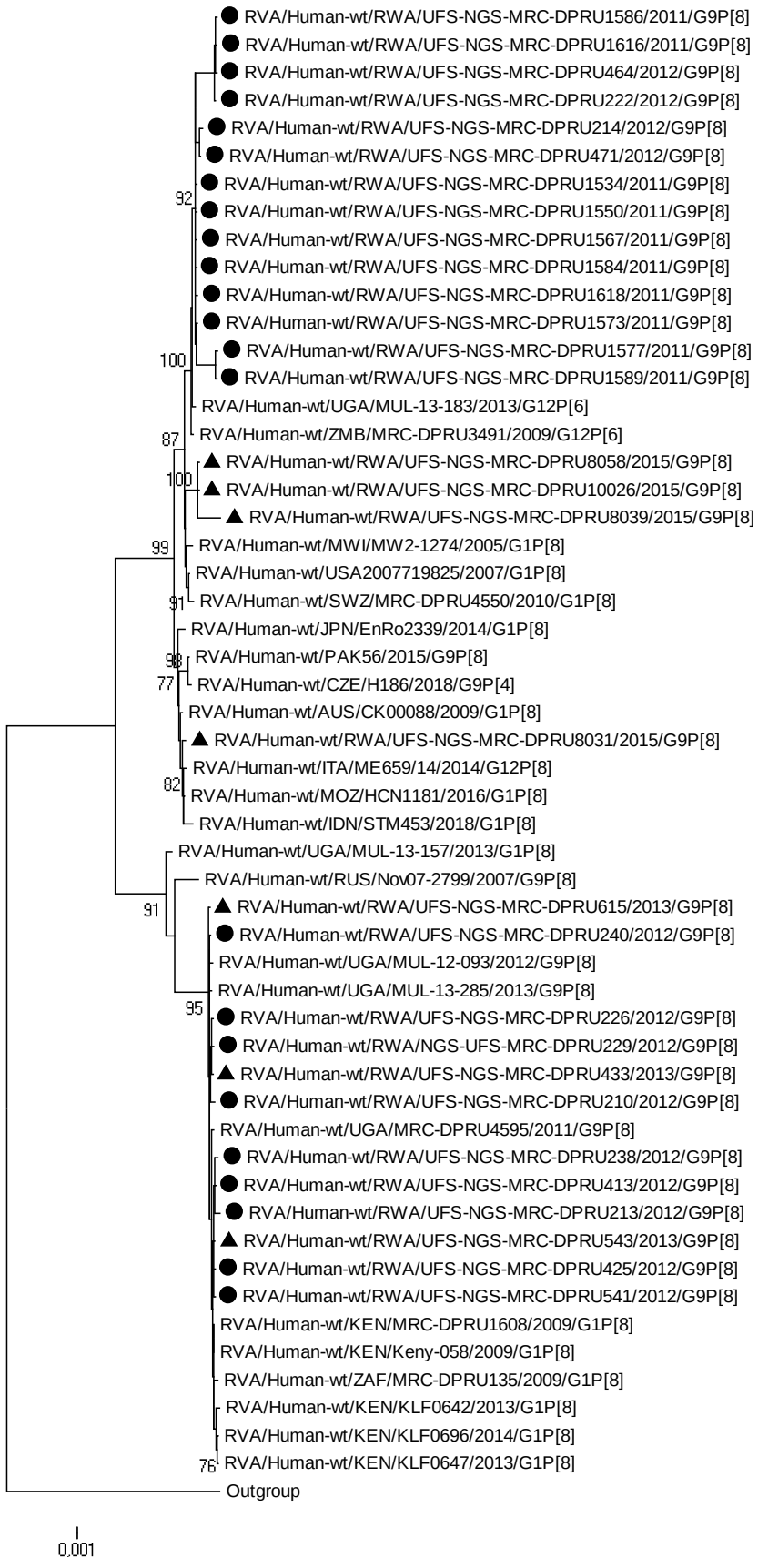
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Outgroup
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99
91
98
77
82
91
95
76
0.001

NSP3


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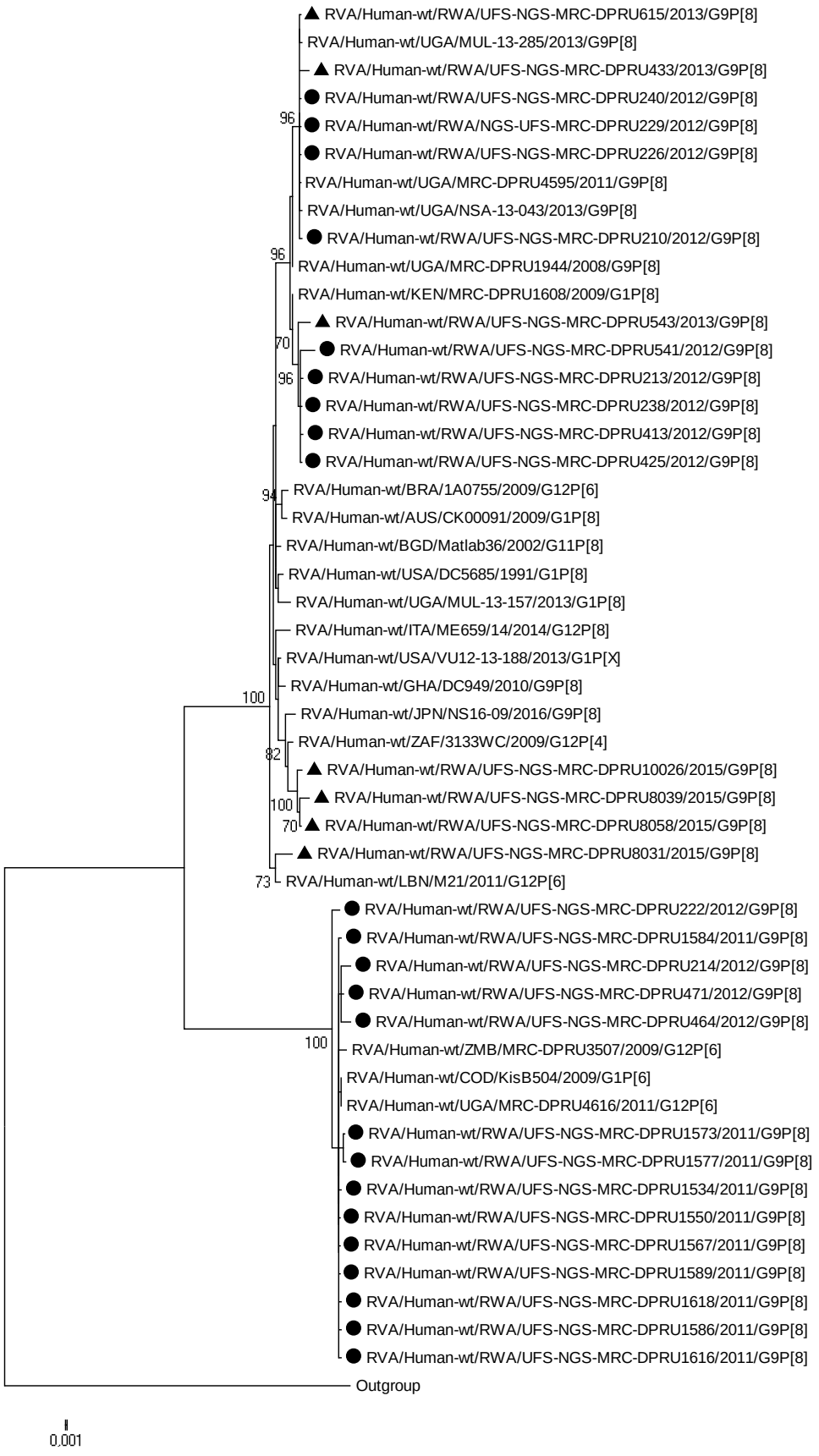
NSP4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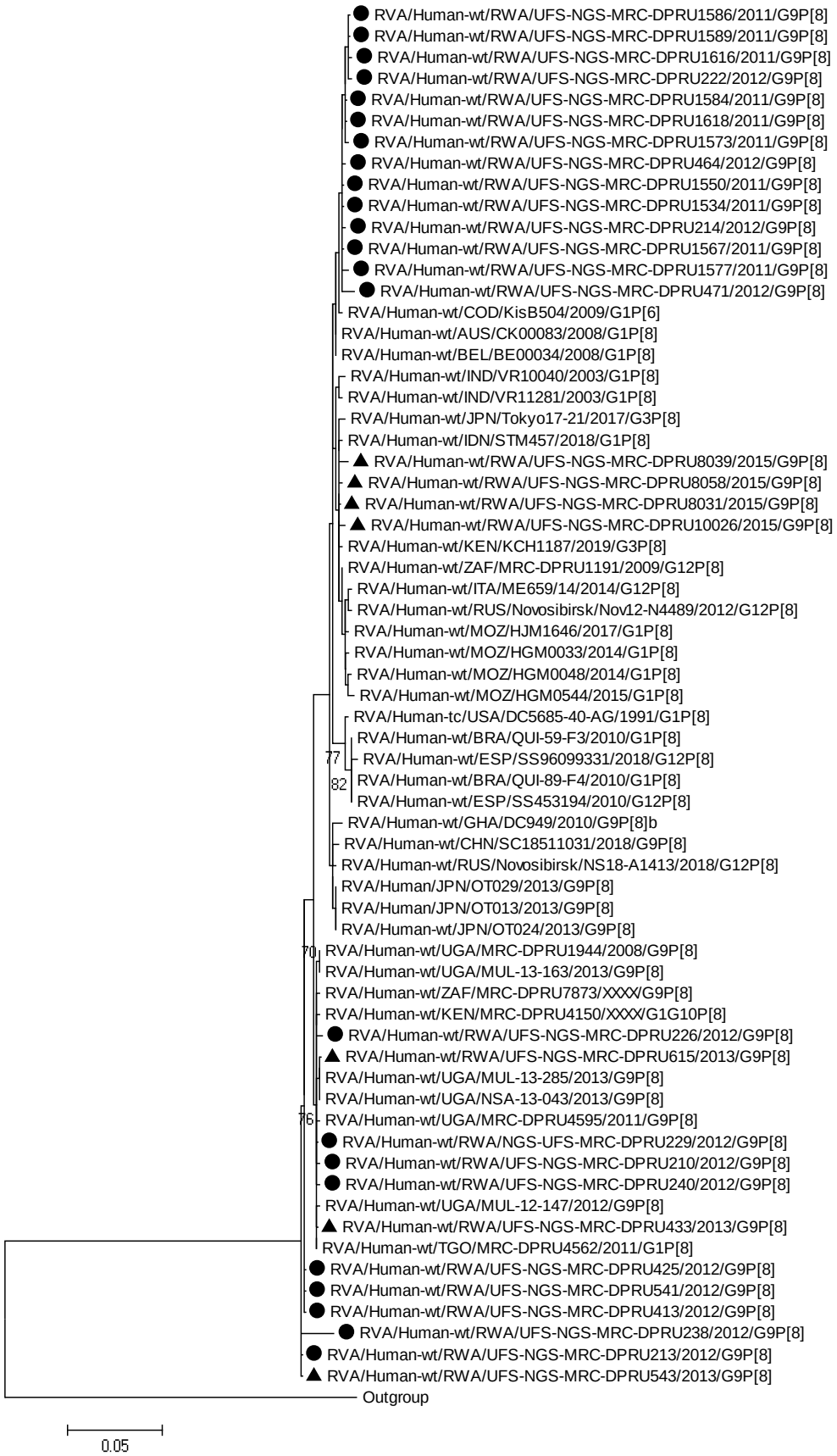
NSP5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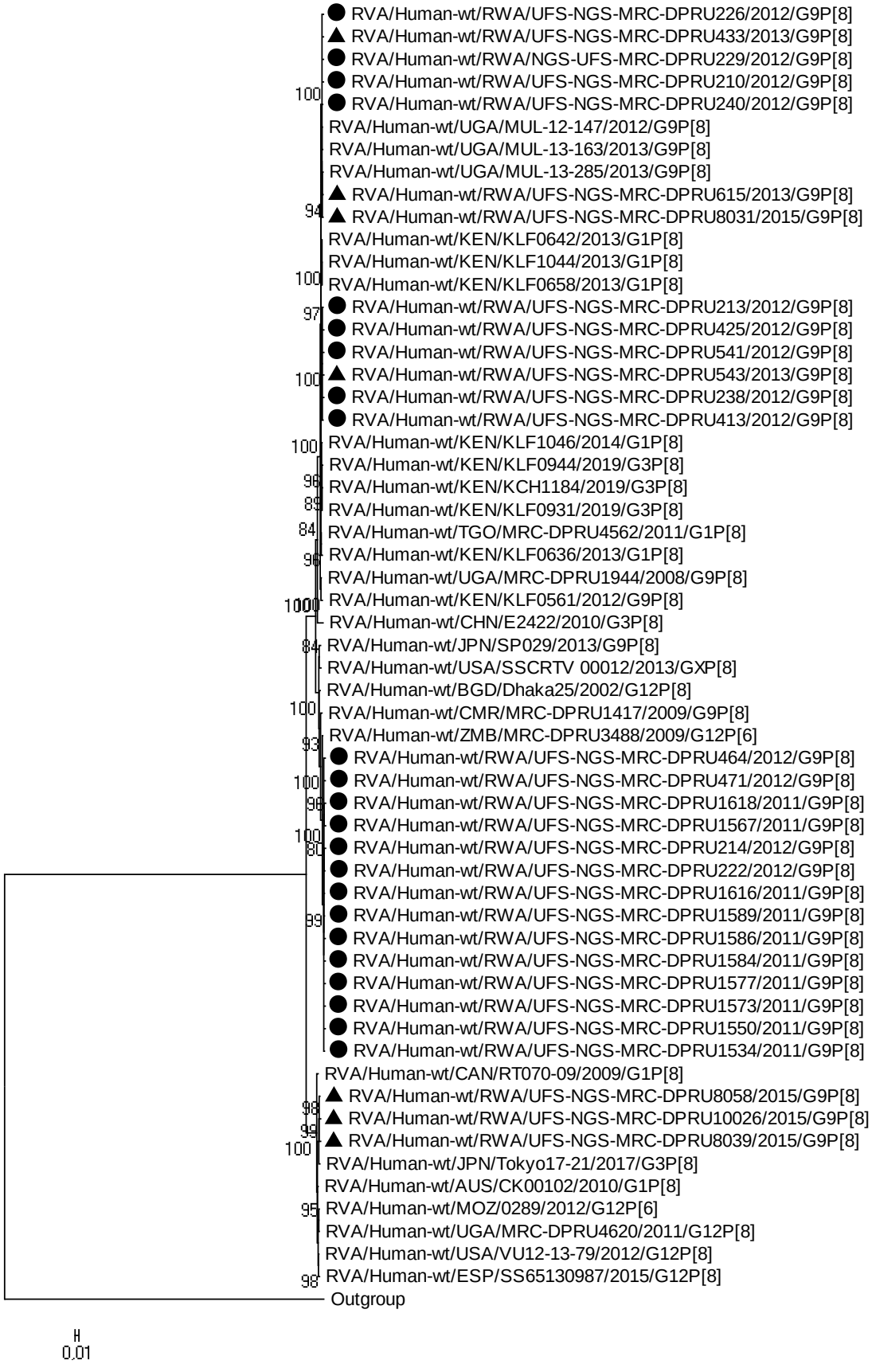
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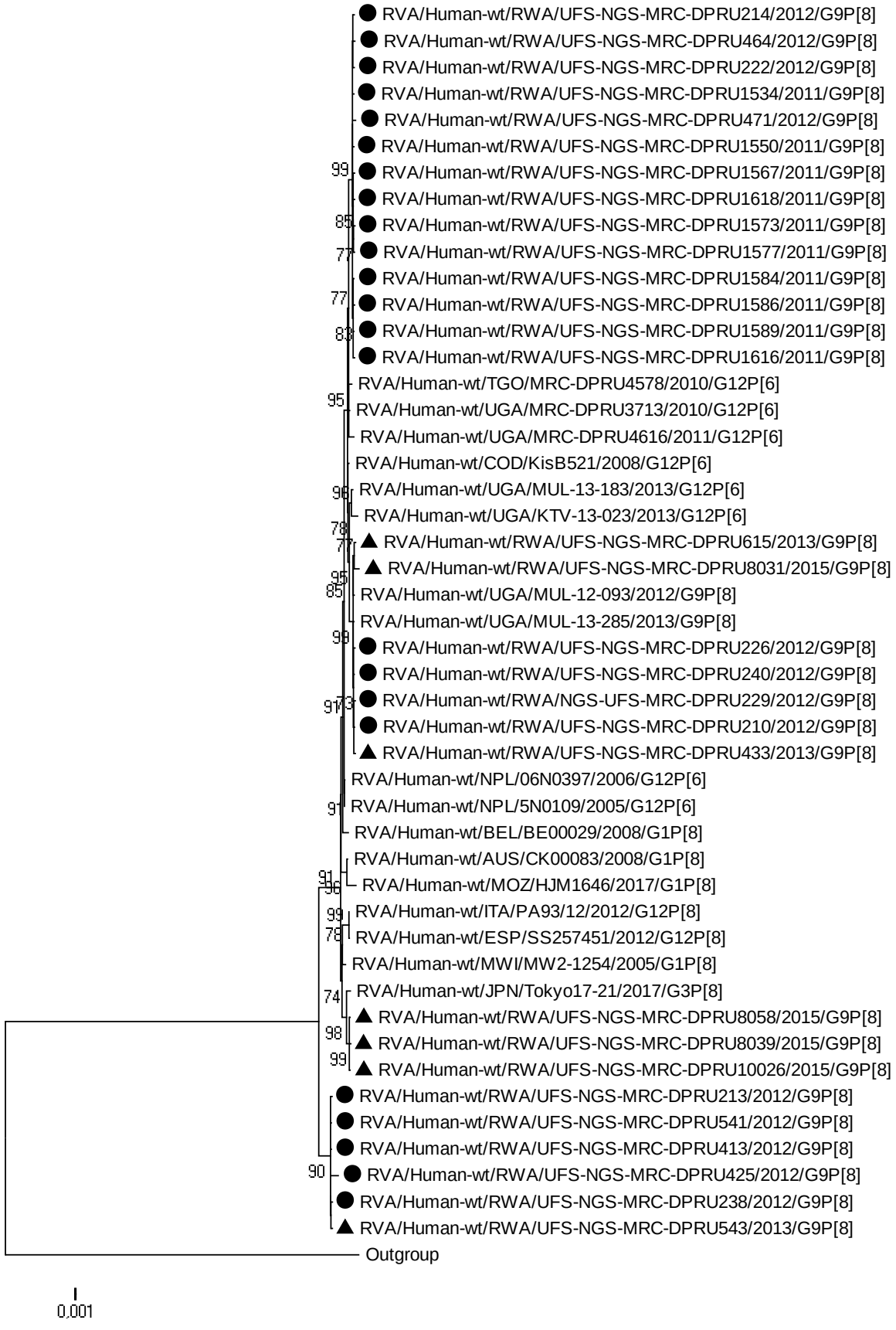
VP2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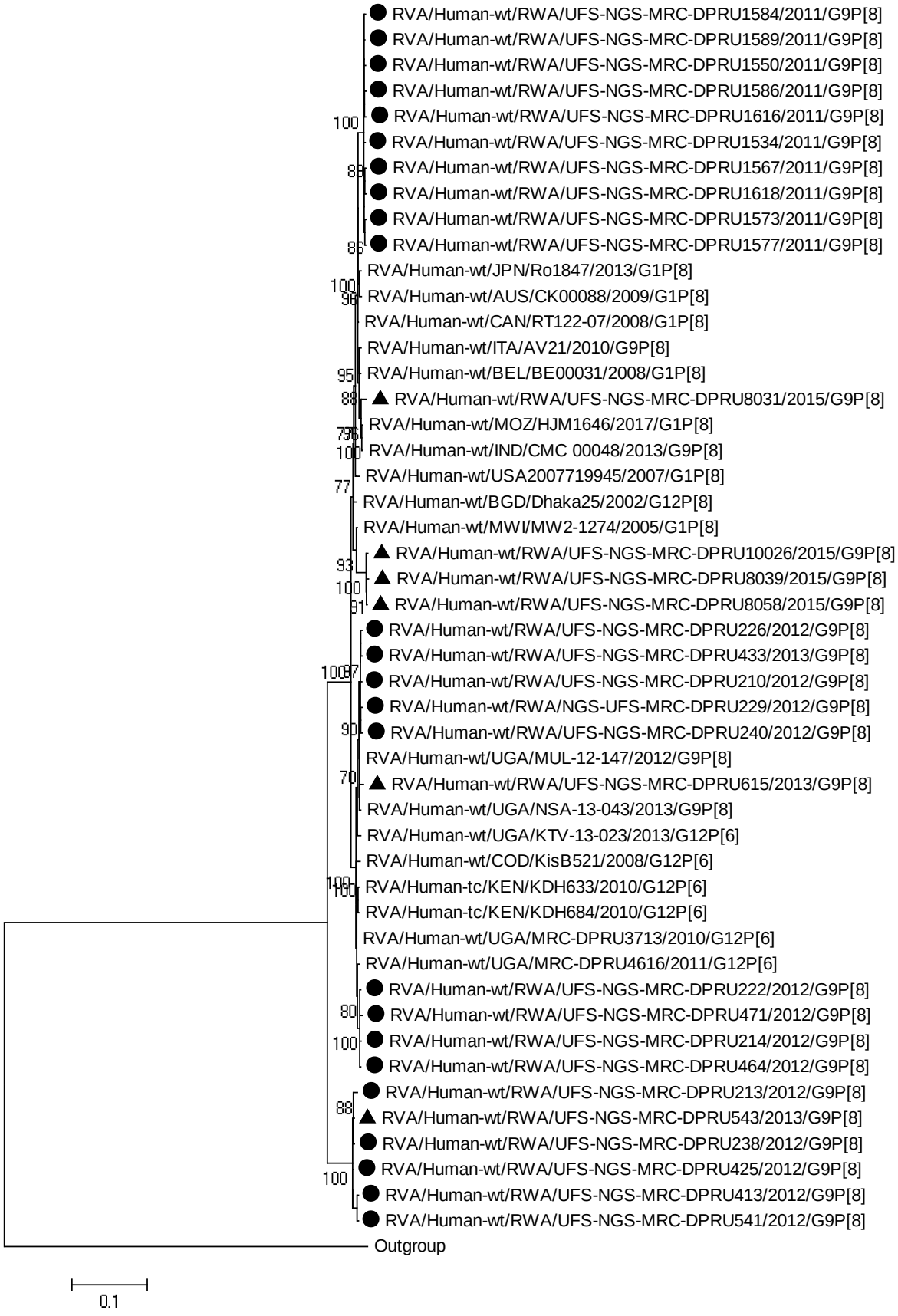
VP3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Outgroup
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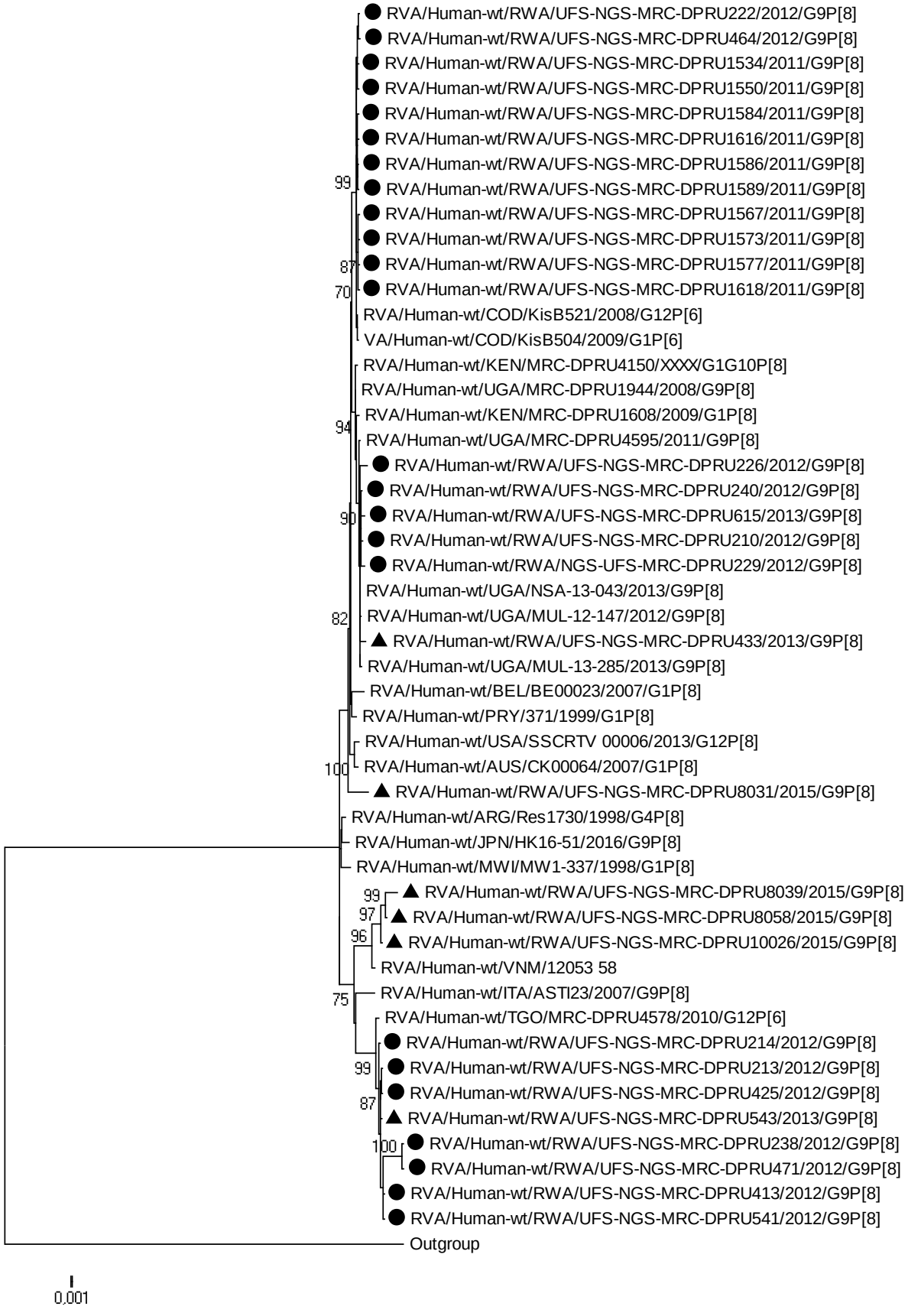
VP6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Outgroup
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Appendix C.3: Pairwise sequence analysis

NSP1

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2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_NSP1_A1	99,9																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_NSP1_A1	99,9	99,9																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_NSP1_A1	99,8	99,9	99,8																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_NSP1_A1	99,9	99,9	99,9	99,8																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_NSP1_A1	99,9	100,0	99,9	99,9	99,9																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_NSP1_A1	99,9	100,0	99,9	99,9	99,9	100,0																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_NSP1_A1	99,9	100,0	99,9	99,9	99,9	100,0	100,0																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_NSP1_A1	100,0	99,9	99,9	99,8	99,9	99,9	99,9	99,9																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_NSP1_A1	99,9	100,0	99,9	99,9	99,9	100,0	100,0	100,0	99,9																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_NSP1	96,8	96,9	96,8	96,7	96,8	96,9	96,9	96,9	96,8	96,9																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_NSP1	97,1	97,2	97,1	97,0	97,1	97,2	97,2	97,2	97,1	97,2	98,6																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_NSP1	99,7	99,7	99,7	99,6	99,7	99,7	99,7	99,7	99,7	99,7	96,7	97,0																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_NSP1	99,6	99,7	99,6	99,5	99,6	99,7	99,7	99,7	99,6	99,7	96,5	96,8	99,4																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_NSP1	96,9	96,9	96,9	96,8	96,9	96,9	96,9	96,9	96,9	96,9	99,8	98,7	96,8	96,6																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_NSP1	96,9	96,9	96,9	96,8	96,9	96,9	96,9	96,9	96,9	96,9	99,9	98,7	96,8	96,6	99,9															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_NSP1	97,0	97,1	97,0	96,9	97,0	97,1	97,1	97,1	97,0	97,1	98,5	99,9	96,9	96,7	98,6	98,6														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_NSP1	96,9	97,0	96,9	96,9	96,9	97,0	97,0	97,0	96,9	97,0	99,9	98,7	96,9	96,6	99,9	99,9	98,7													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_NSP1	97,1	97,2	97,1	97,0	97,1	97,2	97,2	97,2	97,1	97,2	98,6	100,0	97,0	96,8	98,7	98,7	99,9	98,7												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_NSP1	97,1	97,2	97,1	97,0	97,1	97,2	97,2	97,2	97,1	97,2	98,6	100,0	97,0	96,8	98,7	98,7	99,9	98,7	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_NSP1	99,9	100,0	99,9	99,9	99,9	100,0	100,0	100,0	99,9	100,0	96,9	97,2	99,7	99,7	96,9	96,9	97,1	97,0	97,2	97,2										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_NSP1	99,8	99,9	99,8	99,7	99,8	99,9	99,9	99,9	99,8	99,9	96,7	97,0	99,6	99,5	96,8	96,8	96,9	96,9	97,0	97,0	99,9									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_NSP1	97,1	97,2	97,1	97,0	97,1	97,2	97,2	97,2	97,1	97,2	98,6	100,0	97,0	96,8	98,7	98,7	99,9	98,7	100,0	100,0	97,2	97,0								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_NSP1	96,9	96,9	96,9	96,8	96,9	96,9	96,9	96,9	96,9	96,9	99,9	98,7	96,8	96,6	99,9	100,0	98,6	99,9	98,7	98,7	96,9	96,8	98,7							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_NSP1	97,0	97,1	97,0	96,9	97,0	97,1	97,1	97,1	97,0	97,1	98,5	99,9	96,9	96,7	98,6	98,6	99,9	98,7	99,9	99,9	97,1	96,9	99,9	98,6						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_NSP1_A1	97,0	97,1	97,0	96,9	97,0	97,1	97,1	97,1	97,0	97,1	99,7	98,8	96,9	96,7	99,7	99,7	98,7	99,8	98,8	98,8	97,1	96,9	98,8	99,7	98,7					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_NSP1_A1	96,7	96,8	96,7	96,6	96,7	96,8	96,8	96,8	96,7	96,8	95,7	96,0	96,5	96,4	95,8	95,8	96,0	95,9	96,0	96,0	96,8	96,6	96,0	95,8	96,0	96,0				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_NSP1_A1	97,6	97,7	97,6	97,5	97,6	97,7	97,7	97,7	97,6	97,7	96,6	96,9	97,4	97,5	96,7	96,7	96,9	96,8	96,9	96,9	97,7	97,7	96,9	96,7	96,9	96,9	97,9			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_NSP1_A1	97,5	97,5	97,5	97,4	97,5	97,5	97,5	97,5	97,5	97,5	96,6	96,9	97,4	97,3	96,7	96,7	96,9	96,8	96,9	96,9	97,5	97,5	96,9	96,7	96,9	96,9	97,7	99,9		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_NSP1_A1	97,4	97,5	97,4	97,3	97,4	97,5	97,5	97,5	97,4	97,5	96,6	96,9	97,3	97,2	96,6	96,6	96,8	96,7	96,9	96,9	97,5	97,5	96,9	96,6	96,8	96,8	97,7	99,8	99,8	

NSP2

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_NSP2_N1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_NSP2_N1	100,0																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_NSP2_N1	100,0	100,0																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_NSP2_N1	99,9	99,9	99,9																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_NSP2_N1	99,9	99,9	99,9	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_NSP2_N1	100,0	100,0	100,0	99,9	99,9																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_NSP2_N1	100,0	100,0	100,0	99,9	99,9	100,0																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_NSP2_N1	100,0	100,0	100,0	99,9	99,9	100,0	100,0																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_NSP2_N1	99,9	99,9	99,9	99,8	99,8	99,9	99,9	99,9																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_NSP2_N1	100,0	100,0	100,0	99,9	99,9	100,0	100,0	100,0	99,9																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_NSP2	90,9	90,9	90,9	90,8	90,8	90,9	90,9	90,9	90,8	90,9																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_NSP2	99,6	99,6	99,6	99,5	99,5	99,6	99,6	99,6	99,5	99,6	90,6	90,8																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_NSP2	99,9	99,9	99,9	99,8	99,8	99,9	99,9	99,9	99,8	99,9	90,8	91,0	99,5																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_NSP2	90,9	90,9	90,9	90,8	90,8	90,9	90,9	90,9	90,8	90,9	100,0	99,6	90,6	90,8																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_NSP2	90,8	90,8	90,8	90,7	90,7	90,8	90,8	90,8	90,7	90,8	99,9	99,5	90,5	90,7	99,9															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6	100,0	90,8	91,0	99,6	99,5														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_NSP2	90,9	90,9	90,9	90,8	90,8	90,9	90,9	90,9	90,8	90,9	99,8	99,6	90,6	90,8	99,8	99,8	99,6													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6	100,0	90,8	91,0	99,6	99,5	100,0	99,6												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6	100,0	90,8	91,0	99,6	99,5	100,0	99,6	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_NSP2	100,0	100,0	100,0	99,9	99,9	100,0	100,0	100,0	99,9	100,0	90,9	91,1	99,6	99,9	90,9	90,8	91,1	90,9	91,1	91,1										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_NSP2	99,6	99,6	99,6	99,5	99,5	99,6	99,6	99,6	99,5	99,6	90,5	90,7	99,8	99,5	90,5	90,4	90,7	90,5	90,7	90,7	99,6									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6	100,0	90,8	91,0	99,6	99,5	100,0	99,6	100,0	100,0	91,1	90,7								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_NSP2	90,9	90,9	90,9	90,8	90,8	90,9	90,9	90,9	90,8	90,9	100,0	99,6	90,6	90,8	100,0	99,9	99,6	99,8	99,6	99,6	90,9	90,5	99,6							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_NSP2	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,6	100,0	90,8	91,0	99,6	99,5	100,0	99,6	100,0	100,0	91,1	90,7	100,0	99,6						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_NSP2_N1	91,1	91,1	91,1	91,0	91,0	91,1	91,1	91,1	91,0	91,1	99,8	99,6	90,8	91,0	99,8	99,8	99,6	99,8	99,6	99,6	91,1	90,7	99,6	99,8	99,6					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_NSP2_N1	97,8	97,8	97,8	97,7	97,7	97,8	97,8	97,8	97,7	97,8	91,5	91,7	97,4	97,7	91,5	91,4	91,7	91,5	91,7	91,7	97,8	97,4	91,7	91,5	91,7	91,7				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_NSP2_N1	98,2	98,2	98,2	98,1	98,1	98,2	98,2	98,2	98,1	98,2	90,8	91,0	98,2	98,1	90,8	90,7	91,0	90,8	91,0	91,0	98,2	98,1	91,0	90,8	91,0	91,0	97,5			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_NSP2_N1	98,6	98,6	98,6	98,5	98,5	98,6	98,6	98,6	98,5	98,6	91,1	91,3	98,3	98,5	91,1	91,0	91,3	91,1	91,3	91,3	98,6	98,1	91,3	91,1	91,3	91,3	97,8	99,7		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_NSP2_N1	98,6	98,6	98,6	98,5	98,5	98,6	98,6	98,6	98,5	98,6	91,1	91,3	98,3	98,5	91,1	91,0	91,3	91,1	91,3	91,3	98,6	98,1	91,3	91,1	91,3	91,3	97,8	99,7	100,0	

NSP3

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_NSP3_T1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_NSP3_T1	100,0																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_NSP3_T1	100,0	100,0																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_NSP3_T1	100,0	100,0	100,0																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_NSP3_T1	100,0	100,0	100,0	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_NSP3_T1	100,0	100,0	100,0	100,0	100,0																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_NSP3_T1	99,9	99,9	99,9	99,9	99,9	99,9																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_NSP3_T1	99,9	99,9	99,9	99,9	99,9	99,9	99,8																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_NSP3_T1	99,9	99,9	99,9	99,9	99,9	99,9	99,8	99,8																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_NSP3_T1	100,0	100,0	100,0	100,0	100,0	100,0	99,9	99,9	99,9																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_NSP3	98,7	98,7	98,7	98,7	98,7	98,7	98,6	98,6	98,6	98,7																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_NSP3	100,0	100,0	100,0	100,0	100,0	100,0	99,9	99,9	99,9	100,0	98,7	99,0																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_NSP3	99,7	99,7	99,7	99,7	99,7	99,7	99,6	99,6	99,6	99,7	98,4	98,7	99,7																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_NSP3	99,9	99,9	99,9	99,9	99,9	99,9	99,8	99,8	99,8	99,9	98,8	99,1	99,9	99,6																
16	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_NSP3	98,7	98,7	98,7	98,7	98,7	98,7	98,6	98,6	98,6	98,7	100,0	99,5	98,7	98,4	98,8															
17	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_NSP3	98,7	98,7	98,7	98,7	98,7	98,7	98,6	98,6	98,6	98,7	100,0	99,5	98,7	98,4	98,8	100,0														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5	100,0	99,0	98,7	99,1	99,5	99,5													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_NSP3	98,7	98,7	98,7	98,7	98,7	98,7	98,6	98,6	98,6	98,7	100,0	99,5	98,7	98,4	98,8	100,0	100,0	99,5												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_NSP3	99,8	99,8	99,8	99,8	99,8	99,8	99,7	99,7	99,7	99,8	98,5	98,8	99,8	99,5	99,7	98,5	98,5	98,8	98,5											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5	100,0	99,0	98,7	99,1	99,5	99,5	100,0	99,5	98,8										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5	100,0	99,0	98,7	99,1	99,5	99,5	100,0	99,5	98,8	100,0									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_NSP3	98,9	98,9	98,9	98,9	98,9	98,9	98,8	98,8	98,8	98,9	99,4	99,9	98,9	98,6	99,0	99,4	99,4	99,9	99,4	98,7	99,9	99,9								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5	100,0	99,0	98,7	99,1	99,5	99,5	100,0	99,5	98,8	100,0	100,0	99,9							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_NSP3	99,0	99,0	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,0	99,5	100,0	99,0	98,7	99,1	99,5	99,5	100,0	99,5	98,8	100,0	100,0	99,9	100,0						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_NSP3_T1	98,6	98,6	98,6	98,6	98,6	98,6	98,5	98,5	98,5	98,6	99,9	99,4	98,6	98,2	98,7	99,9	99,9	99,4	99,9	98,4	99,4	99,4	99,2	99,4	99,4					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_NSP3_T1	93,4	93,4	93,4	93,4	93,4	93,4	93,3	93,3	93,3	93,4	93,7	93,5	93,4	93,0	93,5	93,7	93,7	93,5	93,7	93,2	93,5	93,5	93,4	93,5	93,5	93,5				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_NSP3_T1	92,4	92,4	92,4	92,4	92,4	92,4	92,3	92,3	92,3	92,4	92,9	92,8	92,4	92,0	92,5	92,9	92,9	92,8	92,9	92,1	92,8	92,8	92,6	92,8	92,8	92,8	98,9			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_NSP3_T1	92,5	92,5	92,5	92,5	92,5	92,5	92,4	92,4	92,4	92,5	93,0	92,9	92,5	92,1	92,6	93,0	93,0	92,9	93,0	92,3	92,9	92,9	92,8	92,9	92,9	92,9	99,0	99,9		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_NSP3_T1	92,5	92,5	92,5	92,5	92,5	92,5	92,4	92,4	92,4	92,5	93,0	92,9	92,5	92,1	92,6	93,0	93,0	92,9	93,0	92,3	92,9	92,9	92,8	92,9	92,9	92,9	99,0	99,9	100,0	

NSP4

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_NSP4_E1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_NSP4_E1	100,0																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_NSP4_E1	100,0	100,0																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_NSP4_E1	99,8	99,8	99,8																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_NSP4_E1	99,8	99,8	99,8	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_NSP4_E1	99,8	99,8	99,8	99,6	99,6																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_NSP4_E1	99,8	99,8	99,8	99,6	99,6	99,6																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_NSP4_E1	100,0	100,0	100,0	99,8	99,8	99,8	99,8																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_NSP4_E1	99,8	99,8	99,8	99,6	99,6	99,6	99,6	99,8																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_NSP4_E1	100,0	100,0	100,0	99,8	99,8	99,8	99,8	100,0	99,8																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_NSP4	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_NSP4	82,1	82,1	82,1	81,8	81,8	82,4	81,8	82,1	81,8	82,1	99,0																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_NSP4	99,8	99,8	99,8	99,6	99,6	99,6	99,6	99,8	99,6	99,8	82,9	81,8																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_NSP4	99,4	99,4	99,4	99,2	99,2	99,2	99,2	99,4	99,2	99,4	83,4	82,4	99,2																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_NSP4	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2	100,0	99,0	82,9	83,4																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_NSP4	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2	100,0	99,0	82,9	83,4	100,0															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_NSP4	82,1	82,1	82,1	81,8	81,8	82,4	81,8	82,1	81,8	82,1	99,0	100,0	81,8	82,4	99,0	99,0														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_NSP4	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2	100,0	99,0	82,9	83,4	100,0	100,0	99,0													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_NSP4	82,1	82,1	82,1	81,8	81,8	82,4	81,8	82,1	81,8	82,1	99,0	100,0	81,8	82,4	99,0	99,0	100,0	99,0												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_NSP4	82,1	82,1	82,1	81,8	81,8	82,4	81,8	82,1	81,8	82,1	99,0	100,0	81,8	82,4	99,0	99,0	100,0	99,0	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_NSP4	99,8	99,8	99,8	99,6	99,6	99,6	99,6	99,8	99,6	99,8	83,4	82,4	99,6	99,2	83,4	83,4	82,4	83,4	82,4	82,4										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_NSP4	99,8	99,8	99,8	99,6	99,6	99,6	99,6	99,8	99,6	99,8	83,4	82,4	99,6	99,2	83,4	83,4	82,4	83,4	82,4	82,4	99,6									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_NSP4	82,1	82,1	82,1	81,8	81,8	82,4	81,8	82,1	81,8	82,1	99,0	100,0	81,8	82,4	99,0	99,0	100,0	99,0	100,0	100,0	82,4	82,4								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_NSP4	81,8	81,8	81,8	81,5	81,5	82,0	81,5	81,8	81,5	81,8	98,4	99,4	81,5	82,0	98,4	98,4	99,4	98,4	99,4	99,4	82,0	82,0	99,4							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_NSP4	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2	100,0	99,0	82,9	83,4	100,0	100,0	99,0	100,0	99,0	99,0	83,4	83,4	99,0	98,4						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_NSP4_E1	83,2	83,2	83,2	82,9	82,9	83,4	82,9	83,2	82,9	83,2	100,0	99,0	82,9	83,4	100,0	100,0	99,0	100,0	99,0	99,0	83,4	83,4	99,0	98,4	100,0					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_NSP4_E1	84,3	84,3	84,3	84,1	84,1	84,6	84,1	84,3	84,1	84,3	97,3	97,1	84,1	84,6	97,3	97,3	97,1	97,3	97,1	97,1	84,6	84,6	97,1	96,5	97,3	97,3				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_NSP4_E1	83,1	83,1	83,1	82,9	82,9	83,4	82,9	83,1	82,9	83,1	96,5	96,3	82,9	83,4	96,5	96,5	96,3	96,5	96,3	96,3	83,4	83,4	96,3	95,7	96,5	96,5	97,3			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_NSP4_E1	83,1	83,1	83,1	82,9	82,9	83,4	82,9	83,1	82,9	83,1	96,5	96,3	82,9	83,4	96,5	96,5	96,3	96,5	96,3	96,3	83,4	83,4	96,3	95,7	96,5	96,5	97,3	100,0		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_NSP4_E1	82,9	82,9	82,9	82,6	82,6	83,1	82,6	82,9	82,6	82,9	96,3	96,1	82,6	83,1	96,3	96,3	96,1	96,3	96,1	96,1	83,1	83,1	96,1	95,4	96,3	96,3	97,1	99,8	99,8	

NSP5

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_NSP5_H1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_NSP5_H1	100,0																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_NSP5_H1	100,0	100,0																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_NSP5_H1	100,0	100,0	100,0																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_NSP5_H1	100,0	100,0	100,0	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_NSP5_H1	100,0	100,0	100,0	100,0	100,0																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_NSP5_H1	100,0	100,0	100,0	100,0	100,0	100,0																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_NSP5_H1	100,0	100,0	100,0	100,0	100,0	100,0	100,0																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_NSP5_H1	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,8																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_NSP5_H1	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,7																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_NSP5	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,1	98,1																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_NSP5	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,6	97,6	99,1																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_NSP5	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,7	99,7	98,1	97,6																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_NSP5	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,7	99,7	98,1	97,6	99,7																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_NSP5	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,7	97,7	99,7	98,8	97,7	97,7																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_NSP5	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,1	98,1	100,0	99,1	98,1	98,1	99,7															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_NSP5	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,6	97,6	99,1	100,0	97,6	97,6	98,8	99,1														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_NSP5	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,1	98,1	100,0	99,1	98,1	98,1	99,7	100,0	99,1													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_NSP5	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,7	97,7	99,3	99,8	97,7	97,7	99,0	99,3	99,8	99,3												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_NSP5	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,7	97,7	99,3	99,8	97,7	97,7	99,0	99,3	99,8	99,3	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_NSP5	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,8	99,7	99,7	98,1	97,6	99,7	98,1	97,6	98,1	97,7	97,7												
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_NSP5	99,7	99,7	99,7	99,7	99,7	99,7	99,7	99,7	99,5	99,5	97,9	97,4	99,5	99,5	97,6	97,9	97,4	97,9	97,6	97,6	99,5									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_NSP5	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,9	97,7	97,7	99,3	99,8	97,7	97,7	99,0	99,3	99,8	99,3	100,0	100,0	97,7	97,6								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_NSP5	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,7	97,6	97,6	99,1	100,0	97,6	97,6	98,8	99,1	100,0	99,1	99,8	99,8	97,6	97,4	99,8							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_NSP5	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,3	98,1	98,1	100,0	99,1	98,1	98,1	99,7	100,0	99,1	100,0	99,3	99,3	98,1	97,9	99,3	99,1						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_NSP5_H1	98,1	98,1	98,1	98,1	98,1	98,1	98,1	98,1	97,9	97,9	99,8	99,0	97,9	97,9	99,5	99,8	99,0	99,8	99,1	99,1	97,9	97,7	99,1	99,0	99,8					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_NSP5_H1	99,3	99,3	99,3	99,3	99,3	99,3	99,3	99,3	99,1	99,1	98,6	98,1	99,1	99,1	98,3	98,6	98,1	98,6	98,3	98,3	99,1	99,0	98,3	98,1	98,6	98,5				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_NSP5_H1	99,1	99,1	99,1	99,1	99,1	99,1	99,1	99,1	99,0	99,0	98,4	97,9	99,0	99,0	98,1	98,4	97,9	98,4	98,1	98,1	99,0	98,8	98,1	97,9	98,4	98,3	99,8			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_NSP5-H1	99,1	99,1	99,1	99,1	99,1	99,1	99,1	99,1	99,0	99,0	98,5	97,9	99,0	99,0	98,1	98,5	97,9	98,5	98,1	98,1	99,0	98,8	98,1	97,9	98,5	98,3	99,8	99,7		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_NSP5_H1	99,3	99,3	99,3	99,3	99,3	99,3	99,3	99,3	99,1	99,1	98,6	98,1	99,1	99,1	98,3	98,6	98,1	98,6	98,3	98,3	99,1	99,0	98,3	98,1	98,6	98,5	100,0	99,8	99,8	

VP1

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP1_R1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP1_R1	99,7																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP1_R1	99,8	99,8																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP1_R1	99,8	99,8	99,9																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP1_R1	99,8	99,9	99,9	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP1_R1	99,9	99,8	99,9	99,9	100,0																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP1_R1	99,9	99,8	99,9	99,9	100,0	100,0																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP1_R1	100,0	99,7	99,8	99,8	99,8	99,9	99,9																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP1_R1	99,9	99,8	99,9	99,9	100,0	100,0	100,0	99,9																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP1_R1	99,8	99,8	99,8	99,9	99,9	99,9	99,9	99,8	99,9																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP1	96,6	96,5	96,6	96,6	96,6	96,7	96,7	96,6	96,7	96,6																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP1	96,4	96,3	96,4	96,4	96,5	96,5	96,5	96,4	96,5	96,4	99,3																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP1	99,8	99,8	99,8	99,9	99,9	99,9	99,9	99,8	99,9	99,9	96,6	96,4																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP1	99,8	99,7	99,8	99,8	99,8	99,9	99,9	99,8	99,9	99,8	96,6	96,4	99,9																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP1	96,7	96,5	96,6	96,6	96,7	96,7	96,7	96,7	96,7	96,6	100,0	99,3	96,6	96,6																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP1	96,6	96,5	96,6	96,6	96,6	96,7	96,7	96,6	96,7	96,6	99,9	99,3	96,6	96,6	100,0															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP1	96,5	96,4	96,5	96,5	96,5	96,6	96,6	96,5	96,6	96,5	99,4	99,9	96,5	96,5	99,4	99,4														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP1	96,6	96,5	96,6	96,6	96,6	96,7	96,7	96,6	96,7	96,6	99,9	99,3	96,6	96,6	99,9	99,9	99,4													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP1	96,4	96,4	96,5	96,5	96,5	96,5	96,5	96,4	96,5	96,5	99,3	99,8	96,5	96,5	99,4	99,3	99,9	99,3												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP1	96,5	96,4	96,5	96,5	96,5	96,6	96,6	96,5	96,6	96,5	99,4	99,9	96,5	96,5	99,4	99,4	99,9	99,4	99,9											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP1	99,7	99,7	99,7	99,8	99,8	99,8	99,8	99,7	99,8	99,8	96,6	96,4	99,8	99,8	96,6	96,6	96,5	96,6	96,5	96,5										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP1	99,7	99,6	99,7	99,7	99,8	99,8	99,8	99,7	99,8	99,7	96,5	96,3	99,7	99,7	96,5	96,5	96,4	96,5	96,4	96,4	99,6									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP1	96,5	96,4	96,5	96,5	96,6	96,6	96,6	96,5	96,6	96,5	99,4	99,9	96,5	96,5	99,4	99,4	100,0	99,4	99,9	100,0	96,5	96,4								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_VP1	96,5	96,4	96,5	96,5	96,6	96,6	96,6	96,5	96,6	96,5	99,4	99,9	96,5	96,5	99,4	99,4	100,0	99,4	99,9	100,0	96,5	96,4	100,0							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP1	96,7	96,5	96,6	96,6	96,7	96,7	96,7	96,7	96,7	96,6	100,0	99,3	96,6	96,6	100,0	100,0	99,4	99,9	99,4	99,4	96,6	96,5	99,4	99,4						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP1	96,4	96,3	96,4	96,4	96,4	96,4	96,4	96,4	96,4	96,4	99,7	99,1	96,4	96,4	99,7	99,7	99,1	99,7	99,1	99,1	96,4	96,3	99,2	99,2	99,7					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP1_R1	96,4	96,3	96,4	96,4	96,4	96,5	96,5	96,4	96,5	96,4	99,7	99,1	96,4	96,4	99,7	99,7	99,1	99,7	99,1	99,1	96,4	96,3	99,2	99,2	99,7	99,8				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP1_R1	93,7	93,7	93,7	93,7	93,7	93,8	93,8	93,7	93,8	93,7	93,9	93,9	93,7	93,7	93,9	93,8	93,9	93,9	93,8	93,9	93,7	93,5	93,9	93,9	93,9	93,8	93,7			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP1_R1	93,7	93,6	93,7	93,6	93,7	93,7	93,7	93,7	93,7	93,6	93,8	93,9	93,6	93,6	93,8	93,8	93,8	93,9	93,8	93,8	93,6	93,5	93,8	93,8	93,8	93,7	93,7	99,9		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP1_R1	93,7	93,6	93,7	93,7	93,7	93,7	93,7	93,7	93,7	93,7	93,9	93,9	93,7	93,7	93,8	93,8	93,8	93,8	93,8	93,8	93,7	93,5	93,9	93,9	93,8	93,8	93,7	100,0	99,9	

VP2

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP2_C1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP2_C1	99,8																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP2_C1	99,9	100,0																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP2_C1	99,9	100,0	100,0																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP2_C1	99,8	99,9	100,0	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP2_C1	99,9	99,7	99,7	99,7	99,7																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP2_C1	100,0	99,8	99,8	99,8	99,8	99,9																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP2_C1	100,0	99,8	99,9	99,9	99,8	99,9	100,0																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP2_C1	99,9	99,9	99,9	99,9	99,9	99,8	99,9	99,9																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP2_C1	99,9	99,9	99,9	99,9	99,9	99,8	99,9	99,9	100,0																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP2	98,9	98,9	98,9	98,9	98,9	98,8	98,9	98,9	99,0	99,0																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP2	96,5	96,4	96,4	96,4	96,4	96,4	96,4	96,5	96,5	96,5	96,2																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP2	99,8	99,8	99,8	99,8	99,8	99,7	99,8	99,8	99,9	99,9	98,9	96,4																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP2	99,8	99,8	99,8	99,8	99,8	99,7	99,8	99,8	99,9	99,9	98,9	96,5	99,9																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP2	99,0	98,9	99,0	99,0	99,0	98,9	98,9	99,0	99,1	99,1	99,9	96,2	98,9	98,9																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP2	98,9	98,9	98,9	98,9	98,9	98,8	98,9	98,9	99,0	99,0	100,0	96,2	98,9	98,9	100,0															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP2	96,4	96,3	96,4	96,4	96,4	96,3	96,4	96,4	96,4	96,4	96,1	100,0	96,3	96,5	96,2	96,2														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP2	98,9	98,9	98,9	98,9	98,9	98,8	98,9	98,9	99,0	99,0	99,9	96,2	98,9	98,9	100,0	99,9	96,2													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP2	96,4	96,3	96,4	96,4	96,4	96,3	96,4	96,4	96,4	96,4	96,1	100,0	96,3	96,5	96,2	96,2	99,9	96,2												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP2	96,5	96,4	96,4	96,4	96,4	96,4	96,4	96,5	96,5	96,5	96,2	100,0	96,4	96,5	96,2	96,2	100,0	96,2	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP2	99,7	99,7	99,7	99,7	99,7	99,6	99,7	99,7	99,8	99,8	98,8	96,3	99,7	99,7	98,8	98,8	96,2	98,8	96,2	96,3										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP2	99,7	99,6	99,7	99,7	99,6	99,5	99,6	99,7	99,7	99,7	98,7	96,2	99,6	99,6	98,8	98,8	96,2	98,8	96,2	96,2	99,5									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP2	96,5	96,4	96,4	96,4	96,4	96,4	96,4	96,5	96,5	96,5	96,2	100,0	96,4	96,5	96,2	96,2	100,0	96,2	100,0	100,0	96,3	96,2								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_VP2	96,4	96,3	96,4	96,4	96,4	96,3	96,4	96,4	96,4	96,4	96,1	100,0	96,3	96,5	96,2	96,2	100,0	96,2	99,9	100,0	96,2	96,2	100,0							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP2	98,9	98,8	98,9	98,9	98,9	98,8	98,8	98,9	98,9	98,9	99,7	96,1	98,8	98,8	99,8	99,8	96,1	99,8	96,1	96,1	98,7	98,7	96,1	96,1						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP2	98,9	98,9	98,9	98,9	98,9	98,8	98,9	98,9	99,0	99,0	100,0	96,2	98,9	98,9	100,0	100,0	96,2	99,9	96,2	96,2	98,8	98,8	96,2	96,2	99,8					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP2_C1	98,6	98,5	98,6	98,6	98,6	98,5	98,6	98,6	98,6	98,6	99,4	95,9	98,6	98,5	99,5	99,5	95,8	99,5	95,8	95,9	98,4	98,4	95,9	95,8	99,5	99,5				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP2_C1	98,3	98,4	98,4	98,4	98,4	98,2	98,3	98,3	98,4	98,4	98,3	96,8	98,3	98,4	98,3	98,2	96,7	98,3	96,7	96,8	98,2	98,2	96,8	96,7	98,2	98,2	98,0			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP2_C1	98,3	98,3	98,4	98,4	98,4	98,2	98,3	98,3	98,4	98,4	98,2	96,7	98,3	98,3	98,3	98,3	96,7	98,3	96,7	96,7	98,1	98,2	96,7	96,7	98,2	98,3	97,9	100,0		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP2_C1	98,3	98,3	98,4	98,4	98,4	98,2	98,3	98,3	98,4	98,4	98,2	96,7	98,3	98,3	98,2	98,3	96,7	98,3	96,7	96,7	98,1	98,2	96,7	96,7	98,2	98,3	97,9	100,0	99,9	

VP3

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP3_M1																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP3_M1	99,8																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP3_M1	99,8	99,9																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP3_M1	99,8	100,0	99,9																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP3_M1	99,8	100,0	100,0	100,0																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP3_M1	99,8	99,8	99,8	99,8	99,8																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP3_M1	99,8	99,8	99,8	99,8	99,8	100,0																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP3_M1	100,0	99,8	99,8	99,8	99,8	99,8	99,8																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP3_M1	99,8	100,0	100,0	100,0	100,0	99,8	99,8	99,8																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP3_M1	99,9	99,9	99,9	99,9	100,0	99,9	99,9	99,9	100,0																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP3	97,1	97,2	97,2	97,2	97,2	97,2	97,2	97,1	97,2	97,2																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP3	93,0	93,1	93,1	93,1	93,2	93,1	93,1	93,0	93,2	93,1	93,3																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP3	97,0	97,0	97,0	97,0	97,0	97,0	97,0	97,0	97,0	97,1	99,0	93,4																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP3	97,0	97,0	97,0	97,0	97,1	97,1	97,1	97,0	97,1	97,1	99,1	93,3	99,7																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP3	97,1	97,2	97,2	97,2	97,2	97,2	97,2	97,1	97,2	97,2	100,0	93,3	99,0	99,1																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP3	97,0	97,1	97,0	97,1	97,1	97,1	97,1	97,0	97,1	97,1	99,9	93,2	98,8	98,9	99,9															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP3	93,0	93,3	93,2	93,3	93,2	93,1	93,1	93,0	93,2	93,2	93,4	99,8	93,4	93,3	93,4	93,3														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP3	97,1	97,2	97,1	97,2	97,2	97,2	97,2	97,1	97,2	97,2	99,9	93,2	98,9	99,0	99,9	99,8	93,3													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP3	93,2	93,3	93,3	93,3	93,4	93,3	93,3	93,2	93,4	93,3	93,6	99,8	93,6	93,5	93,6	93,4	99,8	93,4												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP3	93,1	93,3	93,3	93,3	93,3	93,2	93,2	93,1	93,3	93,3	93,5	99,8	93,6	93,5	93,5	93,4	99,8	93,4	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP3	97,0	97,1	97,1	97,1	97,1	97,1	97,1	97,0	97,1	97,2	99,0	93,4	99,8	99,8	99,0	98,9	93,5	99,0	93,7	93,6										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP3	96,8	96,9	96,8	96,9	96,9	96,9	96,9	96,8	96,9	96,9	98,8	93,3	99,5	99,6	98,8	98,7	93,5	98,8	93,6	93,5	99,6									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP3	93,2	93,3	93,3	93,3	93,4	93,3	93,3	93,2	93,4	93,3	93,6	99,8	93,6	93,5	93,6	93,4	99,8	93,4	100,0	100,0	93,7	93,6								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP3	97,1	97,2	97,2	97,2	97,2	97,2	97,2	97,1	97,2	97,2	100,0	93,3	99,0	99,1	100,0	99,9	93,4	99,9	93,6	93,5	99,0	98,8	93,6							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G98_VP3	93,1	93,3	93,3	93,3	93,3	93,2	93,2	93,1	93,3	93,3	93,5	99,9	93,5	93,4	93,5	93,3	99,9	93,3	99,9	99,9	93,6	93,5	99,9	93,5						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP3_M1	96,8	96,9	96,9	96,9	96,9	96,9	96,9	96,8	96,9	97,0	99,4	93,1	98,6	98,7	99,4	99,3	93,1	99,4	93,3	93,2	98,7	98,5	93,3	99,4	93,2					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP3_M1	98,2	98,2	98,2	98,2	98,3	98,3	98,3	98,2	98,3	98,3	97,1	93,0	96,9	97,0	97,1	97,0	93,0	97,1	93,1	93,1	97,0	96,8	93,1	97,1	93,1	96,8				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP3_M1	97,0	97,1	97,0	97,1	97,1	97,1	97,1	97,0	97,1	97,1	97,3	92,5	97,0	97,1	97,3	97,2	92,6	97,3	92,6	92,6	97,0	96,9	92,6	97,3	92,6	96,9	96,9			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP3_M1	96,9	97,1	97,0	97,1	97,0	97,0	97,0	96,9	97,0	97,1	97,2	92,4	97,0	97,0	97,2	97,2	92,6	97,3	92,5	92,6	97,0	96,8	92,5	97,2	92,6	96,9	96,9	100,0		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP3_M1	97,0	97,0	97,0	97,0	97,1	97,1	97,1	97,0	97,1	97,1	97,4	92,6	97,1	97,2	97,4	97,2	92,6	97,3	92,7	92,7	97,1	96,9	92,7	97,4	92,7	97,0	96,9	99,9	99,9	

VP4

		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
1	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP4_P8																														
2	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP4_P8	99,7																													
3	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP4_P8	100,0	99,8																												
4	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP4_P8	99,8	99,7	99,9																											
5	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP4_P8	99,8	99,7	99,8	99,8																										
6	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP4_P8	99,6	99,8	99,7	99,6	99,7																									
7	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP4_P8	99,7	99,9	99,7	99,6	99,7	99,9																								
8	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP4_P8	99,7	100,0	99,7	99,7	99,7	99,8	99,8																							
9	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP4_P8	99,8	99,8	99,9	99,8	99,9	99,8	99,8	99,9																						
10	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP4	99,8	99,9	99,8	99,8	99,8	99,8	99,8	99,9	100,0																					
11	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP4	97,0	97,1	97,1	96,9	97,0	96,9	97,0	97,0	97,1	97,1																				
12	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP4	99,3	99,4	99,3	99,2	99,3	99,3	99,3	99,3	99,4	99,4	96,7																			
13	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP4	99,4	99,5	99,4	99,3	99,3	99,3	99,4	99,4	99,5	99,5	96,7	99,4																		
14	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP4	99,6	99,7	99,7	99,5	99,6	99,6	99,7	99,7	99,7	99,7	96,9	99,4	99,3																	
15	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP4	96,2	96,3	96,3	96,2	96,2	96,1	96,2	96,3	96,4	96,3	99,2	96,3	96,3	96,1																
16	RVA/Human-w/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP4	97,0	97,0	97,0	96,9	96,9	96,9	96,9	97,0	97,1	97,0	100,0	96,6	96,6	96,9	99,1															
17	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP4	99,6	99,7	99,6	99,5	99,5	99,5	99,6	99,6	99,7	99,7	96,9	99,7	99,3	99,7	96,1	96,9														
18	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP4	97,0	97,1	97,1	96,9	97,0	96,9	97,0	97,0	97,1	97,1	99,7	96,7	96,8	96,9	99,0	99,7	96,9													
19	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP4	99,6	99,7	99,7	99,5	99,6	99,6	99,7	99,7	99,7	99,7	97,0	99,7	99,3	99,7	96,2	96,9	100,0	97,0												
20	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP4	99,4	99,5	99,4	99,3	99,3	99,3	99,4	99,4	99,5	99,5	96,8	99,9	99,5	99,5	96,3	96,7	99,7	96,8	99,8											
21	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP4	99,6	99,7	99,7	99,5	99,6	99,6	99,7	99,7	99,7	99,7	96,9	99,4	99,3	99,7	96,1	96,9	99,7	96,9	99,7	99,5										
22	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP4	99,7	99,8	99,7	99,7	99,7	99,7	99,7	99,8	99,9	99,9	97,0	99,3	99,4	99,7	96,2	96,9	99,6	97,0	99,7	99,4	99,7									
23	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP4	99,6	99,7	99,7	99,5	99,6	99,6	99,7	99,7	99,7	99,7	97,0	99,7	99,3	99,7	96,2	96,9	100,0	97,0	100,0	99,8	99,7	99,7								
24	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_VP4	99,4	99,5	99,4	99,3	99,3	99,4	99,4	99,4	99,5	99,5	96,8	99,8	99,4	99,5	96,3	96,7	99,8	96,8	99,8	99,9	99,5	99,4	99,8							
25	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP4	96,8	96,8	96,8	96,7	96,7	96,6	96,7	96,8	96,8	96,8	99,7	96,8	96,8	96,6	99,3	99,7	96,7	99,5	96,7	96,9	96,6	96,7	96,7	96,8						
26	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP4_P8	96,8	96,8	96,8	96,8	96,8	96,8	96,8	96,9	97,0	96,9	99,3	96,8	96,8	96,7	98,9	99,2	96,7	99,3	96,8	96,9	96,7	96,8	96,8	96,9	99,4					
27	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP4_P8	96,8	96,9	96,8	96,8	96,8	96,8	96,8	96,9	97,0	96,9	99,3	96,7	96,7	96,7	98,8	99,2	96,7	99,3	96,8	96,8	96,7	96,8	96,8	96,8	99,3	99,2				
28	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP4_P8	98,9	98,8	98,9	98,8	98,9	98,8	98,8	98,9	99,0	99,0	96,8	98,4	98,5	98,7	96,0	96,8	98,6	96,8	98,7	98,5	98,7	99,0	98,7	98,5	96,5	96,7	96,7			
29	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP4_P8	98,9	98,8	98,9	98,7	98,8	98,7	98,8	98,8	98,9	98,9	96,8	98,4	98,5	98,7	96,0	96,8	98,6	96,8	98,7	98,5	98,7	98,9	98,7	98,5	96,5	96,6	96,6	99,9		
30	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP4_P8	98,9	98,8	98,8	98,8	98,9	98,7	98,7	98,8	99,0	98,9	96,8	98,3	98,4	98,6	96,0	96,7	98,6	96,8	98,6	98,4	98,6	98,9	98,6	98,4	96,5	96,6	96,6	100,0	99,9	

VP6

		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
1	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP6_I1																														
2	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP6_I1	99,7																													
3	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP6_I1	99,7	100,0																												
4	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP6_I1	99,7	100,0	100,0																											
5	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP6_I1	99,7	100,0	100,0	100,0																										
6	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP6_I1	100,0	99,7	99,7	99,7	99,7																									
7	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP6_I1	100,0	99,7	99,7	99,7	99,7	100,0																								
8	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP6_I1	100,0	99,7	99,7	99,7	99,7	100,0	100,0																							
9	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP6_I1	99,8	99,9	99,9	99,9	99,9	99,8	99,8	99,8																						
10	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP6_I1	99,8	99,9	99,9	99,9	99,9	99,8	99,8	99,8	100,0																					
11	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP6	98,6	98,7	98,7	98,7	98,7	98,6	98,6	98,6	98,8	98,8																				
12	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP6	95,2	95,1	95,1	95,1	95,1	95,2	95,2	95,2	95,2	95,2	95,0																			
13	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP6	95,5	95,4	95,4	95,4	95,4	95,5	95,5	95,5	95,5	95,5	95,3	99,7																		
14	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP6	99,7	99,7	99,7	99,7	99,7	99,7	99,7	99,7	99,8	99,8	98,6	95,1	95,4																	
15	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP6	98,7	98,8	98,8	98,8	98,8	98,7	98,7	98,7	98,9	98,9	99,7	95,1	95,4	98,7																
16	RVA/Human-w/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP6	98,7	98,8	98,8	98,8	98,8	98,7	98,7	98,7	98,9	98,9	99,7	95,1	95,4	98,7	99,8															
17	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP6	95,3	95,2	95,2	95,2	95,2	95,3	95,3	95,3	95,3	95,3	95,1	99,9	99,8	95,2	95,2	95,2														
18	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP6	98,7	98,8	98,8	98,8	98,8	98,7	98,7	98,7	98,9	98,9	99,7	95,2	95,5	98,7	99,8	99,8	95,3													
19	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP6	95,3	95,2	95,2	95,2	95,2	95,3	95,3	95,3	95,3	95,3	95,1	99,9	99,8	95,2	95,2	95,2	100,0	95,3												
20	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP6	95,2	95,3	95,3	95,3	95,3	95,2	95,2	95,2	95,4	95,4	95,2	99,8	99,7	95,3	95,3	95,3	99,9	95,4	99,9											
21	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP6	99,6	99,7	99,7	99,7	99,7	99,6	99,6	99,6	99,7	99,7	98,5	94,9	95,2	99,7	98,6	98,6	95,0	98,6	95,0	95,1										
22	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP6	95,5	95,4	95,4	95,4	95,4	95,5	95,5	95,5	95,5	95,5	95,3	99,7	100,0	95,4	95,4	95,4	99,8	95,5	99,8	99,7	95,2									
23	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP6	95,3	95,2	95,2	95,2	95,2	95,3	95,3	95,3	95,3	95,3	95,1	99,9	99,8	95,2	95,2	95,2	100,0	95,3	100,0	99,9	95,0	99,8								
24	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_VP6	95,3	95,2	95,2	95,2	95,2	95,3	95,3	95,3	95,3	95,3	95,1	99,9	99,8	95,2	95,2	95,2	100,0	95,3	100,0	99,9	95,0	99,8	100,0							
25	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP6	98,9	99,0	99,0	99,0	99,0	98,9	98,9	98,9	99,1	99,1	99,7	95,3	95,6	98,9	99,8	99,8	95,4	99,8	95,4	95,5	98,8	95,6	95,4	95,4						
26	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP6_I1	98,6	98,6	98,6	98,6	98,6	98,6	98,6	98,6	98,7	98,7	99,6	95,1	95,2	98,6	99,7	99,7	95,1	99,7	95,1	95,1	98,5	95,2	95,1	95,1	99,7					
27	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP6_I1	97,3	97,4	97,4	97,4	97,4	97,3	97,3	97,3	97,5	97,5	97,2	94,8	95,0	97,3	97,3	97,3	94,9	97,3	94,9	94,9	97,2	95,0	94,9	94,9	97,4	97,1				
28	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP6_I1	95,0	95,1	95,1	95,1	95,1	95,0	95,0	95,0	95,2	95,2	95,0	95,2	95,5	95,1	95,2	95,1	95,3	95,2	95,3	95,4	94,9	95,5	95,3	95,3	95,1	95,1	94,7			
29	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP6_I1	95,4	95,5	95,5	95,5	95,5	95,4	95,4	95,4	95,6	95,6	95,4	95,6	95,9	95,5	95,5	95,5	95,7	95,6	95,7	95,8	95,3	95,9	95,7	95,7	95,5	95,5	95,0	99,7		
30	RVA/Human-w/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP6_I1	95,4	95,5	95,5	95,5	95,5	95,4	95,4	95,4	95,6	95,6	95,4	95,6	95,9	95,5	95,5	95,5	95,7	95,6	95,7	95,8	95,3	95,9	95,7	95,7	95,5	95,5	95,0	99,7	100,0	

VP7

		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
1	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1616/2011/G9P8_VP7_G9																														
2	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1589/2011/G9P8_VP7_G9	99,9																													
3	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1586/2011/G9P8_VP7_G9	100,0	99,9																												
4	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1584/2011/G9P8_VP7_G9	99,9	99,8	99,9																											
5	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1577/2011/G9P8_VP7_G9	100,0	99,9	100,0	99,9																										
6	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1618/2011/G9P8_VP7_G9	99,8	99,7	99,8	99,7	99,8																									
7	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1573/2011/G9P8_VP7_G9	100,0	99,9	100,0	99,9	100,0	99,8																								
8	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1567/2011/G9P8_VP7_G9	97,2	97,1	97,2	97,1	97,2	97,5	97,2																							
9	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1550/2011/G9P8_VP7_G9	100,0	99,9	100,0	99,9	100,0	99,8	100,0	97,2																						
10	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU1534/2011/G9P8_VP7_G9	100,0	99,9	100,0	99,9	100,0	99,8	100,0	97,2	100,0																					
11	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU210/2012/G9P8_VP7	97,3	97,2	97,3	97,2	97,3	97,1	97,3	94,4	97,3	97,3																				
12	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU213/2012/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8																			
13	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU214/2012/G9P8_VP7	99,8	99,7	99,8	99,7	99,8	99,6	99,8	97,0	99,8	99,8	97,1	99,8																		
14	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU222/2012/G9P8_VP7	99,9	99,8	99,9	99,8	99,9	99,7	99,9	97,1	99,9	99,9	97,2	99,7	99,9																	
15	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU226/2012/G9P8_VP7	97,2	97,1	97,2	97,1	97,2	97,0	97,2	94,2	97,2	97,2	99,7	96,7	97,0	97,1																
16	RVA/Human-wt/RWA/NGS-UFS-MRC-DPRU229/2012/G9P8_VP7	97,4	97,3	97,4	97,3	97,4	97,2	97,4	94,5	97,4	97,4	99,9	97,0	97,2	97,3	99,8															
17	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU238/2012/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8	100,0	99,8	99,7	96,7	97,0														
18	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU240/2012/G9P8_VP7	95,1	95,0	95,1	95,0	95,1	94,9	95,1	92,5	95,1	95,1	97,7	94,7	94,9	95,0	97,4	97,6	94,7													
19	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU413/2012/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8	100,0	99,8	99,7	96,7	97,0	100,0	94,7												
20	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU425/2012/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8	100,0	99,8	99,7	96,7	97,0	100,0	94,7	100,0											
21	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU464/2012/G9P8_VP7	99,8	99,7	99,8	99,7	99,8	99,6	99,8	97,0	99,8	99,8	97,1	99,6	99,8	99,9	97,0	97,2	99,6	94,9	99,6	99,6										
22	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU471/2012/G9P8_VP7	99,7	99,6	99,7	99,6	99,7	99,5	99,7	96,9	99,7	99,7	97,0	99,5	99,7	99,8	96,8	97,1	99,5	94,8	99,5	99,5	99,7									
23	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU541/2012/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8	100,0	99,8	99,7	96,7	97,0	100,0	94,7	100,0	100,0	99,6	99,5								
24	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU433/2013/G9P8_VP7	97,4	97,3	97,4	97,3	97,4	97,2	97,4	94,5	97,4	97,4	99,9	97,0	97,2	97,3	99,8	100,0	97,0	97,6	97,0	97,0	97,2	97,1	97,0							
25	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU543/2013/G9P8_VP7	99,6	99,5	99,6	99,5	99,6	99,4	99,6	96,8	99,6	99,6	96,8	100,0	99,8	99,7	96,7	97,0	100,0	94,7	100,0	100,0	99,6	99,5	100,0	97,0						
26	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU615/2013/G9P8_VP7	97,3	97,2	97,3	97,2	97,3	97,1	97,3	94,4	97,3	97,3	99,6	96,8	97,1	97,2	99,5	99,7	96,8	97,5	96,8	96,8	97,1	97,2	96,8	99,7	96,8					
27	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8031/2015/G9P8_VP7_G9	96,8	96,7	96,8	96,7	96,8	96,6	96,8	93,9	96,8	96,8	99,0	96,4	96,6	96,7	98,9	99,1	96,4	96,8	96,4	96,4	96,6	96,7	96,4	99,1	96,4	99,4				
28	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8039/2015/G9P8_VP7_G9	96,8	96,7	96,8	96,7	96,8	96,6	96,8	93,9	96,8	96,8	95,9	96,4	96,6	96,7	96,2	96,0	96,4	93,7	96,4	96,4	96,6	96,5	96,4	96,0	96,4	95,9	95,2			
29	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU8058/2015/G9P8_VP7_G9	96,7	96,6	96,7	96,6	96,7	96,5	96,7	93,8	96,7	96,7	95,8	96,3	96,5	96,6	96,1	95,9	96,3	93,6	96,3	96,3	96,5	96,4	96,3	95,9	96,3	95,8	95,1	99,8		
30	RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU10026/2015/G9P8_VP7_G9	96,8	96,7	96,8	96,7	96,8	96,6	96,8	93,9	96,8	96,8	95,9	96,4	96,6	96,7	96,2	96,0	96,4	93,7	96,4	96,4	96,6	96,5	96,4	96,0	96,4	95,9	95,2	100,0	99,8	