Zoonotic potential of viral enteric pathogens in South African domesticated animals

by

Neo Segone

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Department of Microbial, Biochemical and Food Biotechnology

Faculty of Natural and Agricultural Sciences

University of the Free State

Supervisor: Prof H.G. O'Neill

Co-Supervisor: Dr A. Strydom

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Declaration

I, Neo segone, declare that the Master's Degree research dissertation that I herewith submit for the Master's Degree qualification at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution

Signature: 

Neo Segone

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List of abbreviations

A

Adenovirus (AdV)

Agarose Gel Electrophoresis (AGE)

Astrovirus (Astv)

Avastrovirus (AAstV)

Avian nephritis virus (ANV)

B

Basic Local Alignment Search Tool (BLAST)

Bat astrovirus (BAstV)

Bottlenose dolphin astrovirus 1 (BdAstV-1)

Bovine astrovirus (BAstV)

Brome mosaic virus (BMV)

C

California sea lion astrovirus 1 (CsIAstV-1)

California sea lion astrovirus 2 (CsIAstV-2)

Canine astrovirus 1 (CaAstV-1)

Capreolus capreolus astrovirus 2 (CcAstV-2)

Capreolus capreolus astrovirus 1 (CcAstV-1)

D

Double stranded ribonucleic acid (dsRNA)

Double-stranded DNA (dsDNA)

Duck astrovirus 1 (DAstV-1)

E

Electron Microscopy (EM)

F

FTD (Fast Track Diagnostics)

Feline astrovirus (FAstV)

Feline rotaviruses (FRVs)

H

Human adenoviruses (HAdV)

Human adenovirus 2 (HAdV-2)

Human astroviruses (HAstVs)

Human classical astroviruses (HAstV 1-8)

Human astrovirus Melbourne (HAstV-MLB1)

Human astrovirus-Virginia 1 (HAsV-VA1)

Human astrovirus-Virginia 2 (HAsV-VA2)

Human enteric adenoviruses (HEAdVs)

Human-mink-ovine astrovirus A (HMOAstV-A)

Human-mink-ovine astrovirus B (HMOAstV-B)

Human-mink-ovine astrovirus C (HMOAstV-C)

I

International committee for the taxonomy of viruses (ICTV)

Internal control (IC)

K

Kobuvirus (KV)

M

Mamastrovirus (MAstV)

Maximum likelihood (ML)

Mink astrovirus 1 (MiAstV-1)

Multiple Sequence Comparison by Log Expectation (MUSCLE)

L

Lithium chloride (LiCl)

N

Next generation sequencing (NGS)

Non-structural protein (NSP)

Norovirus (NoV)

O

Open reading frame (ORF)

Ovine astrovirus 1 (OAstV-1)

P

Parechovirus (PeV)

Picobirnavirus (PBV)

Polyacrylamide gel electrophoresis (PAGE)

Polymerase Chain Reaction (PCR)

Positive control (PC)

Porcine adenovirus (PAdV)

Porcine astrovirus (PAstV)

Porcine astrovirus 2 (PAstV-2)

R

Rat astrovirus (RaAstV)

Real-time PCR (qPCR)

Reverse transcriptase PCR (RT-PCR)

Real-time RT-PCR (RT-qPCR)

ribosomal RNA (rRNA)

RNA-dependent RNA polymerase (RdRp)

Room temperature (RT)

Rotavirus (RV)

Rotavirus Classification Working Group (RCWG)

S

Sapovirus (SaV)

Sapoviruses (SaVs)

Severe Acute Respiratory Syndrome (SARS-Cov)

Single stranded RNA (ssRNA)

T

Terminal protein (TP)

Turkey astrovirus 1 (TAstV-1)

Turkey astrovirus 2 (TAstV-2)

Turkey astrovirus 3 (TAstV-3)

U

University of the Free State Next Generation Unit (UFS-NGS)

Untranslated region (UTR)

V

Viral protein (VP)

Virus Pathogen Database Analysis Resource (ViPR)

Abstract

Diarrhoea is the second leading cause of death in children less than the age of five years of age. Enteric viruses are the major aetiological agents associated with acute diarrhoea. The occurrence of enteric viruses in humans and animals highlights the importance of the One Health approach and interest in investigating the possible potential for zoonosis to occur. Common human enteric viruses include Rotavirus (RV), Sapovirus (SaV), Norovirus (NoV), Adenovirus (AdV) and Astrovirus (AstV). Rotaviruses have been extensively studied as compared to other enteric viruses and there is evidence that rotavirus is a zoonotic virus. In light of the current COVID-19 pandemic, which was caused by a zoonotic transmission, it is vital to investigate and know the zoonotic status of other viruses. In this study, canine (n = 104) and porcine (n = 118) stool samples were screened for human enteric viruses, to identify viral agents with the possible potential for zoonosis. Three water samples from the porcine pen were also evaluated for the presence of enteric viruses. Electropherotyping was used as a primary method for detecting RV. In the canine samples, none (0/104) were positive for RV, whereas for porcine, 13.56% (16/118) were recorded positive for RV. Of the three water samples, none were positive for RV. Real-time RT-PCR (RT-qPCR) was used to expand the study to detect other enteric viruses mentioned above. RT-qPCR was able to identify three more RV positive porcine samples that were not detected by electropherotyping. Therefore, a total of 19/118 (16.10%) porcine samples were positive for RV. Of the 19 RV positive samples, 16 were confirmed to have sufficient RNA by agarose gel electrophoresis and therefore selected for whole genome sequencing. The whole genomes of fifteen group A rotavirus (RVA) strains were determined using the Illumina Miseq platform. One sample displayed a G5P[13] genotype combination, two G5P[6]P[13], three G5P[13]P[23], and nine G5P[23] combinations. One of the fifteen samples also had a co-infection with group C rotavirus (RVC). The average coverage for the RVC strain was too low for phylogenetic analysis but a BLASTn search was used to identify close relatives. Eight of the fifteen samples, were co-infections with picobirnavirus. A group B (RVB) strain was also identified and genotyped. The zoonotic potential of the detected RVAs was determined by phylogenetic analysis. The phylogenetic analysis revealed that study strains are similar to one another and clustered with the South African porcine strains. Exceptions were P[6] and NSP5/6 which were closely related to human strains identified in GenBank. These results suggest possible zoonotic potential for some of the RV strains evaluated. The two P[13]-containing study strains, RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] and RVA/Pig-wt/ZAF/UFS-BOC035/G5P[6]P[13] were distinct with nucleotide sequence identities of 83% and 83.55% to RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13], respectively, suggesting possible reassortment. The presence of

these rotavirus strains on one farm in South Africa, calls for more investigation on the farm and overall surveillance of porcine RV strains in Africa.

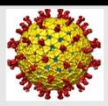
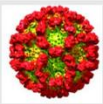
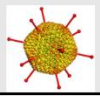
Keywords: One Health, enteric viruses, zoonosis, porcine rotavirus, diarrhoea, electropherotyping, RT-qPCR, next generation sequencing, phylogenetic analysis

1. Chapter 1: Zoonotic potential of human enteric viruses

1.1 Introduction

Diarrhoea was reported as the second leading cause of death in children aged 0-59 months in 2015, making it one of the most common causes of mortality (Liu et al. 2016a), particularly, in children under 5 years of age from developing countries (Kotloff et al. 2013). Although bacteria and parasites contribute to diarrhoeal cases, viruses are the most common pathogens known to cause diarrhoea worldwide (Platts-Mills et al. 2018). Common viral agents associated with enteric infections are rotavirus (RV), astrovirus (Astv), norovirus (Nov), sapovirus (SaV), adenovirus (AdV) (Table 1.1) and to a lesser extent, picobirnavirus (PBV), parechovirus (PeV) and kobuvirus (KV) (Bishop et al. 1973, Chiba et al. 1980, Kapikian et al. 1972, Madeley et al. 1975, Malik et al. 2014, Morris et al. 1975, Sircar et al. 2016).

Table 1.1 Morphological structure of enteric viruses, family classification and their respective genomes

Virus	Structure		Family	Genome	Size (kb)	References
Rotavirus		• Icosahedral • Spikes on the surface	*Reoviridae*	Segmented (11 genes) Non-enveloped dsRNA	18.5	Estes and Greenberg 2013
Norovirus		• Icosahedral	*Caliciviridae*	Non-enveloped Positive sense ssRNA (+ssRNA)	7.3 – 8.5	Clarke and Lambden 2002
Sapovirus		• Small and Icosahedral • Cup shaped depressions on the surface	*Caliciviridae*	Positive sense ssRNA (+ssRNA)	7.1 – 7.7	Oka et al. 2015
Astrovirus		• Icosahedral • Spikes on the surface	*Astroviridae*	Non-enveloped Positive sense ssRNA (+ssRNA)	6.8 - 7.3	Méndez and Arias 2013
Adenovirus		• Icosahedral	*Adenoviridae*	Linear dsDNA	26 - 45	Benko et al. 2005

Amongst the common viruses causing gastroenteritis, rotaviruses (RVs) are the leading cause of viral diarrhoeal mortality and morbidity in children less than the age of 5 years globally (Clark et al. 2017, Makimaa et al. 2020). Noroviruses (NoVs) are best known to cause infection in all age groups, and are associated with 18% of diarrhoeal disease worldwide (Makimaa et al. 2020, Pires et al. 2015,). Sapoviruses (SaVs) cause a similar illness as NoVs, more especially in young children and the elderly. The prevalence of SaVs is, in most cases, similar to that of NoVs (Makimaa et al. 2020). Astroviruses are more diverse, also a common cause of gastroenteritis, accounting for 5% of diarrhoeal cases worldwide (Makimaa et al. 2020, Vu et al. 2017). Lastly, adenoviruses are mostly responsible for diarrhoea in children under the age of 2 years (Blacklow and Greenberg 1991), but can also cause disease in adults (Eckardt and

Baumgart 2011). Adenoviruses contribute approximately 15% of diarrhoeal cases in public health care (Cunliffe et al. 2010), specifically 1.5 to 5.4% in adults (Eckardt and Baumgart 2011). These enteric viruses compromise the health of children, resulting in fatalities. There are no antiviral treatment options available against these viral agents, although rehydration therapy has been regarded as one of the most efficient treatments for diarrhoea (Bányai et al. 2018). Currently, preventative vaccination is only available for RV (Bányai et al. 2018, Desselberger 2017).

Having mentioned the prevalence of enteric viruses in humans, it is also important to note that humans and animals live in close proximity, and some of the enteric viruses have been detected both in humans and animals. This introduces a concept known as zoonotic infection, which is a concern to both human and animal health. Zoonotic infection is defined as a natural infection transmitted between animals and humans (Bidaisee and Macpherson 2014). Rotavirus for instance, is diversified and its epidemiology has been widely studied (Malik et al. 2014, Verma et al. 2018). The zoonotic potential of rotavirus has, therefore, been reported in numerous studies, including one of porcine to human zoonotic transmission (Mukherjee et al. 2009), and a rotavirus strain of canine origin detected in humans (Wu et al. 2012). The possible interspecies transmission of other viruses is not well understood. However, the open reading frame 2 (ORF2) of porcine AstV has shown a close relationship to the human ORF2 (Ulloa and Gutiérrez 2010). Bat AstVs have also indicated zoonotic potential, but further investigation is needed (Chu et al. 2010a). In the *Caliciviridae* family (NoV and SaV), only co-infections with other viral enteric viruses have been reported so far, with possible recombinants within the same species (Menon et al. 2013).

1.2 One Health Approach

One Health is defined by the One Health Commission, a globally non-profitable organization dedicated to implement One Health actions around the world (https://www.onehealthcommission.org/), as “the collaborative effort of multiple disciplines to obtain optimal health for people, animals, and our environment” (Bidaisee and Macpherson 2014). The One Health concept aims to understand the interaction between humans, animals and the environment, and how these interactions affect the emergence of infectious diseases, amongst others (Zinsstag et al. 2012). To attain this aim, One Health is promoting collaboration between veterinary, medical and ecological disciplines for the diagnosis, surveillance and control of emerging infectious diseases (Gebreyes et al. 2014).

Approximately 61% of known infectious diseases are commonly attributed to having originated from animals (Liu et al. 2014). Emerging infectious diseases are, therefore, also often associated with zoonotic transmission (Taylor et al. 2001 , Greger 2007, Graham et al. 2008).

Our planet has experienced a paradigm of deadly zoonotic or vector borne global outbreaks over the last 20-25 years, caused by both viral and bacterial pathogens. Viruses that caused disease outbreaks include hantavirus, Ebola virus, highly pathogenic influenza A viruses, severe acute respiratory syndrome (SARS-CoV), Middle East Respiratory Syndrome (MERS), and West Nile virus (Dhama et al 2013). Currently, the world is experiencing a pandemic caused by SARS-CoV-2, thought to have originated from bats, an RNA virus that is closely related to a group of SARS-like coronaviruses (Gao et al. 2020, Wu et al. 2020). Bacterial agents such as *Escherichia coli* O157H7 and *Bacillus anthracis* have also been associated with large outbreaks (Kumar et al. 2013, Gebreyes et al. 2014). However, the majority of disease outbreaks reported in the past two decades are of viral origin and, specifically, RNA viruses. These viruses are highly genetically variable due to, in part, low fidelity of the RNA-dependent RNA polymerase (Gebreyes et al. 2014).

Viral enteric pathogens, known to cause gastroenteritis, are mostly RNA viruses (Gebreyes et al. 2014). Taking a lesson from the records and reports of deadly emerging infectious diseases, it is important to study existing RNA viruses, especially those of which the pathogenicity, epidemiology and zoonotic potential are not well understood (Verma et al. 2018). These studies will help in the availability of recorded information and data in preparation for the future.

Emerging infectious diseases in animals highlight the health and economic impacts across many countries as is evident by the current global pandemic caused by SARS-CoV-2. Several factors are associated with zoonotic infectious diseases, including increased contact with wildlife through poaching, climate change, human and animal population densities, and poverty (Greger 2007). All these factors explain the collaborative aspect that One Health wants to bring out to the world's perspective because these diseases are not a uni-discipline crisis (Dahal and Kahn 2014). Therefore, the surveillance, epidemiology, and collaborative efforts of different disciplines need to be explored to control and prevent the transmission of zoonoses through animals as carriers and *vice versa*.

1.3 Rotaviruses

The first records of rotaviruses (RVs) were from diarrheic mice (Adams and Kraft 1963), monkeys (Adams and Kraft 1963), and cattle (Stair et al. 1973). The viral particles obtained from the intestinal tissue of mice, a rectal swab of monkeys and faecal samples of diarrheic cattle all resembled reovirus/orthoreovirus. Bishop and colleagues first described rotaviruses in 1973. The human viral particles were obtained from the biopsy of the duodenal mucosa of children presenting with acute gastroenteritis at a hospital in Australia (Bishop et al. 1973).

Rotavirus particles were observed by an electron microscope and subsequently designated rotavirus, (rota meaning wheel in Latin) because of its appearance.

Five years after RV was first described, it was recognized as the most common cause of acute diarrhoea in infants and young children (Parashar et al. 1998). The seasonality for RV infection differs per country, in temperate countries infections are more prominent during winter months whereas in tropical countries RV infection is not greatly affected by the seasonality. The RV infections in tropical countries occur throughout the year (Desselberger 2017, Patel et al. 2013). The typical route of transmission for RV is through the faecal-oral route (Estes and Greenberg 2013). The stability of the virus in the environment has also provided a possibility for water and foodborne outbreaks (Gallimore et al. 2006). Safe hygiene practice is advised to reduce the high risk of RV infection (Verma et al. 2018). To control the rate of mortality and morbidity caused by RV, two oral live attenuated vaccines were licenced in 2006, Rotarix™ and RotaTeq™(Desselberger 2014, Ruiz-Palacious et al. 2006). Since the introduction of Rotarix™ and RotaTeq™, specifically, the number of children deaths and hospitalization due to diarrhoea caused by RVs has decreased across the world (Burnett et al. 2017). Additional two vaccines, Rotavac™ and Rotasiil™ have been pre-qualified by the WHO for global use, making it a total of four RV vaccines (https://www.who.int/immunization/diseases/rotavirus/en/).

1.3.1 Genome and viral structure

Rotaviruses are 70-75 nm in diameter, icosahedral, triple-layered and non-enveloped. The genome of RV consists of 11 segments of double-stranded ribonucleic acid (dsRNA) (Estes and Greenberg 2013). These dsRNA segments encode for six structural (VP1-VP4, VP6 and VP7) and six non-structural (NSP1-NSP6) proteins (Esters and Greenberg 2013). Each of the genome segments encodes for a single protein except for genome segment 11 which encodes two proteins (Estes and Greenberg 2013). The genome is associated with VP1 (RNA-dependent RNA polymerase), and VP3 (guanyltransferase) proteins (Figure 1.1). This structure is encased in a core made up of VP2 protein. The core is surrounded by a middle layer made up of the VP6 protein, the most abundant protein in the RV structure. VP4 and VP7 are the outer capsid proteins, which facilitates attachment and internalization during virus replication, and make up the third layer of the RV structure (Jayaram et al. 2004). These two proteins are known to induce the production of neutralizing antibodies. They form the basis of the dual RV classification system, the P-type and G-type, derived from the function of each protein. VP4 is a **p**rotease inhibitor and VP7, a **g**lycosylation protein (Figure 1.1). To date, 36 G and 51 P-types have been reported worldwide (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification).

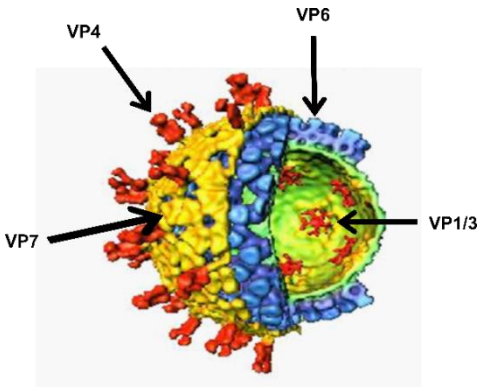


Figure 1.1 The three rotavirus layers of the virion are indicated. The outer layer proteins consist of the spike protein VP4 (red) and VP7 (yellow). The middle layer is made up of VP6 (blue), and the core consists of VP2 (green) which associates with VP1 and VP3 proteins (red) (Jayaram et al. 2004)**.**

The positive-sense dsRNA segment contains a guanidine cap at the 5' end followed by a set of conserved sequences which form part of the untranslated region (UTR). The UTR is followed by an open reading frame (ORF) which codes for the protein product and ends with a stop codon (Estes and Greenberg 2013). Another UTR follows with a subset of conserved terminal 3' sequences and ending with 3' terminal cytidines. The mature mRNA produced during RV infection lack a polyadenylation signal. The conserved terminal sequences contain cis-acting signals important for transcription, RNA translation, RNA transport, replication and assembly (Figure 1.2) (Estes and Greenberg 2013).

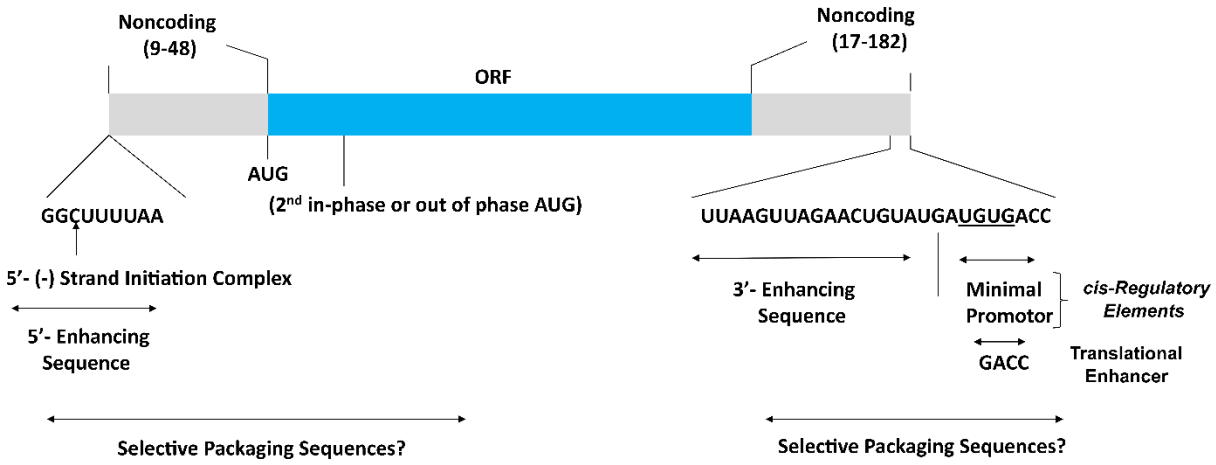


Figure 1.2 Rotavirus genome structure. The 5' -end and 3' -end consist of conserved consensus sequences. Variations in the conserved ends are indicated by underlined nucleotides. There is an open reading frame (ORF) which codes for a protein product. The cis-regulatory elements are indicated by two pointed arrows, which are essential for replication of transcripts. Untranslated regions are shown in grey shaded blocks at both termini. Adapted from Estes and Greenberg 2013.

1.3.2 Classification of rotaviruses

Rotaviruses are classified within a single genus under the family *Reoviridae* (Estes and Greenberg 2013). The VP6 protein is the basis for the classification of RVs into various groups (Matthijnssens et al. 2012). According to the International Committee on Taxonomy of Viruses (ICTV), nine RV groups (group-A, B, C, D, F, G, H, I, and J) have been established (https://talk.ictvonline.org/taxonomy/). Group A (RVA) rotavirus infects birds and mammals, while RVB, RVC, RVH, and RVI have been identified mainly in domesticated mammals. In contrast, RVD, RVF and RVG have been identified only in birds (Matthijnssens and Ranst 2012). Among all the RVs groups, RVA is the most widespread and occurs in most, if not all mammalian hosts, and is therefore, a significant public health concern (Martella et al. 2010).

The increasing availability of sequence data for RVA genomes encoding proteins other than genome segments encoding VP4 and VP7 has allowed the expansion of the genotyping system to the remaining 9 genome segments. The classification system is now based on the whole genome of RV. In this system, the notations Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx are used to represent the genotypes for genome segments encoding VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6, respectively (Matthijnssens et al. 2008b). On the basis of the whole genome classification system and complete RVA genome sequence comparisons, two major genotype constellations have been shown to circulate worldwide among humans, the Wa-like (I1-R1-C1-M1-A1-N1-T1-E1-T1-E1-H1) and the DS-1-like (I2-R2-C2-M2-A2-N2-T2-E2-H2). A third (minor) genotype constellation, the AU-1 like group (I3-R3-C3-M3-A3-N3-T3-E3-H3) has also been shown to circulate in animals (Matthijnssens et al. 2008b).

1.3.3 Human and animal host

Global surveillance studies have identified the most common G- and P- type combinations, which are G1P[8], G2[P4], G3P[8], and G9P[8] (Figure 1.3) constituting 74.70% of the human RV strains circulating globally (Bányai et al. 2012, Doro et al. 2015). Noteworthy, before 1995, G1P[8], G2P[4], G3P[8], and G4P[8] were the common circulating genotypes in humans. The G9 and G12 genotypes were only reported as emerging genotypes after 1995 and currently are also recorded as globally important RV genotypes in combination with the P[8] genotype (Matthijnssens et al. 2009).

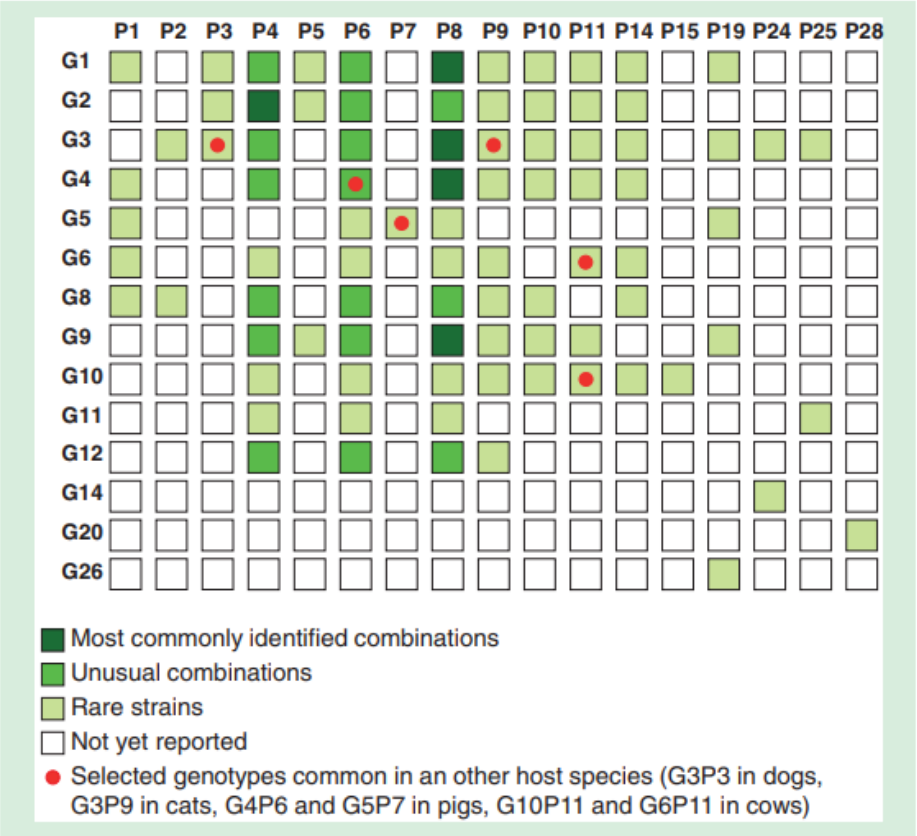


Figure 1.3 The G- and P-type combinations of human rotaviruses and a few selected genotypes common in host species other than humans. Common human rotavirus combinations are shown by dark green blocks, the unusual combinations shown by bright green blocks and rare strains shown by light green blocks. Genotypes common in other hosts are shown by red filled dots. All the blank blocks are genotypes that have not yet been reported at the time of publishing (Dóró et al. 2015).

Group A rotaviruses have been reported in the various animal hosts including bovine, swine, equine, ovine, caprine, canine, feline and avian. Diarrhoea caused by RV in livestock animals, particularly in swine and cattle is a major problem causing significant economic losses due to mortality and morbidity caused by RV (Martella et al. 2010). Porcine and bovine rotaviruses are important pathogens due to their large economic impact on the swine and cattle industry. The most common bovine RV genotypes are G6, G8 and G10 in combination with P[1], P[5] and P[11] (Dóró et al. 2015, Matthijnssens et al. 2009, Santos and Hoshino 2005). Among porcine, the common circulating genotypes include G3, G4, G5, G9 and G11 in association with P[6] and P[7] (Matthijnssens et al. 2011). Feline and canine, similar to domestic livestock, also suffer from diarrhoea induced by RV infection. The G3 and G6 genotypes in association with P[9] are typical for canine and feline rotaviruses (German et al. 2015, Papp et al. 2015).

1.3.4 Interspecies transmission and zoonotic potential

Rotavirus diversity is driven by several factors including interspecies transmission, point mutations, recombination and reassortment (Bányai et al. 2012, Jain et al. 2014). Analysis of

the rotavirus whole genome is a good method to study viral strain diversity and evolution. It has been demonstrated that Wa-like human rotaviruses (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) and porcine rotaviruses (G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1: Gottfried strain used as an example) have a common origin and that DS-1-like human rotaviruses (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2) have a common origin with that of bovine rotaviruses (G6-P[1]-I2-R2-C2-M2-A3-N2-T6-E2-H3: BRV033 strain used as an example) (Matthijnssens et al. 2008a).

Countless studies have been done on RVA, and it has been reported that human RVA strains share genetic and antigenic features with animal origin RVA (Doro et al. 2015). To name a few, a human G11 have shown a significant similarity with G11 genotypes detected in animals (Matthijnssens et al. 2010); RV G3P[3], of canine origin, was found to infect humans (Luchs et al. 2012), and the G5P[6], of porcine origin, was detected in Brazilian children suffering from diarrhoea (Gouvea et al. 1994). Interspecies transmission has also been considered an important mechanism of RV evolution, and has been observed in two bovine strains (NIC522 and B12) possessing the G8P[1] genotype as a direct transmission of bovine rotavirus to humans (Bányai et al. 2009). This is not the only animal to human rotavirus transmission that was reported; the lapine rotavirus (strain B4106) with the genotype G3P[14] and porcine rotavirus with G9P[6] (strain BE2001) were also reported as a direct transmission from animal rotavirus to humans (De Leener et al. 2004, Zeller et al. 2012). A recent study, described a co-infection of a human Wa-like G12P[8] with a GXP[14] strain which clustered with animal strains in a phylogenetic analysis, suggesting a typical bovine strain (Strydom et al. 2019). Reassortment events between human and animal species have also been documented. One such event occurred in India where a G1P[19] strain resulted from a human-porcine reassortment (Chitambar et al. 2009), and another in Bulgaria where a rare G5P6] rotavirus was detected (Mladenova et al. 2012). One study in Uganda reported an interesting finding, where a bovine strain with all its genome segments closely related to human, suggesting human to bovine transmission (Bwogi et al. 2017). This study highlights the high potential for reassortment as a result of interspecies transmission of RV, which also confirms RV as a zoonotic virus.

1.4 Astroviruses

The first astroviruses (AstVs) were identified in 1975 by electron microscopy in children suffering from diarrhoea (Madeley and Cosgrove 1975). Since then, the enteric infections in humans caused by AstVs have been reported worldwide, mainly in infants and children. The AstVs outbreaks are mainly associated with the winter season (Verma et al. 2010). Soon after AstVs were identified in humans, AstVs-like particles were described and reported in domestic animals (Woode and Bregder 1978). The first report on animal AstVs was from lambs and

calves suffering from diarrhoea (Snodgrass and Gray 1977, Woode and Bridger 1978). Astrovirus infections are commonly known as the causative agents of diarrhoea, however, in chickens, cats and ducks, the infection is also associated with intestinal nephritis, pyrexia, and acute hepatitis, respectively (Gough et al. 1984, Hoshino et al. 1981, Yamaguchi et al. 1979). Therefore, AstVs can bypass the gastroenteritis tract and infect other tissues and organs.

1.4.1 Genome and viral structure

The genome of AstVs is a single-stranded RNA (ssRNA), positive sense genome with a size of about 6.8 to 7.3 kb (Méndez and Arias 2013). The genome consists of three overlapping ORFs (ORF1a, ORF1b and ORF2) (Figure 1.4). ORF1a and ORF1b are located at the 5' end of the genome, encoding for the viral protease and the polymerase, respectively. ORF2 is situated at the 3' end of the genome and encodes the capsid protein precursor (Cortez et al. 2017, De Benedictis et al. 2011). The ORF1b, from a genetic point of view, is the least divergent and ORF2 the most divergent among the different ORFs (Strain et al. 2008).

Astroviruses are small; the name is derived from a Greek word "Astron" which means star and describes the five/six-pointed star-like projections of the virion by negative staining transmission electron microscopy (Madeley and Cosgrove 1975, Dong et al 2011). However, these projections are pH-dependent and can only be visible in less than 10% of the population (Caul and Appleton 1982). Therefore, in some instances, the AstVs are misidentified as astrovirus-like particles or enteroviruses.

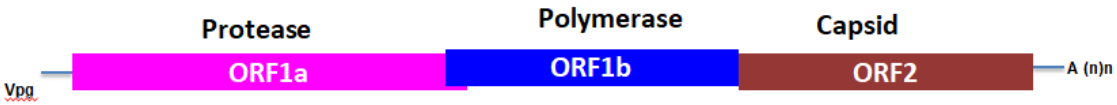


Figure 1.4 Genome organization of typical human astroviruses. The length of ORFs might differ between species, but the genome organization is similar. The 5' end consists of the OF1a and ORFb which encode for viral protease and the polymerase, respectively. Attached to the 5' end is the genome linked viral protein. At the 3' end of the genome, ORF2 encodes for a capsid protein which is translated from a subgenomic RNA, and attached to the 3' end is the poly A tail. (Adapted from Bosch et al. 2014).

1.4.2 Classification of Astroviruses

Astroviruses are taxonomically classified within the family *Astroviridae*. The family is divided into two genera, *Avastrovirus* (AAstV) and *Mamastrovirus* (MAstV) which are known to infect avian and mammalian species, respectively (Méndez and Arias 2013). The international committee for the taxonomy of viruses (ICTV) has officially classified a wide range of the AstV species (Bosch et al. 2012) (Figure 1.5). The wide range of AstVs species capable of infecting

a wide range of animal species indicate how important these viruses are for the economy and public health.

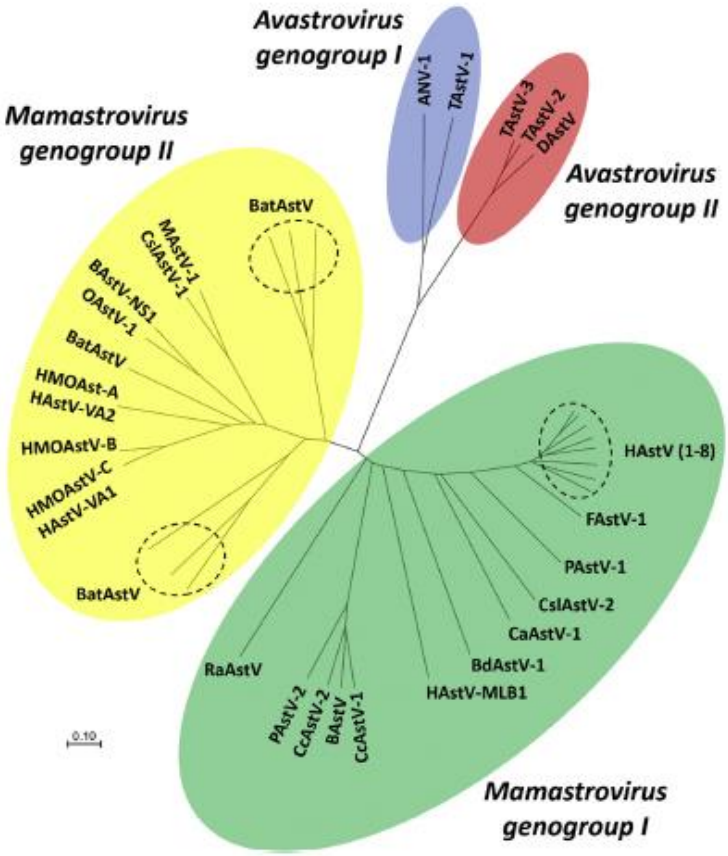


Figure 1.5 Phylogenetic relationships of astroviruses. The AstVs are divided into two genera, *Mamastrovirus* (MAstV) and *Avastrovirus* (AAstV). Each genera is divided into two genogroups, genogroup I and genogroup II. Genogroup I of AAstV include the turkey astrovirus 1 (TAstV-1), and avian nephritis virus (ANV), while genogroup II include turkey astrovirus 2 (TAstV-2), turkey astrovirus 3 (TAstV-3), and duck astrovirus 1 (DAstV-1). The mamastrovirus consists of numerous mammalian species. Genogroup I includes human astroviruses (HAstV 1-8), feline astrovirus (FAstV), porcine astrovirus (PAstV), California sea lion astrovirus 2 (CsIAstV-2), canine astrovirus 1 (CaAstV-1), bottlenose dolphin astrovirus 1 (BdAstV-1), human astrovirus Melbourne (HAstV-MLB1), porcine astrovirus 2 (PAstV-2), *Capreolus capreolus* astrovirus 2 (CcAstV-2), bovine astrovirus (BAstV), *Capreolus capreolus* astrovirus 1 (CcAstV-1), and rat astrovirus (RaAstV). Genogroup II includes bat astrovirus (BAstV), mink astrovirus 1 (MiAstV-1), California sea lion astrovirus 1 (CsIAstV-1), bat astrovirus NS1 (BAstV-NS1), ovine astrovirus 1 (OAstV-1), human-mink-ovine astrovirus A (HMOAstV-A), human astrovirus-Virginia 2 (HAsV-VA2), human-mink-ovine astrovirus B (HMOAstV-B), human-mink-ovine astrovirus C (HMOAstV-C), and human astrovirus-Virginia 1 (HAsV-VA1) (Wohlgemuth et al. 2019).

Human astroviruses are considered one of the major cause of gastroenteritis in children (Desselberger 2017). As suggested by Bosch and co-workers, AstVs infecting mammals are diverse. Table 1.2 lists genotypes that are found in each MAstV genogroup (Donato and Vijaykrishna 2017).

Table 1.2 The Mamastrovirus species and respective genotypes

Genotypes	Species of origin
GI.A	Human
GI.B	Feline
GI.C	Porcine
GI.D	California Sea lion
GI.E	Canine
GI.F	Human
GI.G	Bottlenose dolphin
GII.A	Human
GII.B	Humam
GII.C	Mink
GII.D	California Sea lion
GII.E	Bat
GII.F	Ovine
GII.G to GII.L	Bat

Although AstV classification was originally based on the species in which the virus was discovered (Krishnan 2014), the detection of unrelated AstVs in the same species suggested that this system should be revised. In humans, eight serotypes, known as classic serotypes (HAstVs1-8) have been described (Chu et al. 2010b, Finkbeiner et al. 2009). Based on these findings, two proposals were submitted in 2010 by the *Astroviridae* study group for re-classification of AstVs. Both these proposals take into consideration genetic criteria based on the full-length sequencing of ORF2 encoding for a viral capsid protein. According to these new proposed classifications there are now three species of AAstVs and nineteen species of MAstVs that have been recognized (Figure 1.5, Table 1.2) (Bosch et al. 2012, Donato and Vijaykrishna 2017). The official classification of AstVs is based on the percentage nucleotide and amino acid similarity of ORF2 (Donato and Vijaykrishna 2017). The nucleotide and amino acid cut-off percentage identity is 75%, where different strains of the same AstV species should share >75% identity (Bosch et al. 2012).

1.4.3 Human and animal hosts

Human astroviruses (HAstVs) predominantly affects children under two years of age and immunocompromised individuals (Kirkwood et al. 2005, Bosch et al. 2012). The HAstV-1 is the most common circulating serotype worldwide, followed by HAstV-2 to HAstV-5 and HAstV-8 occasionally, depending on the geographical area (De Grazia et al. 2011).

The first discovery of astroviruses in animals, soon after the description in human beings, was in lambs and calves suffering from diarrhoea (Snodgrass and Gray 1977, Woode and Bridger 1978). The severity of the infection, however, was shown to be subclinical in both species, suggesting why there have been few reports and publications on ovine and bovine astroviruses. The list of animals susceptible to AstV infection has now expanded to include domestic animals, wild animals, avian and mammalian species (De Benedictis et al. 2011). Canine astrovirus-like particles have been reported since 1980 in dogs with and without diarrhoea (Marshall et al. 1984, Vieler and Herbst 1995, Williams 1980). So far astroviruses have been reported in the USA, Germany, Australia, Italy, China and France in human and animal species (Marshall et al. 1984, Toffan et al. 2009, Vieler and Herbst 1995, Williams 1980, Zhu et al. 2011). Feline astrovirus was first described in 1981 (Hoshino et al. 1981). Within a decade, Australia and New Zealand had described feline astrovirus, followed by the USA (Harbour et al. 1987, Herbst and Krauss 1989, Mashall et al. 1987, Rice et al. 1993). Although there is evidence of feline and canine AstV infection in other countries, most if not all African countries, including South Africa, have no data on feline and canine astroviruses. However, South Africa was amongst the first few countries to report porcine astrovirus (Geyer et al. 1994).

Due to the error prone RNA-dependent RNA polymerase that lacks proof-reading activity like all the other RNA viruses, AstVs have a great genetic variability due to introduction of mutations and genetic recombination (Domingo 1997). Recombination events were first described amongst the classic HAstVs (Walter et al. 2001). A human recombinant that contained ORF2 from HAstV-5 and a region from ORF1b from HAstV-3 was detected in one of the studies (Walter et al. 2001). Another human recombinant was identified, with recombination occurring between HAstV-4 and HAstV-1 (Martella et al. 2013). In addition to human astrovirus recombinants, animal recombinants have also been documented in porcine, bovine and canine (Hirashima et al. 2018, Ito et al. 2017, Li et al. 2018). Astrovirus recombination is identified both in humans and animals, which suggests possible recombination between human and animal AstVs. The zoonotic transmission of AstVs is not clear, but there has been a report on a recombination event between the CslAstV strain and HAstV strain suggesting a zoonotic transmission event (Rivera et al. 2010). Further investigation and understanding on AstV heterologous recombination mechanism is required before confirming AstVs as a zoonotic enteric virus.

1.5 Noroviruses

Norovirus (NoV) was first described in 1968 during a gastroenteritis outbreak in Norwalk, Ohio, USA in which it affected children (Kapikian et al. 1972). They are known as the most common non-bacterial cause of foodborne gastroenteritis in persons of all ages and in animals, globally

(Bull et al. 2005, Villabruna et al. 2019). Transmission occurs through the oral-faecal route, person to person transmission and can be foodborne or waterborne (White 2014). The NoV outbreak activity tends to greatly increase during winter but can occur in warmer months as well (Ahmed et al. 2014, Eckardt and Baumgart 2011).

1.5.1 Genome and viral structure

Noroviruses (NVs) are small, non-enveloped with a single-stranded positive-sense RNA genome and a genome size of 7.4 to 7.7 kb (Clarke and Lambden 2002). The linear genome of NVs is organized into three open reading frames (ORFs) (Figure 1.6), encoding both structural and non-structural proteins (Atmar and Estes 2001). The ORF1 encodes the polyprotein containing the viral polymerase, with ORF2 and ORF3, translated from a sub-genomic RNA encoding for the major capsid (VP1) and minor capsid proteins (VP2) (Jiang et al. 1993, Thorne and Goodfellow 2014). At the 5' end, the genome-linked viral protein (VPg) is covalently attached and poly(A) tail covalently attached at 3' end. The untranslated regions at each end contain evolutionarily conserved RNA structures (Simmonds et al. 2008). These are important for viral replication, translation and norovirus pathogenesis (Bailey et al. 2010).

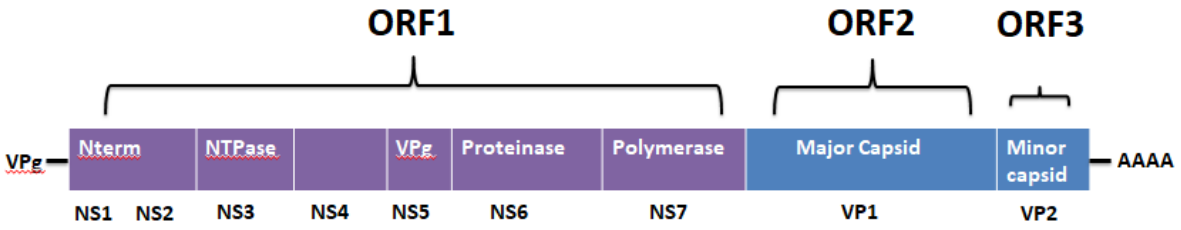


Figure 1.6 The genome organization of noroviruses, which is common to all the noroviruses, except the murine norovirus which has an alternative fourth ORF. The ORF1 encodes for a polyprotein cleaved by viral protease, NS6, to produce non-structural proteins. Open reading frames 2 and 3 are translated from a sub-genomic RNA and encode for VP1 (major capsid) and VP2 (minor capsid). Adopted from Thorne and Goodfellow 2014.

1.5.2 Classification of Noroviruses

Noroviruses are a group of non-enveloped, single-stranded RNA viruses classified into the genera *Norovirus* of the family *Caliciviridae.* They are small, round structured viruses with a diameter of 27 to 35 nm (Glass et al. 2009). A dual nomenclature is used to describe both the polymerase and capsid genotype. The decision on this nomenclature was based on the frequent observation of recombination between ORF1 and ORF2 (Bull et al. 2007, Kroneman et al. 2013). Noroviruses are diverse and are currently divided into seven genogroups (GI-GVII). The genogroups are further subdivided into 40 genotypes (Vinjé 2015).

1.5.2 Human and animal hosts

Although genogroups GI, GII and GIV are primarily known to affect humans, the majority of human norovirus infections and outbreaks are caused by genogroups GI and GII (Vinjé 2015).

The GII.4 genotype has been the most prevalent genotype globally and evolved through the accumulation of mutations and recombination (Siebenga et al. 2009, Wangchuk et al. 2017).

Pigs and cows are the best-studied non-human hosts for norovirus amongst other domestic animals. Within the GII genogroup of norovirus, there are three specific genogroups (GII.11, GII.18 and GII.19) which cause infection in pigs (Zheng et al. 2006). The first carnivore norovirus was detected in a lion cub which had died from severe hemorrhagic enteritis in Italy (Martella et al. 2008). The strain detected in this lion cub had an amino acid percentage similarity of 70% with the human norovirus strain GIV.1. Thereafter, two diarrheic dogs in Lisbon were shown to be infected with a GIV.2 strain. Dogs that shared the same kennel as the diarrheic dogs, also developed diarrhoea (Mesquita and Nascimento 2012). Since the norovirus outbreak in canines, canine norovirus sequences have been detected in faeces of both sick and healthy dogs in South America, Europe and Asia (Caddy 2018, Lyoo et al. 2018, Mesquita et al. 2014). Human strains of norovirus are closely related to animal strains including pigs, dogs and cats. In addition, these animal noroviruses cluster within GII (porcine norovirus) and GIV (feline and canine norovirus) strains (Vinjé 2015). Extensive studies have been done in America, Asia, and Europe on animal norovirus including cattle, pigs, dogs and cats (Farkas et al. 2005, Mattison et al. 2007, Scheuer et al. 2013, Soma et al. 2015). In Africa, including Egypt, Tunisia, Ethiopia and South Africa, animal norovirus has been reported for only cattle and pigs (Hassine-Zaafrane et al. 2012, Mohamed et al. 2017, Taku et al. 2017). No study has reported companion (cats and dogs) norovirus infection in South Africa (Villabruna et al. 2019). The current data from African countries suggest that there is no sufficient published reports on domestic aninmal norovirus.

Numerous studies have been investigating the possibility of human to animal norovirus transmission (Caddy et al. 2015, De Graaf et al. 2016, Mattison et al. 2007, Villabruna et al. 2019). The interspecies transmission investigation was done by screening animal stool samples for human noroviruses. The animal stools were sampled from households with symptomatic individuals. In a study of 92 dogs, 4.3% were norovirus positive, and three possessed GII.4 and one GII.12 genotype. Of the three GII.4 strains detected in canine, one could be linked to a GII.4 strain found in faeces of the owner's dog (Summa et al. 2012). This finding provides a possible risk of interspecies transmission; however, more work is needed to elucidate the zoonotic potential of noroviruses further.

1.6 Sapoviruses

Sapoviruses (SaVs) are another one of the significant causes of acute gastroenteritis in both children and adults (Phan et al. 2004). The first discovery of sapovirus was in 1977 during an acute gastroenteritis outbreak in a home of infants in Sapporo, Japan, hence the virus was

known as Sapporo-like viruses (Chiba et al. 1979). The virus causes infection in a wide range of hosts including pigs, mink, dogs, sea lions and bats (Diez-Valcarce et al. 2018). High prevalence of sapovirus infection is observed in children less than the age of five. The severity of SaVs gastroenteritis is milder than that of norovirus and rotavirus (Zhou et al. 2016). Similar to NoVs, SaVs infection occur mostly in winter than warmer months but is not exclusive to colder months only (Eckardt et al. 2011)

1.6.1 Genome and viral structure

The sapovirus (SV) and norovirus are the only two genera out of five that belong to *Caliciviridae* family and are associated with diarrhoea (Farkas et al. 2004). The genome of SaVs is single-stranded, positive-sense RNA with a size of 7.1-7.7 kb in length and has a polyadenylated 3' end that is important for replication (Oka et al 2006). Most SaV genomes consist of two open reading frames (ORFs, Figure 1.7), but some include a third ORF of which the function is unknown (Soma et al. 2015). Open reading frame one translates into a large polyprotein which gets processed into six non-structural proteins (NS1-5 and NS6-NS7) and the major capsid protein, VP1. The ORF 2 is predicted to translate into a minor structural protein, VP2 (Oka et al. 2015). The virions of SaV are composed of a single structural capsid protein, which has an icosahedral symmetry (Oka et al. 2015).

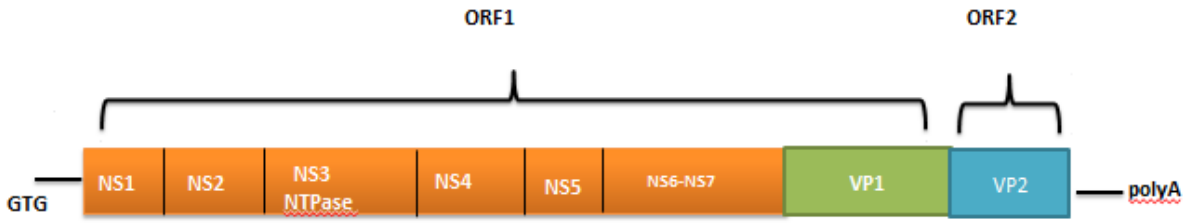


Figure 1.7 Genome organization of sapovirus. The genus sapovirus consists of two open reading frames: ORF1 encodes for a polyprotein consisting major structural protein VP1 (green) and non-structural proteins, NSs, (orange) respectively. The ORF2 encodes a minor structural protein, VP2 (blue). The 5' end of the genome consists of conserved sequence, GTG, and attached to the 3' end is the poly A tail. Adopted from Oka et al. 2015.

1.6.2 Classification of sapovirus

To date, SaVs are classified into fifteen genogroups and further subdivided into 16 genotypes (Farkas et al. 2004, Oka et al. 2012). The classification of SaVs is based on the complete capsid sequence encoding VP1 protein. Genogroups GI, GII, GIV, and GV are known to infect humans and genogroups GIII, GV, GVI,GVII, GIX, GX, GX, and GXI are mostly known to affect pigs, with GXIV affecting bats, GXII mink, GI chimpanzees, GV sea lions and GII along with GXV affect rats (Liu et al. 2016b, Oka et al. 2015, Romani et al. 2012).

1.6.3 Human and animal hosts

Although SaV is recognized as an important etiological agent of acute diarrhoea, research on SaV is less advanced as compared to genera found in the same family, such as norovirus (Oka et al. 2015). The prevalence of SaV was shown to range from 0.3 to 9.3%, and usually has a lower prevalence than NoV infections (Okada et al. 2002, Phan et al. 2004) worldwide. The prevalence of human SaV has been reported only in few Sub-Saharan African countries including Kenya, Tanzania, Malawi, Gabon and Burkina Faso with the lowest prevalence of 5.7% and the highest prevalence of 18% and an average of 7.5% (Dove et al. 2005, Mans et al. 2014, Matussek et al. 2015, Murray et al. 2016). In South Africa, human SaV infection prevalence was 7.7% in children less than 5 years of age between 2009 and 2013 (Page et al. 2016). A variety of SaV strains circulate with GI and GII detected frequently. GI genotype is associated with severe diarrhoeal cases. There is currently limited data on SaV, particularly in African countries. No significant zoonotic potential of the viruses have been reported yet, but it is predicted to be a rare event (Bank-Wolf et al. 2010). Animal sapoviruses comprise of porcine enteric sapovirus, bovine enteric sapovirus, canine enteric sapovirus, bat enteric sapovirus, chimpanzee enteric sapovirus, sea lion enteric sapovirus, rats enteric sapovirus as well as mink enteric sapovirus (Bank-Wolf et al. 2010, Guo et al. 2001, Lauritsen et al. 2015, Oka et al. 2016, Saif et al. 1980). In Africa, animal SaV has been documented in Tanzania between 2001 and 2012, where a prevalence of 1.7% was detected in pigs (Sisay et al. 2016). Another animal SaV study was in Ethiopia in 2013, where animals studies included, spotted hyenas, African lions and bat eared fox; in this study 34.8%, 33.3%, and 22.2% SaV prevalence was recorded, respectively (Olarte-Castillo et al. 2016).

1.7 Adenoviruses

Human adenoviruses (HAdV) are associated with a variety of diseases, including acute respiratory infection, acute gastroenteritis, conjunctiva, hemorrhagic cystitis, hepatitis, hemorrhagic colitis, pancreatitis, and meningoencephalitis (Wold et al. 2007). The HAdVs were first isolated from civilians and army recruits who had a respiratory infection (Hilleman and Werner 1954). Almost a decade later, two AdVs were isolated from bovine and were found to be closely related to but not identical to HAdVs (Klein 1962). As research on AdVs increased, a novel human AdV was identified in an outbreak causing diarrhoea. Such strains were subsequently referred to as human enteric adenoviruses (HEAdVs). The species F of HEAdVs, includes serotypes HAdV-40 and HAdV-41 and is the only species associated with severe diarrhoea in humans (Jones et al. 2007). Adenovirus infections occur throughout the year and have no definite seasonality (Moyo et al. 2014)

1.7.1 Genome and viral structure

Of all the enteric viruses discussed, AdVs have the most complex, largest genome size, and are also the only double-stranded DNA (dsDNA) viruses discussed in this review. The genome size ranges from 26 to 45 kb (Benko et al. 2005). Similar to viruses discussed in the preceding sections, the structure of AdVs is icosahedral shaped. The virions are non-enveloped with a diameter of 70-90 nm. The outer side of the capsid consists of hexons (II) and penton bases (III). Attached to penton bases are fibres (IV) protruding from the virion surface. Polypeptide IX is located between hexons in the centre of each facet. Underneath the vertex region of pentons are 2 monomers of IIIa. Pentons are formed by a penton base and a fibre. Underneath the hexons are multiple copies of the protein VI, forming a ring. The inner surface of the hexons consists of protein VIII. The core is a complex of the DNA genome, proteins, V, VII, X, and the terminal protein (Figure 1.8 A) (Russell 2009, Martin 2012).

The genome organization of AdVs, particularly, Mastadenovirus include a terminal protein (TP) linked to the 5' end of the genome. Products of early (E) genes include E1 to E4. The E1 and E4 are involved in the modulation of host cell's transcriptional machinery while E2 is associated with the virus DNA replication complex. The late (L) gene products, L1 to L5, are responsible for virion assembly and maturation (Brown et al. 1996).

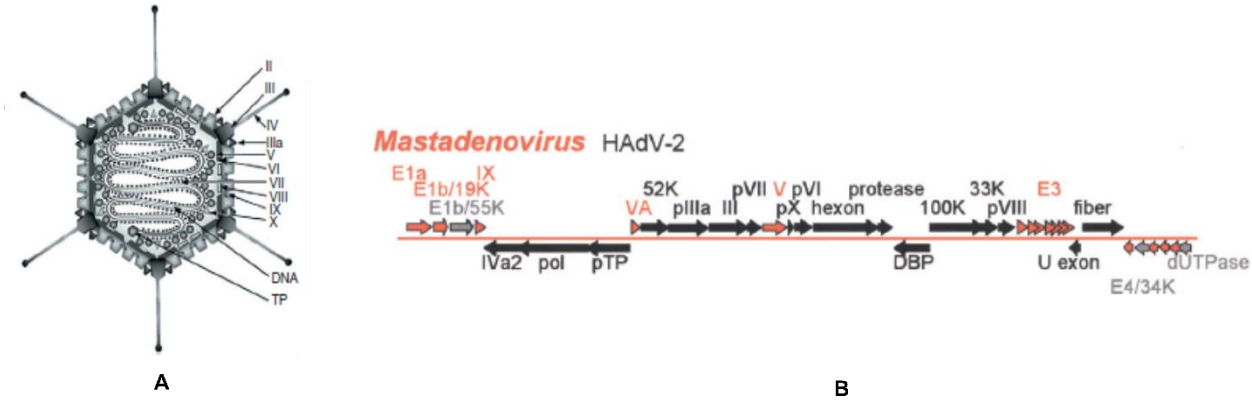


Figure 1.8 Human adenovirus two (HAdV-2) virion structure (A) and genome organization (B). The capsid contains proteins II, III, IIIa, IV, VI, VIII, and IX. The core comprises proteins V, VIII, X, and terminal protein (TP) (A). The black arrows indicate conserved genes, while the grey arrows indicate genes present in more than one genus and red arrows show genus specific genes (B) (King et al. 2012).

1.7.2 Classification of adenoviruses

Adenoviruses (AdVs) belong to the family *Adenoviridae*, which comprises five genera, *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus*, and *Ichtadenovirus*. Of these genera, we will only focus on the classification of Mastadenovirus which only affects mammalian species, including bats, dogs, ruminants, horses, humans, swine and mice. The

initial method of classification for AdVs was based on serology, with members of each genus sharing a common antigen (Harrach et al. 2011, Jones et al. 2007,). Currently, AdVs are classified according to genome organization and phylogenetic relationships between viruses (Kaján 2016, Kaján et al. 2017). To date, 51 human adenovirus (HAdV) serotypes and seven species (HAdV-A to HAdV-G) have been characterized and classified in the genus *Mastadenovirus* under the family *Adenoviridae* (Jones et al. 2007).

1.7.3 Human and animal hosts

Adenovirus infection causing gastroenteritis in humans is common (Desselberger 2017). Reported data on AdVs and its association in causing gastroenteritis infection in animals is limited. Currently, there is potentially no published work on the transmission of the HAdV to animals or *vice versa*. However, recently, AdV infection in pigs and dogs associated with acute diarrhoea has been reported (Alves et al. 2018, Kumthip et al. 2019). In Brazil, a prevalence of 4.9% was recorded in association with diarrhoea and vomiting in dogs (Alves et al. 2018). The infection was caused by canine adenovirus type 1 (cAdV-1), which is commonly known as an aetiological agent for hepatitis (Pratelli et al. 2001). This finding suggests that, even when a pathogen is commonly known to cause a particular disease, clinical signs like diarrhoea should also be accounted for in diagnostics. Another study in Thailand, investigated enteric viruses in pigs, including porcine adenovirus (pAdV); which is mostly not considered a possible pathogen responsible for acute diarrhoea. In this study, 16.9% of diarrhoea in pigs was due to pAdV (Kumthip et al. 2019). Extensive evolutionary relationships and phylogenetic studies on enteric AdVs are unclear, and more research needs to be conducted.

1.8 Detection of viral enteric pathogens

Electron Microscopy (EM) is a common method for the detection of all the enteric viruses listed in this chapter. In fact, it was the initial method in which aetiological agents causing diarrhoea were identified with (Bishop et al. 1973, Madeley et al. 1975) (Table 1.3). The enteric viruses were detected by observing the morphology of the virus under an EM characterizing the pathogen according to established guidelines of classification. The downfall for the use of EM is that it requires a qualified microscopist, it is expensive and can be less sensitive for virus particles that are shed in lower concentrations (Hamza et al. 2011). Another possible method for detection is cell culture in which the virus can be propagated in suitable cells and the behaviour of the virus can be studied *in vitro* (Firth et al. 2014). The method can be time consuming, as sometimes it takes longer periods before pathogen detection. Also, cell culture is susceptible to bacterial contamination. It is currently not possible to propagate human viral species, like SaVs, NoVs and AdVs in cell culture, while human rotaviruses are also

notoriously difficult to adapt to cell culture (Arnold et al. 2009, Bhar and Jones 2019, Cromeans et al. 2015, Oka et al. 2018, Sisay et al. 2016).

Enzyme assays are more sensitive and efficient than EM and cell culture and are used for antigen detection (Kidd and Brandt 1988). However, enzyme assays can be challenging when an antigenically diverse viral strain is under investigation, like AstVs and SaVs. Electropherotyping is essentially important for RV detection as it involves separation of RV genome segments providing characteristic migration pattern for each group (Holmes 1996). There are two types of electropherotyping that have been employed for RV detection, the agarose gel electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE) (Dubal et al. 2015, Herring et al. 1982). Although PAGE is more sensitive and allows for appropriate separation of the 11 RV genome segments, AGE can also distinguish between different RV groups (Chudzio et al. 1989). Agarose gel electrophoresis take superiority because it is cheaper, less laborious and a tool to identity non-group A strains (Rodger and Holmes 1979). Adenovirus nucleic acid can also be separated by PAGE. On the gel the AdVs will be recognized by characteristic high molecular weight band. Electropherotyping can, however, be less sensitive and is not sufficient alone to determine RV serotypes. Polymerase Chain Reaction (PCR), reverse transcriptase PCR (RT-PCR) for RNA viruses is the common, widely used tool for all the enteric viruses outlined in this study. Nowadays, real-time PCR (qPCR) is becoming the golden tool for its rapid turn out, specificity, sensitivity and broad reactivity (Desselberger 2017, Higgins et al. 2020, Morillo et al. 2011).

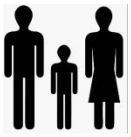
Table 1.3 Methods used for detection of viral enteric pathogens with listed advantages and disadvantages

Methods	Viruses	Advantages	Disadvantages
Electron Microscopy	• Rotavirus (RV) • Norovirus (Nov) • Sapovirus (SaV) • Astrovirus (AstV) • Adenovirus (AdV)	• Gold standard for determining viral agents as the aetiology causing diarrhoea	• Expensive, poor sensitivity and requires professional training for the use of the equipment
Cell culture	• AdV • RV	• Study the behaviour of the virus *in vitro*	• Long period before detection, poor sensitivity, susceptible to bacterial contamination, some viruses cannot replicate
Enzyme linked assays	• RV • NoV • SaV • AstV • AdV	• Better sensitivity	• Cross reactivity
Electropherotying	• RV • AdV	• Aids in determining RV groups other than RVA • Inexpensive	• Less sensitive
Reverse transcriptase Polymerase Chain Reaction (RT-PCR)	• RV, AstV, NoV, SaV	• Sensitive • Easy to set up	• Contamination • False positive results
Reverse Transcriptase real-time Polymerase Chain Reaction (RT-qPCR	• RV, NoV, AstV, SaV, AdV	• More sensitive • Rapid	• Prone to contamination

1.9 Problem Statement

Domesticated animals such as companion animals and livestock live in close proximity with humans. These animals can harbour many pathogens capable of infecting humans (Delahoy et al. 2018). Many of these pathogens are transmissible through animal faeces, and because

of the close interaction, there is a high disease association and potential risk for zoonotic transmission (Li et al. 2011). These pathogens include parasites, bacteria and viruses and some are associated with enteric infection leading to acute gastroenteritis (Malik and Matthijnssens 2014).




Rotavirus	DS-1-like Wa-like AU-like	DS-1-like G6, G8, G9 and P[1], P[5], P[11]	Wa-like G3, G4, G5, G9, G11 and P[6], P[7]	AU-like G3, G6 and P[9]
Norovirus	GI, **GII (GII.4**) and **GIV**	GIII	**GII**.11, **GII**.18, **GII**.19	**GIV**.2, GVI, GVII
Sapovirus	GI, GII, GIV, and GV		GIII, GVI and GVII	
Astrovirus	**GI**.A		**GI**.C	**GI**.B, **GI**.E
Adenovirus	HAdVF serotypes 40 and 41			

Figure 1.9 A summary of typical enteric virus genotypes circulating in humans and animals. Genogroups in bold show common genogroups found in different species (Chu et al. 2010, Méndez and Arias 2013).

Of all the enteric viruses discussed in the preceding sections, summarized in Figure 1.9, RVA, NoV, and AstV have been detected both in companion animals and livestock, with SaV mainly detected in porcine (Oka et al. 2015, Li et al. 2017). Group A rotaviruses are not regarded as major enteric pathogens of cats and dogs. However, RV-like particles have been detected at low frequencies from both asymptomatic and symptomatic domestic animals. Although RV vaccines are available for livestock animals, the ONE Health approach is concerned about the role of animals during disease transmission. This is mainly because small children and adults and their pets are usually in close contact. Human RVs with genetic homology to feline rotaviruses (FRVs) have been isolated from Japan, Israel, Tunisia, and the United States (Fredj et al. 2013, Nakagomi et al. 1985, Nakagomi and Nakagomi 2000). In South Africa, no study has reported FRV in humans. A number of reports on potential zoonotic infections in people have been established, however, due to the lack of rotavirus surveillance programmes in animals, little is known about the prevalence of potential zoonotic strains in animals (German et al. 2015). In the United Kingdom, one of the few studies on surveillance of RV in domesticated animals indicated a prevalence of 3.0% (n = 1727) in catteries (German et al. 2015). The prevalence of RV was, however, not associated with diarrhoea but season.

In contrast, pigs play a major role in the agricultural sector and the economy, but infectious diarrhoea seem to be one of the devastating conditions experienced by these sectors worldwide (Holland 1990, Salamunova et al. 2018). Viruses can be the causative agents of these infectious diarrhoea cases leading to acute gastroenteritis and can even escalate to causing mortality in neonatal pigs. Viruses associated with diarrhoea in pigs include RV, NoV, SaV, and AstV (Figure 1.9).

To our knowledge, no study has specifically investigated the zoonotic potential of these enteric viruses in South African dogs and pigs.

1.10 Aim and study objectives

The aim of this project was to identify the presence of enteric viruses in domesticated animals and investigate the possible potential for zoonosis of the identified viruses.

Objectives:

1. Identify the presence of human enteric viruses of public health importance in companion animals and livestock (**Chapter 2**)
2. Molecular characterisation of identified viruses (**Chapter 3**)
3. Determination of the zoonotic potential of the identified viruses (**Chapter 3**)

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Chapter 2: Molecular detection of viral enteric pathogens with possible potential for zoonosis in domesticated animals

2.1 Introduction

Diarrhoeal disease commonly affects humans and animals. Enteric viruses are known etiological agents associated with the disease (Bishop and Kirkwood 2008). As discussed in Chapter 1, enteric viruses implicated in animals include rotavirus (RV), norovirus (NoV), sapovirus (SaV), astrovirus (AstV), and enteric adenovirus (AdV) (Bishop et al. 1972, Flewett et al. 1975, Kapikian et al. 1972, Madeley et al. 1975,).

Members of the *Caliciviridae* family, including NoVs and SaVs, are most commonly known to cause food- and water-borne outbreaks (Divizia et al. 2004, Sdiri-Loulizi et al. 2010). The family of *Caliciviridae* affects a wide host range. For NoVs, the close genetic relatedness of porcine NoV GII genogroup to human strains suggests the potential for zoonosis (Martella et al. 2008, Mattison et al. 2007, Wang et al. 2005). Several years ago, a study reported a NoV canine strain (ca/Visseu/C33/2008/2008/PT) which clustered with another NoV canine strain (ca/Bari/91/91/2007/IT) and a NoV human strain (Hu/chiba/2004/JP) on a phylogenetic tree; which could indicate a possible interspecies transmission (Mesquita et al. 2010). Another report suggested that a sick dog caused a NoV outbreak in a retirement home in the United Kingdom (Humphrey et al. 1984).

Animal SaVs constitute of porcine enteric SaV and mink enteric SaV, but pigs are the predominant host. To date, only the Cowden strain within the SaV genus can be propagated in cell culture (Saif et al. 1980, Farkas et al. 2004, Bank-Wolf et al. 2010). No zoonotic transmission has been reported for SaV although a study by Hansman and co-workers described a possible recombination event between human GII, GIV and porcine GIII Sav (Hansman et al. 2005). The possible intergenogroup recombination, which requires co-infection between human and animal SaV highlights a possible route for zoonosis. In contrast, AstVs have a wide host range suggesting a high possibility for interspecies transmission. However, zoonotic infection has not been clearly established. Enteric adenoviruses only infect and cause diarrhoea in humans. Therefore, there has been no report of animal infections and, consequently, the potential for zoonotic transmission to date. As for RV, there is evidence for transmission of RV strains from one host species to another, suggesting that RV is a zoonotic viral pathogen (Doro et al. 2015, Martella et al. 2010). Table 2.1 summarises the enteric viruses mentioned above and their possible potential for zoonosis.

Table 2.1 Enteric viruses and their interspecies transmission along with their zoonotic potential

Virus	Human	Animal	Zoonotic	Transmission
Rotavirus	Yes	Yes	Yes	Human ⇄ Animal
Norovirus	Yes	Yes	Possible	Human ← Animal
Sapovirus	Yes	Yes	Possible	Unknown
Astrovirus	Yes	Yes	Not detected	Unknown
Adenovirus	Yes	No	Not detected	Unknown

Children under the age of 5 years of age are more at risk of contracting enteric viruses from infected companion animals, as they are less cautious about hygiene compared to adults. Furthermore, livestock handlers are also at risk of contracting a virus. The impact of the global pandemic caused by SARS-Cov-2 highlights the importance to understand zoonosis. The aim of this chapter was to screen canine and porcine stool samples for the presence of human enteric viruses to determine the zoonotic potential of these viruses. The samples were screened using two methods namely electropherotyping, targeting mainly rotavirus infections, and reverse transcription real-time polymerase chain reaction (RT-qPCR).

2.2 Materials and Methods

2.2.1 Ethics statement and sample collection

This animal study was conducted with the approval of the Animal Research Ethics committee at the University of the Free State (Ethics number: UFS-AED2018/0030; Appendix A1). Sampling was done in collaboration with veterinary clinics, a rescue centre, and a farm. No invasive action was performed. Instead, faecal matter was directly picked up from the surface, placed in sterile containers and stored at 2-8 °C while transported to the Department of Microbial, Biochemical, and Food Biotechnology, University of the Free State, Bloemfontein, South Africa. Upon arrival, the stool samples were stored at -20 ºC until they were processed. The clinical status of each animal was recorded by assessing the consistency of the faeces. Liquid stool was regarded as symptomatic and solid stool asymptomatic (Appendix A2 and Appendix A3). All samples and waste were handled and stored according to WHO Biosafety Level 2 guidelines.

A total of 104 canine faecal samples, symptomatic (n=2), asymptomatic (n=41) and 61 faecal samples of unknown clinical status, were collected from five sites in Bloemfontein, South Africa (Figure 2.1).

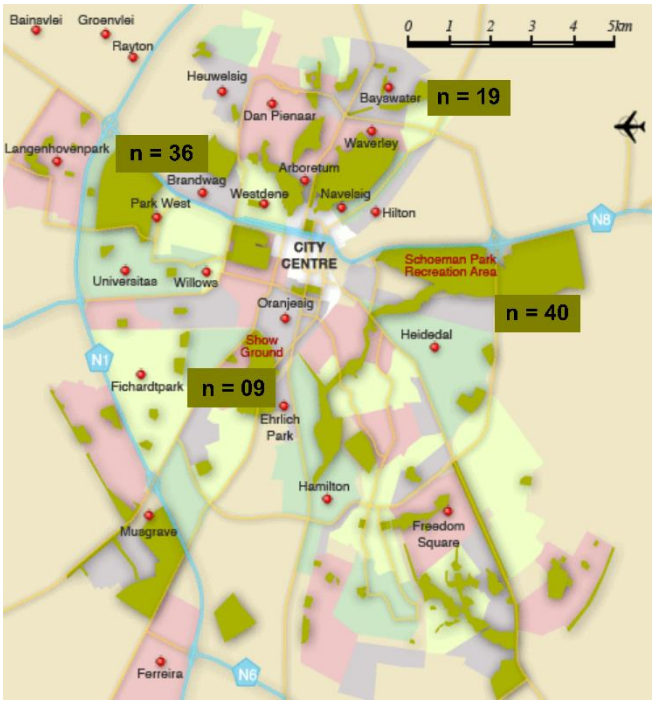


Figure 2.1 A map of Bloemfontein showing geographical locations of clinics and a rescue centre where faecal samples were collected. Samples were collected from four geographical regions: two veterinary clinics in Langenhovenpark (west; n = 36), one veterinary clinic in Bayswater (north; n = 19), another veterinary clinic in Fichardtpark (south; n = 09), and a rescue centre (east; n = 40). Samples were collected from puppies and adult dogs, both symptomatic and asymptomatic (Appendix A2).

A total of 118 porcine faecal samples and 3 water samples were collected during five sampling trips (Figure 2.2, Appendix A3). Faecal samples were collected over a period of 2 years and 2 months (January 2018 to February 2020) from piglets below the age of 39 days (Figure 2.2, Appendix 3). The water samples (drinking water for the pigs) were collected from the pigpen during the stool sampling (Appendix A3).

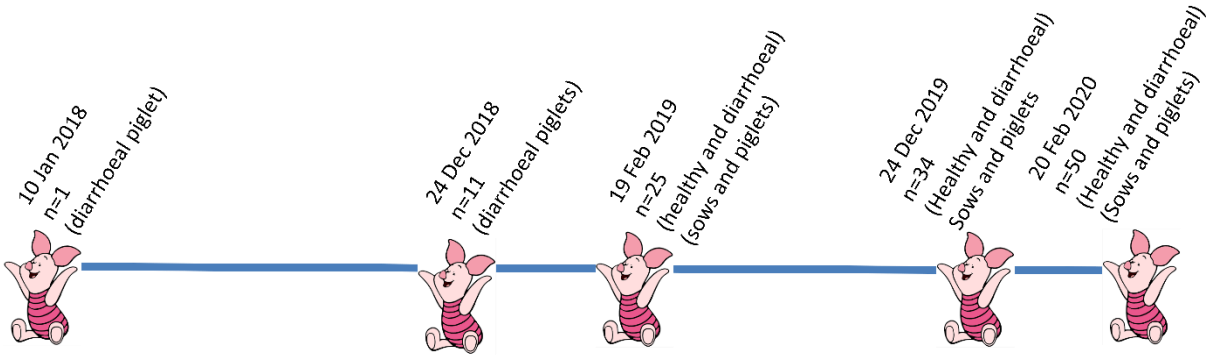


Figure 2.2 Timeline depicting sampling trips. For each trip the sampling date, total number of stool samples collected at the time, clinical status and age category are indicated.

2.2.2 Determination of viral presence with RNA extraction

Total RNA was extracted from 225 samples using TRIzol™ (Sigma Aldrich, United States). Briefly, the stool samples and water samples were thawed at room temperature, 50 µl of stool (same volume for water sample) was suspended in 450 µl molecular grade water, and vortexed. The diluted sample was combined with 900 µl of TRIzol™ and 100 µl VERTREL (Sigma-Aldrich, United States). Three hundred microlitres chloroform (Sigma-Aldrich, United States) was added to the homogenized solution, mixed by inversion and centrifuged at 20 000 *x g* for 15 min at 4 °C (Centrifuge 5804R, Eppendorf, Germany) to allow separation of the solution into three layers: a clear upper aqueous layer, an interphase and an organic layer. The clear upper aqueous layer (containing RNA) was transferred into a new 2 ml microcentrifuge tube and RNA was precipitated by adding 750 µl of isopropanol (Merck, Germany) and centrifugation at 20 000 *x g* for 15 min at 4 °C. Following centrifugation, the isopropanol was drained, the pellet air-dried and resuspended in 95 µl of elution buffer (Qiagen, Germany).

Extracted RNA was examined by gel electrophoresis on a 1% agarose gel prepared with 0.5 x TBE (50mM Tris, 50mM boric acid, 1mM EDTA) and stained with 5 µg/ml ethidium bromide (Sigma Aldrich, United States). Briefly, 5 µl of the sample and 1 µl 6X TriTrack DNA loading dye (Thermo Fisher Scientific, United States) were combined and subjected to electrophoresis for 30 min at 90 V (Bio-Rad Laboratories Inc, United States). The GeneRuler Express DNA Ladder was used as the DNA marker (Thermo Fisher Scientific, United States). The gel was then examined under UV light using a ChemiDoc MP Imaging System (Bio-Rad Laboratories Inc, United States).

2.2.3 Determination of viral presence with RT-qPCR

Total viral RNA was extracted from both the canine (n = 104) and selected porcine (n = 31) stool samples using QIAamp viral RNA kit (Qiagen, Germany) according to the manufacturer's instructions. A negative control sample supplied with the FDT kit was included in the extraction protocol to ascertain that there is no contamination occurring during the extraction process. Briefly, stool samples were thawed at room temperature and a 10% dilution was prepared usingmolecular grade water. The solution was vigorously vortexed and centrifuged at 14 000 *x g* for 15 minutes at 4 °C. Thereafter, 140 µl of the supernatant was added to the lysis buffer of the QIAamp kit. An internal control, brome mosaic virus (BMV) (2 µl) supplied with the FTD Viral Gastro Kit (Fast Track Diagnostics) was added as an extraction and RT-qPCR control. This was followed by loading the solution onto the QIAamp Mini column and centrifuging at 6000 *x g* for 1 min. To eliminate contaminants, the column was washed twice using wash buffers, and total viral RNA was eluted with 60 µl of the elution buffer.

The FTD kit readily contains three master mixes, controls, the enzyme and the buffer. The RT-qPCR is performed in a single tube for each master mix and the presence of a specific pathogen is detected by an increase in fluorescence. The increase in fluorescence is exponential and associated with a threshold cycle (cq) value. Regarding the master mixes, each master mix contains a primer/probe combination which targets a specific sequence. The Noro master mix targets NoV GII, IC (brome mosaic virus, BMV), and NoV GI. The second master mix, referred to as ARA master mix, targets HAstV, RVA and HAdV. Finally, the Sapo master mix targets SaV. In each RT-qPCR reaction, positive controls, a negative control and an internal control (BMV) (added during RNA extraction) are included. The samples were screened with the FTD Viral Gastro Kit (Fast Track Diagnostics) by multiplex RT-qPCR for the presence of the following viruses: norovirus GII (NoV GII), human astrovirus (HAstV), and sapovirus (SaV) at a detection wavelength of 520 nm, rotavirus (RVA) at a detection wavelength of 550 nm, and norovirus GI (NoV GI), human adenovirus (HAdV) at a detection wavelength of 670 nm following the manufacturer's instructions. The positive control, containing plasmids for NoV GI/GII, HAstV, RoV, HAdV and SaV were thawed at room temperature for 20 to 30 min and vortexed thoroughly before use. The final reaction volume was 25 µl, which included 12.5 µl of 2 x RT-PCR buffer (Fast-Track mastermix), 1.5 µl of the primer-probe, 1 µl of 25 x RT-PCR enzyme mix (Fast-track mastermix), and 10 µl of extracted RNA. Each run included a negative control and positive controls. Reaction tubes were inserted into the Rotor-Gene 3000 Q 5 Plex HRM (Qiagen, Germany) and the qPCR conditions were set according to the manufacturer's instructions: 50 ºC for 15 min, 94 ºC for 1 min with 40 cycles of 94 ºC for 8 seconds and 60 ºC for 1 minute.

Q-Rex software v1.1 (www.qiagen.com) with a Q-Rex Basic plug-in v2, compatible with the Rotor-Gene® Q instruments, was used to visualize fluorescence data and determine quantification cycle values (cq).

2.3 Results

2.3.1 Electropherotyping

Amongst all the enteric viruses discussed in chapter 1, the zoonotic nature of RV is well established. Therefore, initial screening focused on the detection of RV. Electropherotyping was used since it is an inexpensive method and can potentially also detect non-group A RV strains (Herring et al. 1982, Kasempimolporn et al. 1988, Zbiden et al. 1992). The genome of RV consists of 11 genome segments which can be separated with gel electrophoresis to form the characteristic 4-2-3-2 electrophoretic pattern of rotavirus group A viruses (Zbinden et al. 1992).

2.3.1.1 Canine samples

A total of 104 canine samples were screened for the presence of RV viral RNA using electropherotyping. None of the canine samples displayed the characteristic electrophoretic pattern of RV strains. However, bands were observed in some of the samples and were mostly below 1000 bp (Figure 2.3).

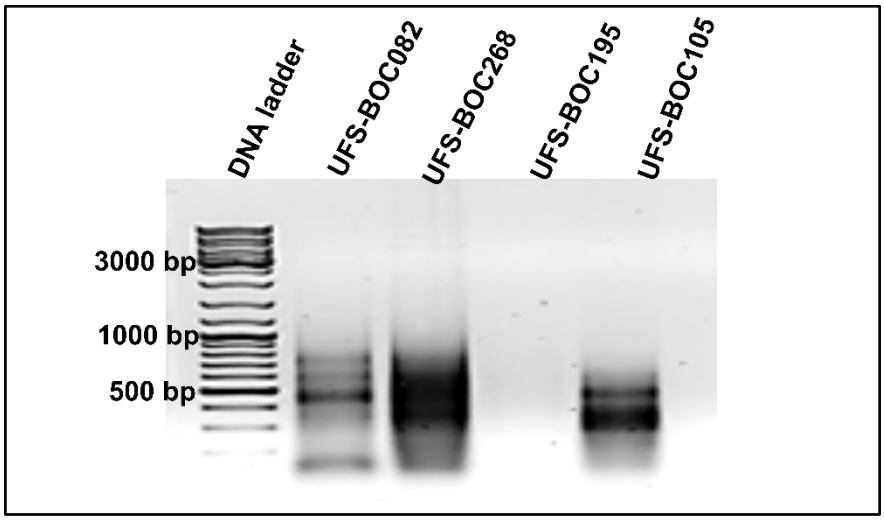


Figure 2.3 Agarose gel electrophoretic analysis of extracted total RNA from canine samples. Sample number: UFS-BOC082 is an example of a canine sample with unidentified, non-RV bands.

2.3.1.2 Porcine Samples

A total of 118 porcine stool samples and 3 water samples were screened by extraction of RNA and visualization on agarose gels (Figure 2.4 A). A variety of RNA species was observed from total RNA extracted from each sample, where ssRNA and degraded ribosomal RNA (rRNA) were in a form of smears and large blobs at the bottom of the gel (Figure 2.4 A). The dsRNA was shown by multiple bands that represented a typical RV profile (Figure 2.4 B). One sample (UFS-BOC050) showed a distinct RV profile (Figure 2.4 B) from the rest of the RV profiles identified (Figure 2.4 A and Figure 2.4 B). Regarding water samples, all 3 water samples were collected from the pen (Appendix A3) and were tested for the presence of RV. None of the 3 water samples indicated the presence of RV.

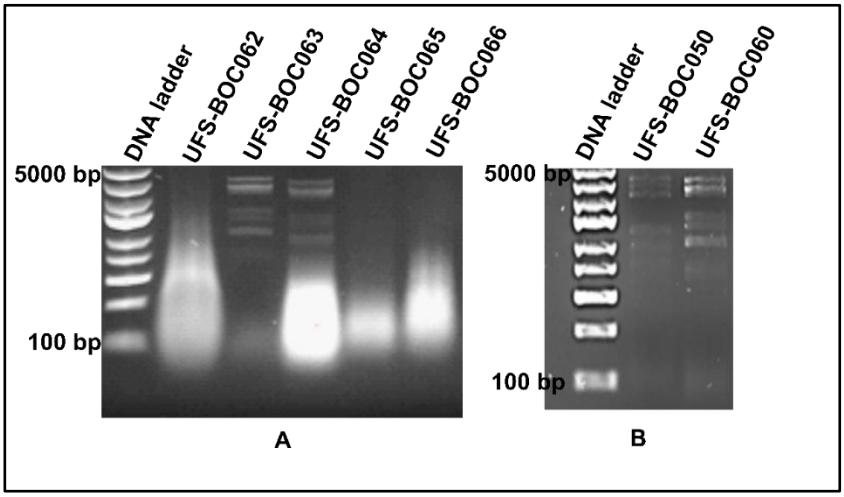


Figure 2.4 Agarose gel electrophoretic analysis of extracted RNA from porcine samples. A**:** RNA extracted from porcine samples showing RV profiles for some samples and porcine samples that were negative for RV. B**:** RNA extracted from porcine samples showing different RV profiles.

Table 2.2 A summary of porcine RV detected by electropherotyping

Date	Samples (n)	Sample name	Host	Stool	Pen	Age (days)	Positive (%)
10/01/2018	1	UFS-BOC001	piglet	diarrhoea	unknown	28	1 (100)
24/12/2018	11	UFS-BOC009	piglet	diarrhoea	unknown	30	1 (9.09)
19/02/2019	25	UFS-BOC035	piglet	diarrhoea	unknown	30	1 (4.00)
24/12/2019	34	UFS-BOC050	piglet	diarrhoea	19248	20	5 (14.7)
		UFS-BOC060	piglet	diarrhoea	19134	05	
		UFS-BOC063	piglet	diarrhoea	19134	05	
		UFS-BOC064	piglet	diarrhoea	19134	05	
		UFS-BOC071	piglet	diarrhoea	19134	05	
20/02/2020	50	UFS-BOC076	piglet	diarrhoea	18202	28	8 (16.0)
		UFS-BOC077	piglet	diarrhoea	18202	28	
		UFS-BOC078	piglet	diarrhoea	18202	28	
		UFS-BOC079	piglet	diarrhoea	18212	28	
		UFS-BOC081	piglet	diarrhoea	19134	30	
		UFS-BOC082	piglet	diarrhoea	19248	30	
		UFS-BOC083	piglet	diarrhoea	19102	30	
		UFS-BOC124	piglet	diarrhoea	19102	30	
Total	121						16 (13.22)

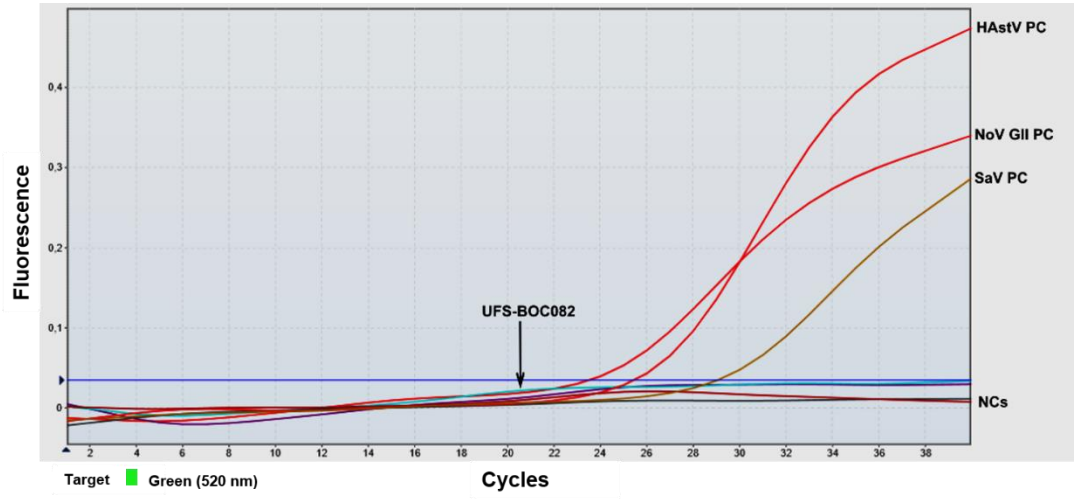
A total of 16 porcine samples (approximately 13%) were positive for RV using electropherotyping (Table 2.2). Of the 16 porcine samples, all were taken from piglets presenting with diarrhoea and were all under the age of 5 weeks. Each sampling trip presented with a sample positive for RV. The number of RV positive samples increased from sampling trip 4 (24/12/2019), which could possibly be influenced by an increase in the sampling size.

2.3.2 RT-qPCR

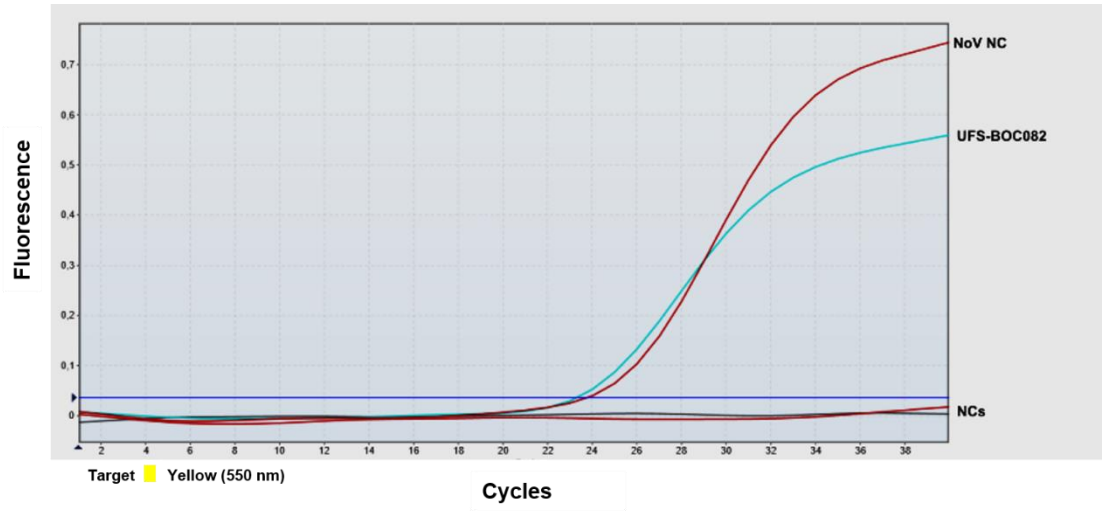
Since no rotavirus was detected in the canine samples, the study was expanded to also evaluate the presence of other human enteric viruses. The samples were tested with RT-qPCR. This is a more sensitive screening method and specifically targeted human enteric viruses: NoV GI, NoV GII, HAstV, RVA, HAdV and SaV.

2.3.2.1 Canine samples

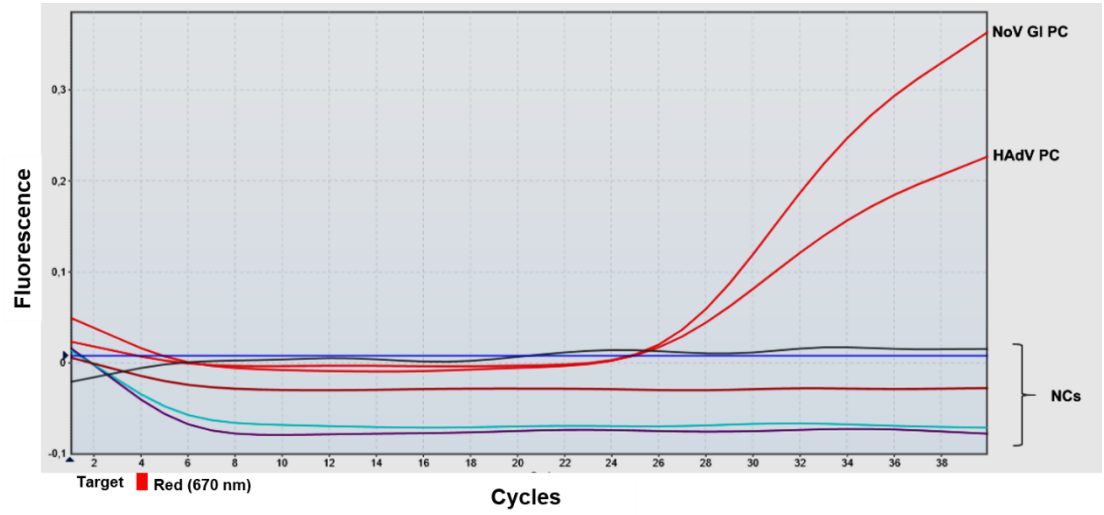
The amplification of all the positive controls was exponential (Figure 2.5) and cq values below 33 were obtained. Negative controls are all the flat lines on the graphs. (Figure 2.5 B and Figure 2.5 C). Amongst all the 104 canine samples that were tested for the presence of the human enteric viruses, none of these viruses were detected with the RT-qPCR. One of the canine samples that showed bands in an agarose gel (UFS-BOC082, Figure 2.3), was also negative for all the five human enteric viruses, indicated by a blue curve on the amplification cycles (Figure 2.5).



A



B



C

Figure 2.5 Amplification cycle of all the targets described in the FTD kit for positive controls and a canine sample (UFS-BOC082). A: the green target depicts amplification cycle for NoV GII, HAstV, and SaV at a detection wavelength of 520 nm. B: the yellow target show amplification cycle for RV and internal control (IC) at a detection wavelength of 550 nm, the curve for UFS-BOC082 sample is in the Noro master mix which targets the IC at the yellow target. C: the red target, indicates amplification cycle for NoV GI and HAdV at a detection wavelength of 670 nm.

2.3.2.2 Porcine samples

Of the 118 porcine stool samples, 31 samples were tested for the presence of human enteric viruses: NoV GI, NoV GII, HAstV, RV, HAdV and SaV. The porcine samples were chosen based on the following criteria: liquidity, indicating that the host was suffering from diarrhoea but was negative using electropherotyping, samples that were negative on the agarose gel but were collected from the same pen as RV positive samples and lastly, for a sow that had an RV positive piglet(s) (Appendix A3).

Exponential curves were generated for the positive controls and the RV target. The amplification cycle indicates an increase in fluorescence (Figure 2.6) which was also reported as cq values, similar to canine samples (Table 2.3).

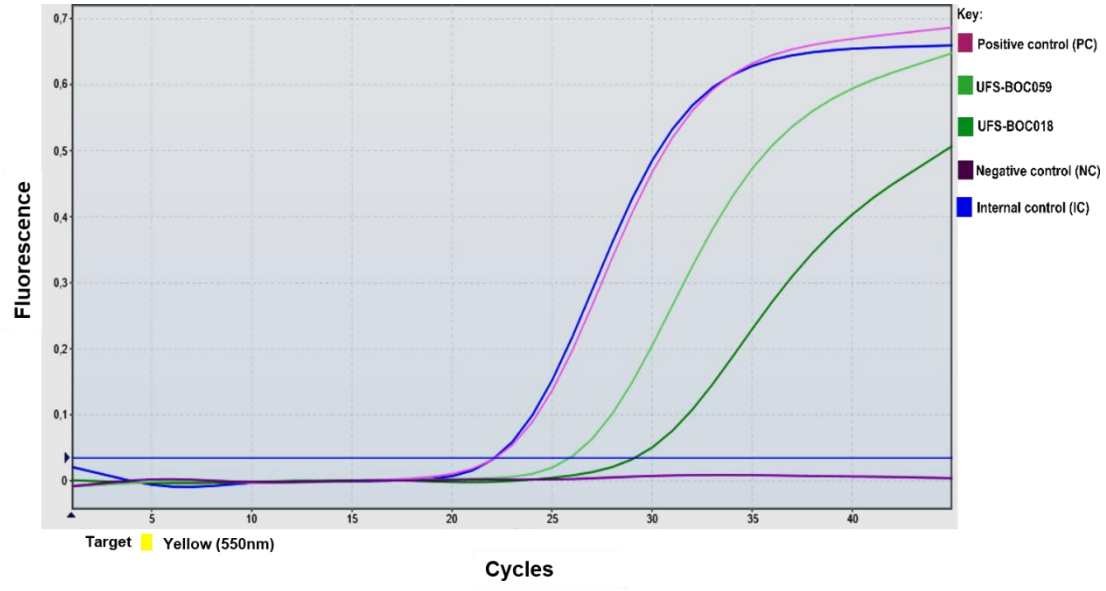


Figure 2.6 Amplification cycle of the yellow target or ARA master mix, amplifying the RVA positive control (PC), Internal control (IC), and porcine RV positive samples, UFS-BOC018 and UFS-BOC059. The presence of RV in the samples is indicated by exponential curves. The purple flat curve is the negative control.

Table 2.3 A summary of porcine RV detected with RT-qPCR

Date (total samples)	Sample name	Agarose gel (RV)	RT-qPCR (RV)	Cq	Host	Stool	Pen
Jan 2018 (1)	UFS-BOC001	**Positive**	**Positive**	25.00	Piglet	Diarrhoea	unknown
Sample trip 2 (11)	UFS-BOC002	Negative	Negative		Piglet	Diarrhoea	unknown
	UFS-BOC004	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC005	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC006	Negative	negative		Piglet	Diarrhoea	unknown
Sample trip 3 (25)	UFS-BOC014	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC018	Negative	**positive**	29.12	Piglet	Diarrhoea	unknown
	UFS-BOC022	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC027	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC032	Negative	negative		Piglet	Diarrhoea	unknown
	UFS-BOC035	**Positive**	**positive**	21.50	Piglet	Diarrhoea	unknown
	UFS-BOC036	Negative	negative		Piglet	Diarrhoea	unknown
Sample trip 4 (34)	UFS-BOC048	Negative	negative		Sow	Normal	19134
	UFS-BOC056	Negative	negative		Piglet	Normal	19248
	UFS-BOC057	Negative	negative		Piglet	Diarrhoea	19102
	UFS-BOC058	Negative	negative		Piglet	Diarrhoea	19102
	UFS-BOC059	Negative	**positive**	25.88	Piglet	Normal	19134
	UFS-BOC061	Negative	negative		Piglet	Diarrhoea	19141
	UFS-BOC071	**Positive**	**positive**	22.39	Piglet	Diarrhoea	19134
	UFS-BOC073	Negative	negative		Piglet	Diarrhoea	19241
Sample trip 5 (50)	UFS-BOC080	Negative	negative		Piglet	Normal	18212
	UFS-BOC078	**Positive**	**positive**	22.75	Piglet	Diarrhoea	18202
	UFS-BOC081	**Positive**	**positive**	20.92	Piglet	Diarrhoea	18119
	UFS-BOC102	Negative	negative		Piglet	Diarrhoea	17286
	UFS-BOC107	Negative	negative		Piglet	Diarrhoea	19286
	UFS-BOC110	Negative	negative		Piglet	Diarrhoea	19182
	UFS-BOC119	Negative	negative		Piglet	Diarrhoea	19242
	UFS-BOC094	Negative	negative		Piglet	Diarrhoea	18381
	UFS-BOC096	Negative	negative		Piglet	Diarrhoea	18135
	UFS-BOC122	Negative	**positive**	29.85	Piglet	Diarrhoea	19169
	UFS-BOC124	**Positive**	**positive**	21.52	Piglet	Diarrhoea	18197
	Controls:						
Positive control				25.63			
Internal control				22.13			

An interesting observation was made for some of the samples that were negative for RV using electropherotyping, where these samples indicated exponential increase in fluorescence using RT-qPCR. These samples include UFS-BOC018, UFS-BOC059, and UFS-BOC122. They all

gave exponential amplification cycles with cq values of 29.12, 25.88, and 29.85, respectively (Table 2.3). The cq values for these samples were all within the threshold of pathogen detection, and, therefore, reported positive for RV. As expected, porcine samples that tested positive with electropherotyping also tested positive using RT-qPCR.

2.4 Discussion

In order to determine the zoonotic potential for human enteric viruses (NoV GGI, NoV GII, HAstV, RVA, HAdV, and SaV) in domesticated animals, the presence of these viruses was investigated in canine and porcine stool samples. Prior to this study, AGE was already an established technique in the research lab, therefore, the canine and porcine stool samples were screened first for RV using electropherotyping. The RNA extracted from the porcine samples indicated different RV profiles using electropherotyping (Figure 2.4), but RNA extracted from canine samples did not indicate the presence of RV in the samples (Figure 2.3). Since no RV was detected in canine samples, the study was expanded to evaluate the presence of other human enteric viruses, which include, NoV GI, NoV GII, HAstV, RV, HAdV and SaV using RT-qPCR.

A few studies have reported canine rotavirus (Fulton et al. 1981, Hackett and Lappin 2014, Sieg et al. 2015), including a study from Brazil that reported the detection of a P[3] RVA genotype from dogs with a prevalence of 3% (Gabbay et al. 2003). A Nov, genotype GIV, was identified in a young dog in Italy, which provides evidence that NoVs are capable of causing infection in pets (Martella et al. 2008). Subsequent studies reported NoV infection in dogs accounting for 40% of NoV infection in symptomatic dogs and 9% infection in asymptomatic dogs (Mesquita et al. 2010). For AstVs, infection was reported in two household dogs housed together from Italy. Although the two dogs were housed together, only one showed clinical signs of diarrhoea. Nonetheless, amongst other species, like AdV, dogs are susceptible to AstV infection (Martella et al. 2012). One study in Italy reported that 2.2% of dogs infected with SaV using RT-qPCR assays (Bodnar et al. 2016), and another study in Japan reported a 2.06% SaV infection in dogs (Soma et al. 2015). The low levels of SaV infection reported provides evidence that SaVs are not major enteric viruses causing diarrhoea in dogs. Enteric adenoviruses have only been reported in humans and environmental samples, to date (Brisebois et al. 2018, Moudjahed et al. 2017), with canine adenovirus type 1 associated with hepatitis, and not necessarily the causative agent of acute diarrhoea (Pratelli et al. 2001).

The detection limit of RT-qPCR used in this study is a cq value ≤ 33 according to the guidelines provided in the FTD Viral gastroenteritis kit. All the samples with cq values > 33 were therefore

considered negative. Moreover, negative controls (without template) showed no amplification, confirming the specificity of the primer/probe combination. For the canine samples (n=104), no human enteric viruses, investigated in this study, were detected, however; with every RT-qPCR run, the positive controls amplified, providing evidence for a successful RT-qPCR run. In South Africa there is no reported study on human enteric viruses in dogs.

Rotavirus in pigs was detected for 16/118 (13.55%) samples by electropherotyping. Of the 118 porcine stool samples, 31 were selected to screen for human enteric viruses using RT-qPCR. Of the 31 porcine samples that were tested, six had already shown RVA profiles on agarose gels, but the remaining samples (n= 25) included for RT-qPCR run did not indicate RV profiles. Despite testing negative using electropherotyping, a further 3 samples were identified using RT-qPCR. This brings to a total of 19/118 (16.10%) samples which tested positive for RV. When comparing cq values obtained for these three samples, 29.12, 29.85, and 25.88 for UFS-BOC018, UFS-BOC122, and UFS-BOC059, respectively, to cq values of samples that were positive with electropherotyping (Table 2.3), we can clearly see that these samples have high cq values. The lower the cq value, the higher the concentration of viral RNA present in the samples. This explains why the three samples could not be detected for RV with electropherotyping. More samples tested positive with RT-qPCR which confirms that RT-qPCR is a more sensitive method than electropherotyping (Liu et al. 2013). No NoV, SaV, AstV or AdVs were detected in the porcine samples. Rotavirus, however, remains a major cause of diarrhoea in piglets, with high incidences of about 83% of porcine RV reported from USA (Marthaler et al. 2014) using RT-qPCR as a diagnostic method. Contaminated water with enteric viruses can be a route for virus transmission (Grabow 2007, Kiulia et al 2010). In this study we included water samples from the pigpens, to investigate if RV was possibly transmitted via contaminated water. None of the water samples indicated the presence of RV.

Porcine samples which tested positive for RV were all from piglets. Considering the season at which sampling took place, this study indicates a low detection rate for RV on the farm since RV prevalence is usually the highest during the cold, winter months. Extending sampling to the winter months could therefore increase detection. The age range was from 05 days to 30 days. The age range is associated with the nursing to weaning period, which is also the age category where most piglets get infected with RV (Saif et al. 1980). All the samples that tested positive for RV were detected from diarrhoeic samples (Table 2.2 and Table 2.3). This is similar to a study in Japan which reported the highest detection rate in symptomatic pigs that were in a weaning period (Miyazaki et al. 2012).

RV positive porcine samples were observed within the same pen number (Table 2.2). This observation leads to a limitation of the study, where samples cannot be connected to a specific

piglet. Therefore, it is not known if RV positive samples from the same pen came from one piglet or multiple piglets. Rotavirus group A, which is known to have zoonotic potential, was detected using electropherotyping and RT-qPCR in porcine samples, but no other human enteric viruses were detected in either the porcine or canine samples. The zoonotic potential of the detected RV samples will be further explored and discussed in Chapter 3.

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Chapter 3: Whole-genome characterization of porcine rotavirus strains from the Western Cape region, South Africa

3.1 Introduction

Rotavirus (RV) is grouped into nine groups (RVA-RVD, and RVF-RVJ) (https://talk.ictvonline.org/taxonomy/) as discussed in section 1.3.2, Chapter 1. Groups RVA, RVB, RVC, and RVH have been described in pigs (Saif and Jiang 1994, Vlasova et al. 2017). A whole-genome classification system that is inclusive of all the 11 RV segments of RVA has been developed by the Rotavirus Classification Working Group (RCWG) (Matthijnssens et al. 2008b). The system was developed based on nucleotide cut-off percentages of different genotypes per genome segment (Matthijnssens et al. 2008b). The RVA percentage identity cut-off values of 80%, 80%, 85%, 83%, 84%, 81%, 79%, 85%, 85%, 85%, and 91% have been established for genome segments VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6, respectively (Matthijnssens et al. 2008a). To date 36 G, 51 P, 26 I, 22 R, 20 C, 20 M, 31 A, 22 N, 22 T, 27 E, and 22 H genotypes have been identified (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/newgenotypes). For non-group A RVs, a whole genome classification system similar to the RVA classification system is yet to be developed by the RCWG (Matthijnssens et al. 2011).

Genetic data for RVB was at first limited to human strains and a murine strain (Matthijnssens et al. 2011, Nagashima et al. 2008, Yamamoto et al. 2010). However, a recent increase in RVB whole genome sequence data has led to the development of a similar classification system for RVB (Marthaler et al. 2012). Similar to RVA, RVB cut-off values of 80%, 81%, 70%, 76%, 75%, 78%, 70%, and 78% for genome segments VP7, VP6, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6, respectively, have been proposed (Hayashi-Miyamoto et al. 2017, Marthaler et al. 2012, Suzuki et al. 2011). Based on these cut-off values, 24 G, 13 I, 4 M, 7 A, 4 N, 4 T, 4 E, and 6 H genotypes have been identified (Hayashi-Miyamoto et al. 2017, Marthaler et al. 2014, Suzuki et al. 2011, Suzuki et al. 2012a, Suzuki et al. 2012b).

Regarding RVC, only a limited number of full-genome sequences have been identified (Yamamoto et al. 2010). Similar to RVH, only a limited number of strains have been described from China (Jiang et al. 2008), Bangladesh (Alam et al. 2007, Nagashima et al. 2008), Japan (Wakuda et al. 2011) Brazil (Molinari et al. 2014, Molinari et al. 2015) and South Africa (Nyaga et al. 2015, Nyaga et al 2016).

According to the RVA classification system, there are 12 G-types (G1-G6, G8, G12, and G26) and 16 P-types (P[1] –P[8], P[11], P[13], P[19], P[23], P[26], P[27], P[32], and P[34]) associated with porcine rotavirus (Amimo et al. 2013, Amimo et al. 2015, Vlasova et al. 2017). The most common genotypes reported have been G3 to G5, G9 and G11 associated with P[5] to P[7], P[13], and P [28], respectively (Okitsu et al. 2011, Papp et al. 2013).

Porcine RV is reported in most parts of the world (Vlasova et al. 2017). However, porcine RVA genotype distribution varies per geographical area. In North America and South America, the most prevalent genotype combination detected is G5P[7] (Parra et al. 2008, Vlasova et al. 2017, Winiarczyk et al. 2002). The P[7] genotype has a high prevalence of 77% (Papp et al. 2013). In addition to the G5P[7] genotype combination, in Europe, the G4P[6] was also found to be a predominant genotype combination in pigs (Van der Heide et al. 2005). This is similar to Asia where G4P[6] was observed as the most predominant combination in a study conducted in Thailand between 2009 to 2010, recording a prevalence of 19.8% (Saikruang et al. 2013). In East Africa, a prevalence of 26% of RVA in pigs was reported in Uganda and Kenya (Amimo et al. 2015). South Africa surveillance study reported a prevalence of 85% of RVA in pigs, but this was more than 20 years ago, therefore, the prevalence of RVA in South African pigs is not clear (Geyer et al. 1996).

The advancement in next generation sequencing (NGS) technologies has allowed for the rapid generation of whole genome sequence data for RVA. Although Sanger sequencing, a first-generation DNA sequencing technique, is still considered the gold standard due to the lower error-rates, the method is time-consuming and not cost-effective when a large number of sequences needs to be generated (Hert et al. 2008). For characterization of RVA strains, many authors combined Sanger sequencing or NGS with targeted PCR-amplification of genome segments or partial genome segments (Matthijnssens et al. 2008a, Rahman et al. 2007). This approach can, however, bias the true consensus sequence of the viral population in the sample. A sequence-independent approach for dsRNA genome characterization was described previously (Potgieter et al. 2009). The sequence-independent amplified material can then be combined with NGS to produce massive parallel sequencing of DNA fragments (Jere et al. 2011, Nyaga et al. 2013). Next generation sequencing platforms, such as the Illumina MiSeq platform, is a second-generation DNA sequencing technology (Bentley et al. 2008). The technology utilizes sequencing-by-synthesis, provides high sequence throughput, is relatively less expensive for sequencing segmented dsRNA genomes like RV and is less time consuming as compared to Sanger sequencing (Kwong et al. 2015).

In Chapter 2, RV was detected in 16.10% (n = 118) porcine samples. Of the 19 porcine samples, the genetic material could only be visualized on an agarose gel in 16 samples. In

this chapter, the whole genome consensus sequences of these viruses were determined using a sequence-independent approach. Phylogenetic analysis was used to determine if any of the characterized viruses had any zoonotic potential.

3.2 Materials and Methods

3.2.1 Double-stranded RNA enrichment and purification

The extracted RNA for the 16 RV positive samples that were analysed with electropherotyping in chapter 2 (section 2.2.2**)** were treated with 2 M LiCl (Sigma Aldrich, Germany) overnight at 4°C to remove single-stranded RNA (ssRNA). Following overnight incubation, the sample was centrifuged at 4°C for 30 min at 20 000 x g, and the supernatant was transferred to a clean microcentrifuge tube. This was followed by treating the sample with DNase. Briefly, a total volume of 9 µl of the 10X reaction buffer (Tris-HCl, pH 8.3, 20mM $MgCl_2$) was added to the RNA. After that, 9 U of DNase I (Sigma Aldrich, United States) was added to the reaction tube, and gently mixed by flicking the tube. The DNase/buffer/RNA mixture was then incubated for 15 min at room temperature (RT). The DNase was inactivated with 3.85 mM EDTA and incubated at 70°C for 10 min. The reaction mixture, free of possible DNA contaminants, was cooled down on the ice and 2 µl RNasin® Plus RNase Inhibitor (Promega, United States of America) was added to the tube. To remove possible residuals of LiCl and ssRNA, the dsRNA was purified with the MinElute Gel Extraction kit (Qiagen, Germany), according to the manufacturer's instructions. The sample was mixed with 330 µl of the QG buffer by vortexing. The solution was then transferred to a spin column and centrifuged for 1 min at 17 900 x g. After that, 750 µl of the PE buffer was added to the column, incubated for 1 min at room temperature (RT). The flow-through was removed by centrifuging for 1 min at 17 900 x g at RT; this step was repeated to remove ethanol residuals from PE buffer. The column was placed in a new tube, and the dsRNA was incubated in 25 µl of the elution buffer for 10 min at RT, after that, centrifuged at 17 900 x g. The purified dsRNA was examined by gel electrophoresis on a 1% agarose gel prepared with buffer 0.5 x TBE (50 mM Tris, 50 mM boric acid, 1 mM EDTA) and stained with 5 µg/ml ethidium bromide (Sigma Aldrich, United States). Electrophoresis was carried out for 30 min at 90 V (Bio-Rad Laboratories Inc, United States). The GeneRuler Express DNA Ladder was used as the DNA marker (Thermo Fisher Scientific, United States). The gel was examined under UV light using a ChemiDoc MP Imaging System (Bio-Rad Laboratories Inc, United States).

3.2.2 Primer ligation

In order to synthesize complete cDNA for all the eleven dsRNA genome segments of RV, an 'anchor, self-annealing primer', PC3-T7 loop (5'–pGGATCCCGGGAATTCGGTAATACGACTCACTATATTTTTATAGTGAGTCGTATTA–OH –

3') was ligated to the dsRNA as previously described (Potgieter et al. 2009). In short, the primer (Integrated DNA Technologies, United States) was ligated in 50 mM of HEPES, pH 8.0 (Sigma-Aldrich, United States), 18 mM $MgCl_2$ (Sigma-Aldrich, United States), 0.01% BSA (Thermo Fisher Scientific, United States), 3 mM DTT (Roche, Switzerland), 1 mM ATP (Thermo Fisher Scientific, United States), 10% DMSO (Thermo Fisher Scientific, United States), 20% PEG_{6000} (Thermo Fisher Scientific, United States), and 10 U T4 ligase (Thermo Scientific, United States) in a final reaction volume of 30 µl. The reaction was vortexed, spun down and incubated for 16 hours at 37°C. The ligation product was purified using the Minelute gel extraction kit (Qiagen, Germany). Briefly, the sample was added to 330 µl of the QG buffer, the sample/QG buffer mix was transferred to a column and centrifuged at 17 900 x g for 1 min. PE buffer (750 µl) was added, and the solution in the column incubated for 1 min at RT. The flow-through was removed by centrifuging at 17 900 x g for 1 min. This step was repeated to remove residual ethanol. The RNA incubated in 15 µl of the elution buffer for 10 min at RT, was then eluted by centrifugation at 17 900 x g for 3 min.

3.2.3 cDNA synthesis

The purified dsRNA was reverse transcribed by using the Maxima H Minus Double-Stranded cDNA kit (Thermo Fisher Scientific, United States), by following the manufacturer's instructions with minor modifications. Briefly, 13 µl of the ligated dsRNA was denatured at 95ºC for 5 minutes, followed by the addition of 1 µl random hexamer primers. The primers were allowed to anneal at 65ºC for 5 minutes in a thermal cycler (Bio-rad Laboratories Inc., United States). First-strand synthesis was performed by adding 5 µl of the first strand reaction mixture along with 1 µl of first-strand enzyme to the mixture. The first strand reaction mixture was incubated for 10 minutes at 25ºC, followed by 2 hours at 50ºC. The reaction was terminated at 85ºC for 5 minutes. Second strand synthesis was performed by adding 55 µl of nuclease-free water, 20 µl of the second strand mixture, and 5 µl of the second strand enzyme to the mixture. The solution was mixed gently, spun down and incubated for 1 hour at 16ºC. Thereafter, the reaction was terminated by adding 6 µl of EDTA (0.5 M, pH 8.0). Possible RNA contaminants were removed by adding 10 µl of RNase, followed by incubation at room temperature for 5 min. The cDNA was purified using the Invisorb® fragment clean-up kit (Stratec Molecular). Briefly, the sample was mixed with 500 µl of the binding buffer and added to the column, followed by centrifugation at 14 000 x g for 1 min. Thereafter 15 µl of the elution buffer was added to the column and incubated for 5 min, and then the column was centrifuged at 11 000 x g for 4 min. Purified cDNA was submitted for sequencing at the University of the Free State Next-Generation sequencing Unit (UFS-NGS), South Africa. To perform whole genome sequencing, an Illumina Miseq sequencer (Illumina, Inc, United States) was used.

Sequencing was performed using Miseq Reagent kit V2 (500 cycles) with 251 x 2 paired-end reads.

3.3 Data analysis

3.3.1 *De-novo* assembly and reference mapping

Data assembly and quality control were done to raw sequencing reads in CLC-Bio genomics workbench version 8.5.1 (Qiagen). The reads were trimmed and assembled into contigs using *de-novo* assembly. Resulting contigs with average coverage above 100 were identified with the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov). The contigs identified as rotavirus were used to identify reference strains for reference mapping. The trimmed reads were mapped against reference sequences retrieved from GenBank (reference sequence strain names provided in (Appendix A4). Rotavirus consensus sequences for each genome segment were derived from reference mapping and identified in BLAST and the Virus Pathogen Database Analysis Resource (ViPR) (Pickett et al. 2012).

3.3.2 RVA Phylogenetic analysis

Phylogenetic analysis was done in MEGA X (Kumar et al. 2018). Alignments of study sequences with open reading frames (ORFs) of at least 80% and reference strains (retrieved from GenBank) were done using Multiple Sequence Comparison by Log Expectation (MUSCLE) (Edgar 2004). Evolutionary model testing was implemented in MEGA X to determine the best models of nucleotide substitution for each genome segment. Maximum likelihood (ML) phylogenetic trees were inferred for each genome segment with 1000 bootstrap replicates using the best-fit model. Pairwise distance matrices were obtained for all the 11 genome segments in MEGA X, using the p-distance model. The resulting ML trees were visualized and edited using Inkscape (inkscape.org).

3.3.3 RVB Genotyping

The RVB genotypes of the study sequences were determined by comparing them to representative sequences for all RVB genotypes that have been determined so far for each segment (Shepherd et al. 2018). Sequences were aligned in MEGA X, and nucleotide distance matrices were computed for each segment using the p-distance model. The genotype with the highest nucleotide percentage identity to the study sequence was used to infer the genotype. Cut-off values were rounded off to one decimal place (Appendix A7).

3.3.4 RVC analysis

The consensus sequences for RVC were extracted from an average coverage of 35.2 and above. This was an exception made for RVC in this study since consensus sequences are

usually extracted from average coverage of 100 and above. The extracted consensus sequences were identified in BLASTn.

3.4 Results

3.4.1 dsRNA electrophoretic patterns

The total RNA extracted was treated with lithium chloride (LiCl) to enrich for dsRNA, and the resulting dsRNA was separated by 1% agarose gel by electrophoresis and stained with ethidium bromide (Figure 3.1 B). The smears expressed on the gel in Figure 3.1 B possibly represent ssRNA, including mRNA or degraded rRNA. The residual ssRNA appears as bright bands at the bottom of the gel, around 100 bp (Figure 3.1 A)**.** Precipitation with LiCl (Figure 3.1 B), as described previously (Potgieter et al. 2009), removed most of the ssRNA, although some traces of mRNA were visible as light smears (Figure 3.1 B).

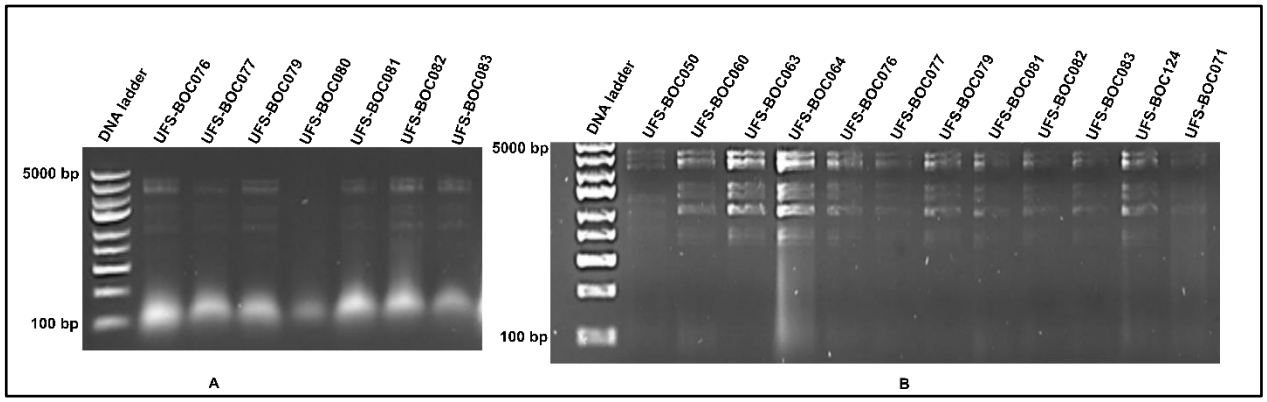


Figure 3.1 Agarose gel electrophoresis of RNA extracted from porcine faecal samples. A shows agarose gel with total RNA, and B shows agarose gel with LiCl treated RNA.

3.4.2 Genome assembly

Paired-end reads were successfully generated for 16 samples using the Miseq (Illumina, Inc) platform. The number of reads generated ranged from 19 248 to 689 830 (Appendix A4). Genome segments ranging from 78.2 to 100% of the ORFs were assembled. The generated sequences had an average coverage ranging from 35.2 to 34 416.9 (Appendix A4) Group A RV, group B RV, group C RV and picobirnavirus were identified among the *de-novo* contigs (Table 3.1). Co-infections with picobirnavirus were detected in 8 of 15 (53.33%) of the RVA positive samples (Table 3.1). In one sample, a co-infection of group A RV and group C RV was identified. In contrast, group B RV was only detected as a single-infection.

Table 3.1 Summary of porcine data collection and viruses identified

Collection date	Pen	Sample	Organism
10/01/2018	unknown	UFS-BOC001	RVA
24/12/2018	unknown	UFS-BOC009	RVA RVC Picobirnavirus
19/02/2019	unknown	UFS-BOC035	RVA Picobirnavirus
24/12/2019	19134	UFS-BOC050	RVB
		UFS-BOC060	RVA
		UFS-BOC063	RVA
		UFS-BOC064	RVA
		UFS-BOC071	RVA
20/02/2020	18202	UFS-BOC076	RVA Picobirnavirus
		UFS-BOC077	RVA Picobirnavirus
		UFS-B0C078	RVA Picobirnavirus
	18212	UFS-BOC079	RVA
	18119	UFS-BOC081	RVA Picobirnavirus
		UFS-BOC082	RVA Picobirnavirus
		UFS-BOC083	RVA
	18197	UFS-BOC124	RVA Picobirnavirus

3.4.3 RVA genome constellation

Genotype constellations were determined for all the RVA strains according to the guidelines provided by the Rotavirus Classification Working Group (RCWG) (Matthijnssens et al. 2011) (Table 3.2). In all 15 samples that were positive for RVA, 5/15 (33.38%) samples contained mixed infections, specifically for the genome segment 4 encoding VP4 protein. All the porcine RVA exhibited the Wa-like backbone (-**I5**-R1-C1-M1-**A8**-N1-**T7**-E1-H1) with a typical porcine genome segment 6 (I5), genome segment 5 (A8) and genome segment 7 (T7) (Table 3.2). A G5 genotype was determined for genome segment 9 encoding for VP7 protein. Three VP4 genotypes were identified (P[6], P[13], and P[23]). Two P[6]P[13] and three P[13]P[23] mixed infections were detected (Table 3.2).

Table 3.2 Genome constellation for South African porcine RVA strains

Collection date	Pen	Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5/6
10/01/2018	unknown	UFS-BOC001	G5	P[13]	I5	R1	C1	M1	A8	N1	T7	E1	H1
24/12/2018	unknown	UFS-BOC009	G5	P[6]P[13]	I5	R1	C1	M1	A8	N1	T7	E1	H1
19/02/2019	unknown	UFS-BOC035	G5	P[6]P[13]	I5	R1	C1	M1	A8	N1	T7	E1	H1
24/12/2019	19134	UFS-BOC060	G5	P[13]*P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC063	G5	P[13]*P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC064	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC071	G5	P[13]P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
20/02/2020	18202	UFS-BOC076	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC077	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-B0C078	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
	18212	UFS-BOC079	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
	18119	UFS-BOC081	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC082	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
		UFS-BOC083	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
	18197	UFS-BOC124	G5	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1

*average coverage below 100

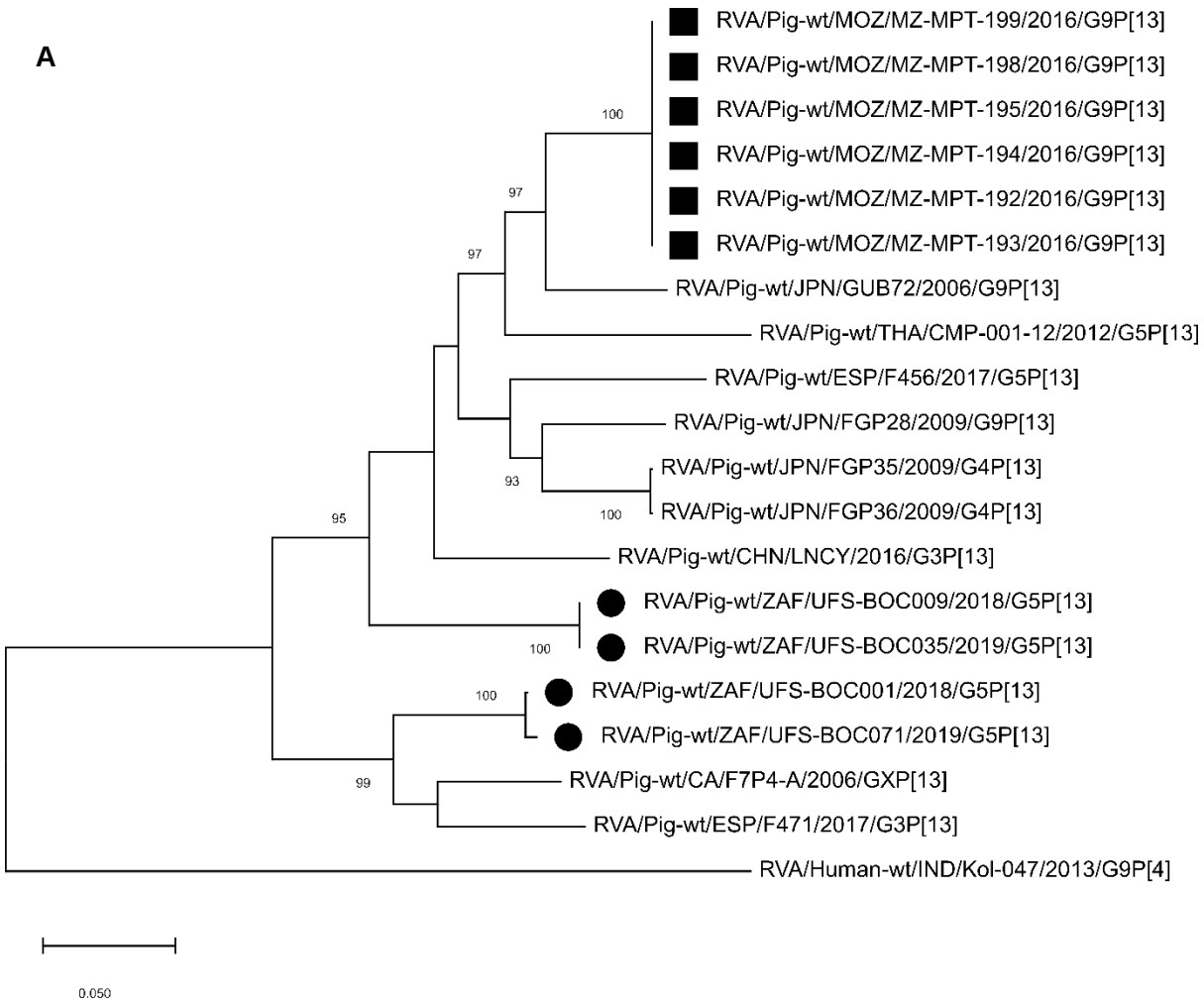
3.4.4 Phylogenetic analysis

In this study, the genetic relationships of the 11 genome segments of RVA were analysed. The four P[13] sequences detected showed diversity. Four because, the other two P[13] strains (US-BOC060 and UFS-BOC063) had ORFs below 80%, and were not included in phylogenetic analysis (Appendix A4). The nucleotide alignment of the P[13] strains; however, showed that the two strains were similar to UFS-BOC001 and UFS-BOC071 (Appendix A8), The UFS-BOC071 strain (RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23]) shared nucleotide sequence identity of 99.53% with the UFS-BOC001 strain (RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]) (Appendix A6). These two P[13] type study strains clustered separately from the UFS-BOC009 strain (RVA/Pig-wt/ZAF/UFS-BOC009/2019/G5P[6]P[13]) and UFS-BOC035 strain (RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]) (Figure 3.2 A), which shared nucleotide sequence identity of 83.16% and 83.55% to the UFS-BOC001 strain, respectively (Appendix A6**)**. Noteworthy, the P[13] type study strains UFS-BOC009 and UFS-BOC035 did not cluster close to any reference strain (Figure 3.2 A). In contrast, UFS-BOC001 and UFS-BOC071 clustered together with porcine strains from Canada (RVA/Pig-wt/CAN/F4P4-A/2006/GXP[13]) and Spain (RVA/Pig-wt/ESP/F471/2017/G3P[13]) (Figure 3.2

A). The Mozambican P[13] type strains clustered together and had a nucleotide sequence identity of 83.55% to UFS-BOC001.

Two P[6] type study strains were also phylogenetically analyzed, where the UFS-BOC035 strain (RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13) shared nucleotide sequence identity of 99.57% with the UFS-BOC009 strain (RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]) (Appendix A6), and clustered together during phylogenetic analysis (Figure 3.2 B). The P[6] study strains shared 94.47% to 94.95% nucleotide sequence identity with human G4P[6] type strains from China and Vietnam available on GenBank. The closest relative for the P[6] study strains was, however, a porcine strain from China (RVA/Pig-wt/CHN/Z84/2007/GXP[6], with a nucleotide percentage sequence identity of 95.5% (Figure 3.2 B, Appendix A6). The P[23] type study strains were identical with 100% nucleotide sequence identity among them (Appendix A6), supporting the phylogenetic clustering of these strains (Figure 3.2 C). The closest strain was a porcine South African strain (RVA/Pig-wt/ZAF/MRC-DPRU/1487/2007/G3G5P[23]) (Figure 3.2 C).

The G5 type study strains clustered together (Figure 3.2 D), with some variation, where nucleotide identities ranged from 91.15% to 99.68% (Appendix A6). The closest relatives, which clustered with G5 type strains, were South African strains with nucleotide identities ranging from 94.20 to 95.96% (Appendix A6). The VP2 study strains also clustered together and presented some variation (Figure 3.2 E), with percentage nucleotide sequence identities ranging from 99.29 to 99.51% (Appendix A6). In contrast to most of the study strains clustering together with South African porcine strains, VP2 clustered with a porcine strain from USA (RVA/Pig-wt/USA/LS00006_OSU/1975/G5P[X]) (Figure 3.2 E). The NSP5/6 study strains did not cluster close to any reference strain (Figure 3.2 F), but a human G5P[6] strain from Japan (RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]) had the highest shared nucleotide percentage identity of 98.32% with the UFS-BOC001 strain (Appendix A7). The rest of the genome segments encoding proteins VP6, VP1, VP3, NSP1, NSP2, NSP3, NSP4, formed similar clustering to the G5 (Appendix A6).


A
RVA/Pig-wt/MOZ/MZ-MPT-199/2016/G9P[13]
RVA/Pig-wt/MOZ/MZ-MPT-198/2016/G9P[13]
RVA/Pig-wt/MOZ/MZ-MPT-195/2016/G9P[13]
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RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13]
RVA/Pig-wt/ESP/F456/2017/G5P[13]
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97
93
100
95
100
100
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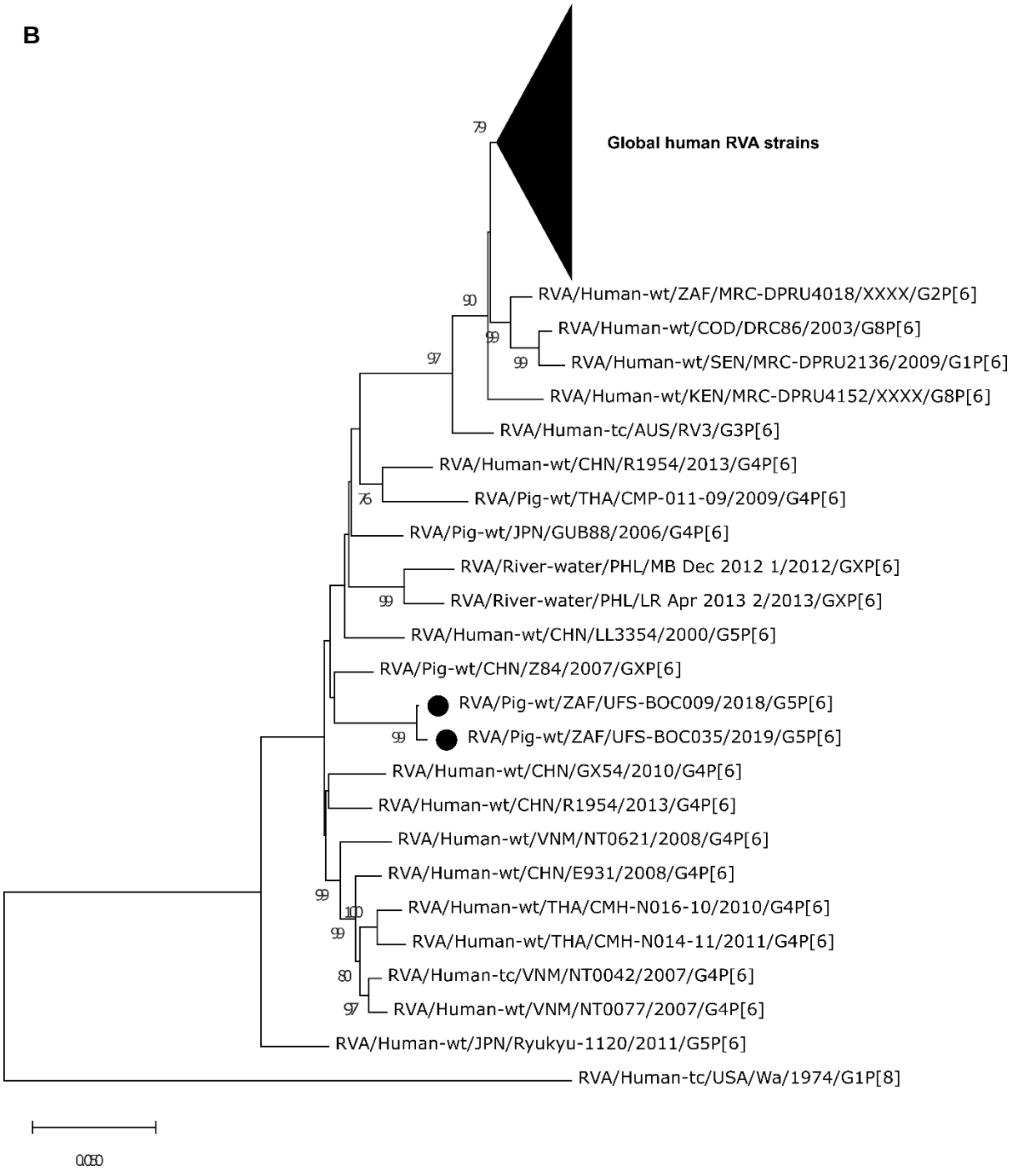
B
Global human RVA strains
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RVA/Human-wt/SEN/MRC-DPRU2136/2009/G1P[6]
RVA/Human-wt/KEN/MRC-DPRU4152/XXXX/G8P[6]
RVA/Human-tc/AUS/RV3/G3P[6]
RVA/Human-wt/CHN/R1954/2013/G4P[6]
RVA/Pig-wt/THA/CMP-011-09/2009/G4P[6]
RVA/Pig-wt/JPN/GUB88/2006/G4P[6]
RVA/River-water/PHL/MB Dec 2012 1/2012/GXP[6]
RVA/River-water/PHL/LR Apr 2013 2/2013/GXP[6]
RVA/Human-wt/CHN/LL3354/2000/G5P[6]
RVA/Pig-wt/CHN/Z84/2007/GXP[6]
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]
RVA/Human-wt/CHN/GX54/2010/G4P[6]
RVA/Human-wt/CHN/R1954/2013/G4P[6]
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RVA/Human-wt/CHN/E931/2008/G4P[6]
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RVA/Human-wt/VNM/NT0077/2007/G4P[6]
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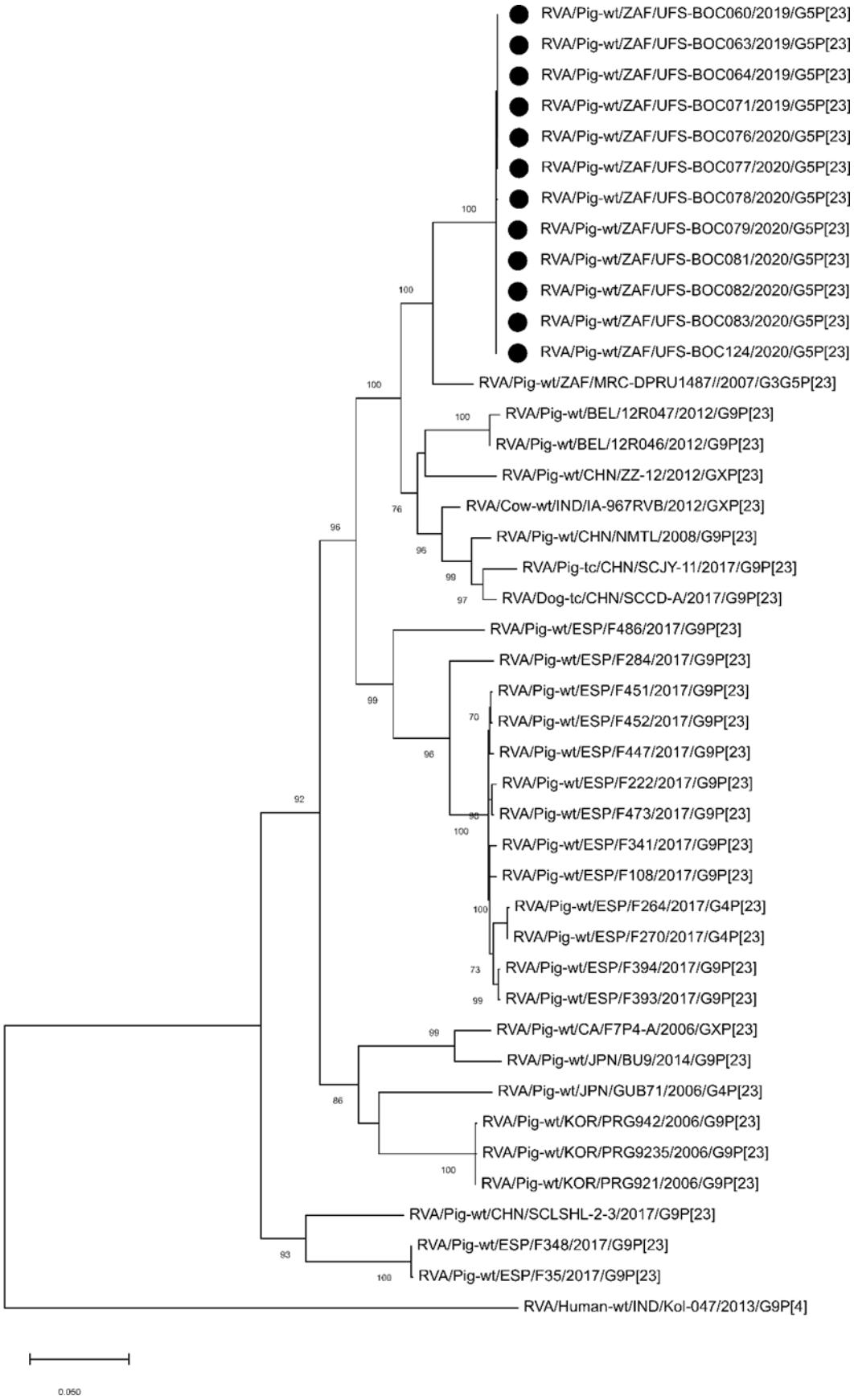
90
99
99
97
76
99
99
100
99
80
97

C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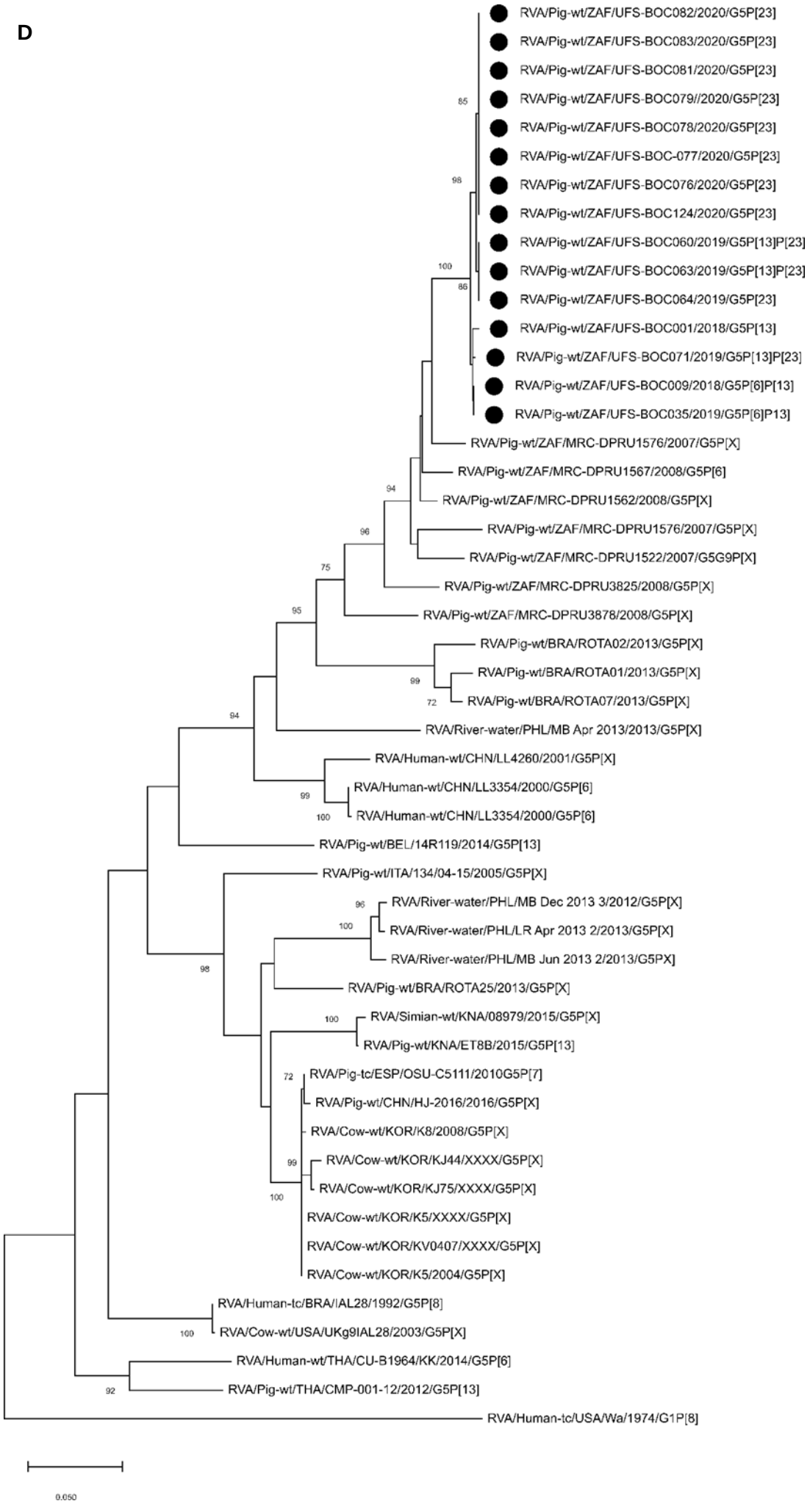
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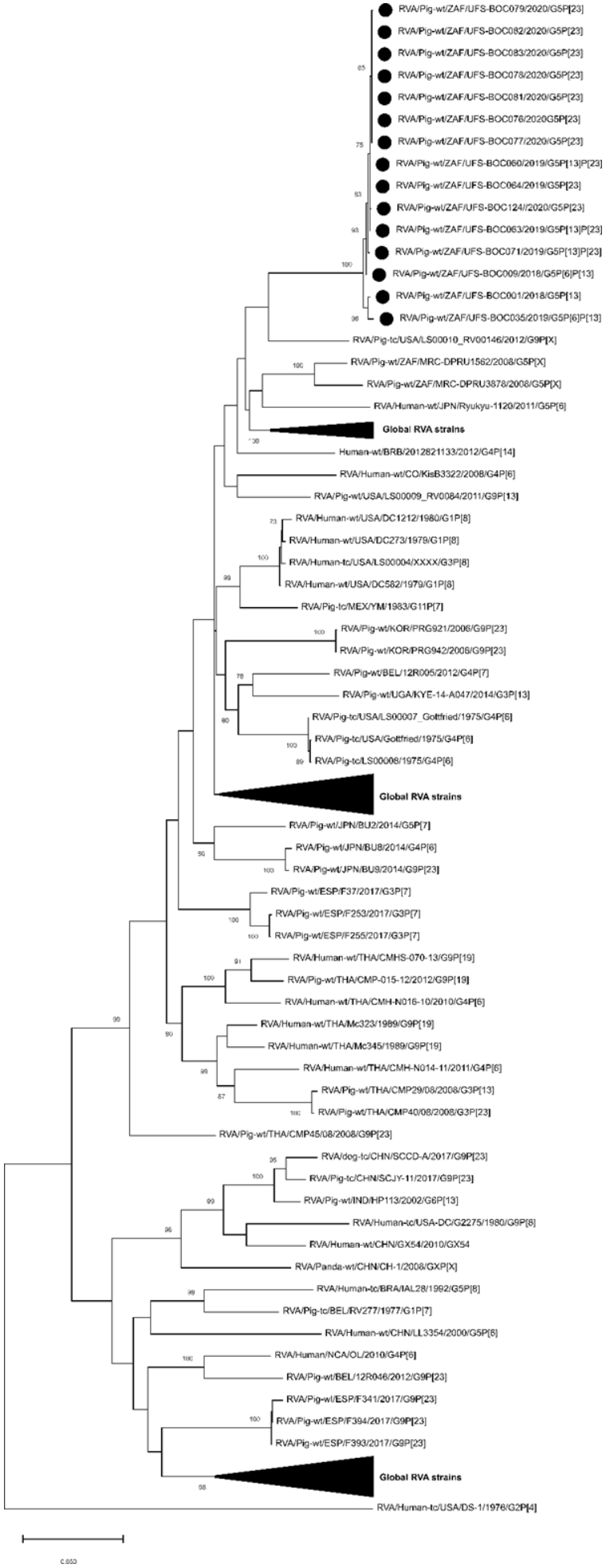
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RVA/Pig-tc/USA/Gottfried/1975/G4P[6]
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Global RVA strains
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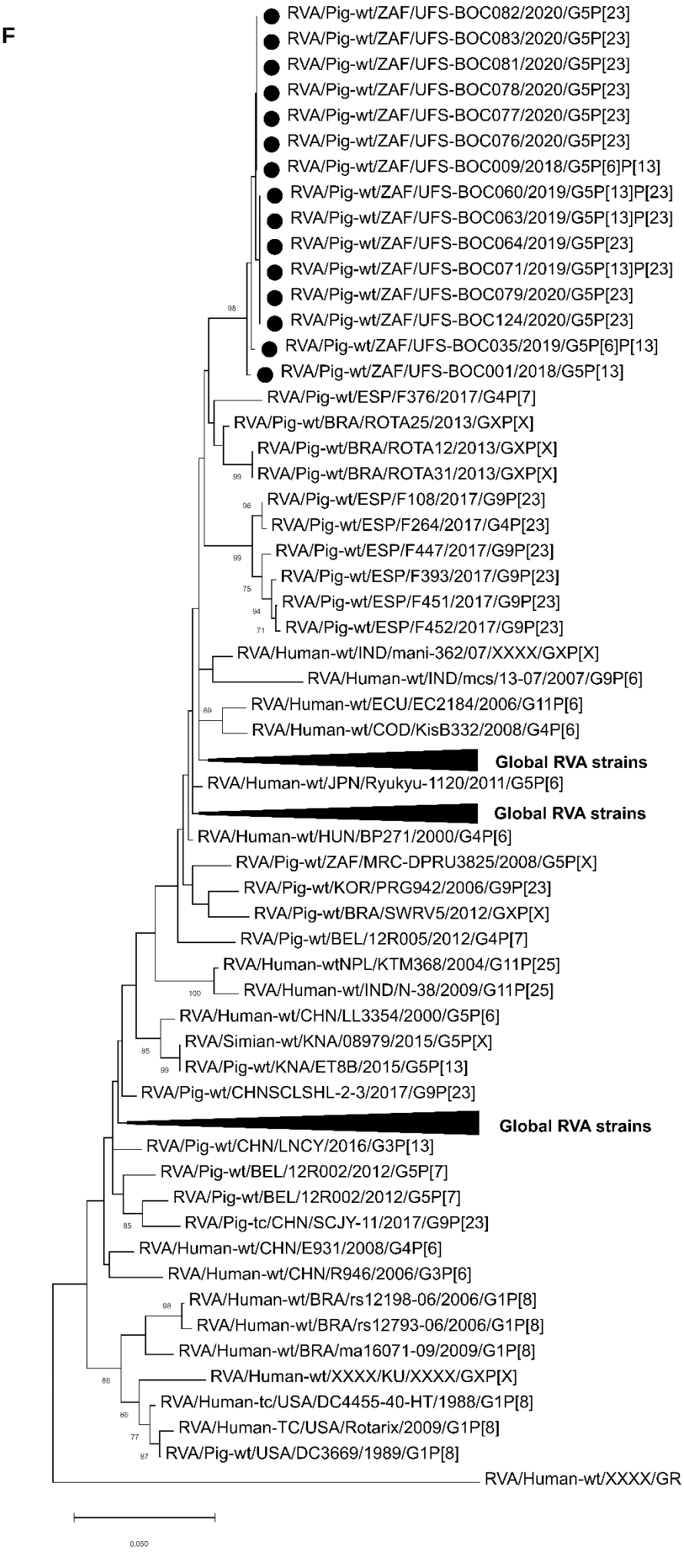
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Global RVA strains
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RVA/Pig-tc/CHN/SCJY-11/2017/G9P[23]
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Figure 3.2 Phylogenetic analysis of genome segments encoding P[13], P[6], P[23], G5, VP2 and NSP5/6 proteins of RVA. Historical evolution is inferred using Maximum Likelihood method General Time Reversible model (Nei and Kumar 2000) for VP4 encoding sequence for P[13] (A), P[23] (C), VP2 (E) and Tamura-3-parameter model (Tamura 1992) for VP4 encoding sequence for P[6] (B), G5 (D) and NSP5/6 (F). Bootstrap values above 70% are shown. Study strains are indicated with black-shaded circles, and for P[13] strains from Mozambique (Boene et al. 2021) strains are shown with black-shaded blocks.

3.4.5 RVB genome constellation

The percentage nucleotide identity values were determined and compared to the recently proposed cut-off values for the genotype classification of RVB (Shepherd et al. 2018). Distance matrices were generated for each segment (Appendix A7) using alignment files. The current study showed that genome segments 4, 1, 2, and 11 with nucleotide identities of 79%, 73%, 78% and 75% respectively, do not fall within the cut-off threshold values of 80%, 78%, 79% and 79%, respectively, as proposed by Shepherd and co-workers (Table 3.3). However, since these values were the highest percentage nucleotide identities identified, they were still used for genotyping the respective genome segments. It is important to note that the cut-off values for the current study were rounded off to one decimal place.

Table 3.3 Nucleotide identity cut-off values of RVB

Genome segment	Currently proposed cut off values %*	Current study percentage nucleotide identity values %
Segment 9	80	80
Segment 4	80	**79#**
Segment 6	81	84
Segment 1	78	**73**
Segment 2	79	**78**
Segment 3	77	83
Segment 5	76	77
Segment 8	83	84
Segment 7	78	78
Segment 10	76	77
Segment 11	79	**75**

*Based on cut-off values as described by Shepherd et al. 2018

#Nucleotide identities below the threshold values are indicated in red

The highest percentage nucleotide identities were obtained for each representative genotype (Shepherd et al. 2018) per genome segment. The strain having the highest percentage identity to our study strain was used to assign a genotype (Appendix A7). Genome constellation for RVB in this study was identified as G14-P[5]-I11-R4-C4-M4-A8-N10-T4-E4-H7 (Table 3.4).

Table 3.4 Genome constellation for RVB strain

Collection date	Pen	Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5/6
24/12/2019	19248	UFS-BOC050	G14	P[5]	I11	R4	C4	M4	A8	N10	T4	E4	H7

3.4.6 RVC strain identity

The average coverage for the group C RV strain was very low for each of the RV segments (Appendix A4). Different to the RVA and RVB detected sequences, consensus sequences were extracted from all segments with average coverage above 35.2. Almost all the genome segments had an average coverage below 100, except for genome segment 3, genome segment 4, and genome segment 7, which had average coverage just above 100 (Appendix A4). Genome segment 10 was the lowest with an average coverage of 35.2. Therefore, the genome segment was not further analysed. Partial genome sequences were extracted (Table 3.5) and analysed with BLASTn, where the BLASTn results indicated that all 10 genome segments for our study strain are closely related to porcine strains from the USA, China, Japan and Vietnam on the basis of percentage identity. The percentage identities ranged from 87.05 to 96.51% (Table 3.5).

Table 3.5 Rotavirus group C (RVC) BLASTn closest strains and their percentage identity

Genome segment	Length (bp)	Closest BLAST results	Percentage nt identity (%)
Segment 9	915/1075	RVC/Pig-wt/USA/MN-265/2015	88.55
Segment 4	2080/2204	RVC/Pig-wt/USA/MN29/2012/G9P[7]	87.16
Segment 6	1167/1352	RVC/Pig-wt/USA/1A46/2012/G6P[5]	92.54
Segment 1	3133/3276	RVC/Pig-wt/CHN/PoRVC_VP1_VIRES_NM02_C/2017	96.51
Segment 2	2359/2727	RVC/Pig-wt/JPN/CJ59-32/2003	89.68
Segment 3	1217/2100	RVC/Pig-wt/USA/OK47/2012/G6P[X]	87.45
Segment 5	1178/1246	RVC/Pig-wt/VNM/12130_S3	86.77
Segment 8	841/939	RVC/Pig-wt/USA/M036/2012/G5P[4]	93.21
Segment 7	1155/1209	RVC/Pig-wt/JPN/87-G2/2008	89.03
Segment 11	502/628	RVC/Pig-wt/VNM/14175_22	87.05

3.5 Discussion

In this study, we characterized the whole genome constellations of 16 RV strains detected in South African pigs, raised on a farm in George, Western Cape Province. Fifteen RVA strains were identified along with one RVC and one RVB strain. Group A rotavirus is one of the major viral agents detected in diarrheic piglets from 1 to 8 weeks of age (Saif et al. 1994). Rotavirus in this study was detected in piglets between 1 and 5 weeks of age, with a prevalence of 16.1%. (including the three RVA positive samples detected with RT-qPCR described in chapter 2). The incidence of RVA reported in this study (16.10%) was lower compared to 26.2% (n = 446) porcine RVA prevalence reported in East Africa (Amimo et al. 2013) but higher compared to 6.5% (n= 292) RVA prevalence reported in Ireland (Collins et al. 2010), 9.4% (n = 371) RVA prevalence reported in the United States (Amimo et al. 2013) and 11.8% (n = 288) RVA prevalence reported in Mozambique (Boene et al. 2021). When we evaluated the frequency with which the different groups were detected, RVA was detected at the highest frequency of 93.75% (15/16) and RVB and RVC at a frequency of 6.25% (1/16) each. This is expected because RVA in pigs is more common (Martella et al. 2007). RVC was, however, detected as a co-infection with RVA. Furthermore, the association of RV with enteric diseases in pigs is evident because RV infection was detected only in pigs which showed clinical signs of diarrhoea.

All the eleven genome segments of RVA were phylogenetically analysed. The P[13] type study strains were more diverse, with UFS-BOC009 strain and UFS-BOC035 strain having nucleotide sequence identities of 83.16% and 83.55%, respectively, with the UFS-BOC001 strain. This indicates that the two P[13] study strains are distinct. Since they clustered separately, it could be that they have not been detected anywhere else. This also suggests a possible reassortment of different P[13] strains. This finding is similar to the porcine P[13] strains detected in east Africa, where they shared 87.6% nucleotide identity with each other (Amimo et al. 2014). Another similar observation was reported in the USA, where the P[13] strains detected in young pigs were found to be more diverse compared to other P-types (Amimo et al. 2013). According to these records, P[13] strains appear to be diverse, and commonly detected in pigs (Ghosh et al. 2007). All the P[13] study strains were expected to cluster with Mozambican P[13] porcine strains due to geographical proximity, but they were closely related to the Canadian and Spanish strains instead.

Regarding the P[6] genotype detected, there is a possibility that it is of human-origin because the phylogenetic relationship between porcine and human strains has been documented, where the P[6] human and porcine strains clustered together (Nyaga et al. 2018). The P[6] strains, however, are known to be endemic to Africa and a common P-type in porcine (Heylen

et al. 2016). Despite that P[6] type study strains clustered with a porcine strain, further analysis using multiple sequence alignment between the study strains and known sequences in GenBank showed that the P[6] study strains were also closely related with human P[6] type strains. This finding may represent previous zoonotic events. In a study done by Amimo and co-workers in East Africa, porcine P[6] study strain was found to be closely related to a human P[6] strain from Congo (Amimo et al. 2013). The genome segment 11 sequences were not clustering to any reference strain but had the human strain from Japan with the highest nucleotide identity to UFS-BOC001. This finding suggests a possible reassortment of genome segment 11 between human and porcine strain. The genome segment 2 encoding VP2 protein, were different as these sequences clustered with a strain from the USA, and not the South Africa strains. All the other study sequences were similar and formed close relatives with South African porcine strains. This result makes sense since the RV porcine strains from this study were isolated from South Africa.

The RVB strain was detected in only one sample (0.84%). A study in the United States reported a high group B occurrence of 46.8% in pigs (n = 173) (Marthaler et al. 2012). Moreover, RVB detected in this study was identified as porcine RVB. All of the genome study sequences were closely related to the USA porcine strain besides genome segment 6 which was closely related to the porcine Japan strain (RVB/Pig-wt/JPN/PB-S24-11/GXP[X]). A G14 genotype assigned to the VP7 RVB is common in porcine, as it was also reported in two porcine samples in Italy, one 3 weeks old and asymptomatic, the other 14 days old with unknown clinical status (Marthaler et al. 2014). Porcine RVC in our study was detected as a mixed infection with RVA. A study in Italy also indicated that porcine RVC occurs most frequently as a mixed infection with other RV groups. This study reported 11% (n = 118) prevalence of RVC/RVA mixed infection (Martella et al. 2007), which differs from the 6.25% reported in this study. Results of the BLASTn search analysis showed that our porcine study strains are closely related to porcine strains from the USA, Vietnam and Japan. Porcine strains reported in South Africa are limited. The last study to report RVB and RVC in porcine was by Geyer and co-workers (Geyer et al. 1996). Therefore, more data on the occurrence of RVC in porcine is needed to understand the pathogenicity and epidemiology of this group, similar to RVB.

In addition to RV detected in this study, picobirnaviruses (PBVs) were detected as a co-infection with RVA. The PBVs are classified under the family *Picobirnaviridae*. Their genome is small in size, non-enveloped, and they have a bi-segmented dsRNA genome (Duquerroy et al. 2009). The genome size ranges from 2200 to 2700 bp for the large genome segment which encodes for a capsid protein, and 1200 to 1900 bp for small genome segments which encodes for the viral RNA-dependent RNA polymerase (RdRp) (Pereira et al. 1988). The PBV

segments could have migrated simultaneously with RV segments which are similar in size as PBVs, explaining why it was not visible on the agarose gels (Figure 3.1). PAGE is also a more sensitive electropherotyping method which could have resulted in differentiation between the RV and PBV bands. However, AGE was utilized in this study. It makes sense to see PBVs in the *de-novo* analysis because, when the samples were prepared for Next Generation Sequencing, the samples were enriched for dsRNA and eliminated single-stranded RNA (ssRNA) by LiCl treatment. This is not the first study to report PBVs in pigs. PBVs were also reported in Argentina and further said to be associated with PBVs detected in humans (Giordano et al. 2011, Martínez et al. 2010). In this study, however, the relatedness of the PBVs to human PBVs was not investigated.

3.6 Conclusion

Stool samples were collected from diarrheic and asymptomatic pigs. In this study, 15 RVA strains with a characteristic Wa-like genome constellation and one RVB genome constellation were determined from diarrheic piglets (≤ 31 days old) showing clinical signs of diarrhoea. Evidence for the presence of RVC was also obtained. Different RV groups were found to be circulating on the farm. However, RVA was recorded as the most prevalent RV group. Furthermore, mixed infections (P[6]P[13] and P[13]P[23]) and co-infections (RVA/RVC and RVA/picobirnavirus) were also detected. Overall, this study adds to the knowledge and data of porcine RVA genotypes in Africa and reveal the occurrence of porcine RVB in South Africa. Most importantly, for the RV strains evaluated, no strains with zoonotic potential were detected during phylogenetic analysis, although the P[6] genotype detected could possibly be of human origin. The non-group A RV genetic data will further contribute to establish whole genome classification systems for non-group A rotaviruses.

3.7 References

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Chapter 4: Concluding remarks

In this study, domesticated animal (canine and porcine) stool samples were analysed to detect human enteric viruses and determine the zoonotic potential thereof. Electropherotyping and RT-qPCR were used to detect the viral agents. Electropherotyping is a cheap method and readily available in most molecular laboratories (Herring et al. 1982). Reverse transcriptase (RT) qPCR was used because it allowed the simultaneous detection of human viral pathogens (RV, NoV, SaV. AstV, and AdV) (multiplex RT-qPCR). Moreover, RT-qPCR is widely used for its rapid turn out, sensitivity and specificity (Higgins et al. 2020).

Rotavirus was detected in porcine samples by electropherotyping, whereas no rotavirus was detected in the canine samples. The canine samples (n = 104) were further evaluated for the presence of human enteric viruses with RT-qPCR. In every run for the RT-qPCR for canine samples, positive controls were exponentially amplified, indicating that the run was a success. Negative controls were also included, and all of the negative controls were not amplified, validating that samples were not contaminated. An RNA extraction control (IC, brome mosaic virus) was also included. In RT-qPCR assays, all internal controls were successfully amplified, indicating successful RNA extraction and absence of PCR inhibitors. However, no human enteric virus was detected in any of the 104 canine samples.

Selected porcine samples (n = 31) were also evaluated with RT-qPCR. As expected, the samples that were positive with electropherotyping were also positive for rotavirus with RT-qPCR. Three of the porcine samples which did not show any RV profiles with electropherotyping, were exponentially amplified and had cq values below 33, thus reported positive for RV. This confirms that RT-qPCR is a more sensitive method compared to electropherotyping, especially when the viral load in the sample analysed is low (Higgins et al. 2020).

In total, 19 porcine samples were positive for RV, but only 16 samples were selected for whole genome sequencing because yield and quality of the dsRNA was sufficient as judged by agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was coupled with ligation of a self-annealing anchor primer to the dsRNA (PC3-T7loop, Potgieter et al. 2009) in order to obtain full-length genome sequence data. Next generation sequencing was performed on an Illumina Miseq platform.

In our study, a total of 16 RV species from porcine were successfully sequenced. *De-novo* assembly was used to identify viruses in the sample by performing BLASTn analysis of the resulting contigs. By utilizing this method, our samples contained RVA, RVB and RVC as well as picobirnavirus. Contigs, identified as RV, were used to identify reference strains for reference mapping. Consensus sequences were extracted from reference mapping for RV. Fifteen RVA strains had G5 genotypes for VP7 and typical porcine backbones (-I5-R1-C1-M1-

A8-N1-T7-E1-H1). One sample contained a single infection of P[13] genotype, two contained a mixed infection of P[6]P[13], three contained a mixed infection of P[13]P[23], and nine contained a single infection of P[23] genotype. The detected group RVs were phylogenetically analysed to investigate possible zoonosis. Most of the study strains clustered together, with close relatives from South African porcine strains. Two distinct P[13] sequences were identified. UFS-BOC009 and UFS-BOC035 strains did not cluster close to any reference strains, indicating that they are distinct and have possibly never been detected anywhere else. UFS-BOC001 and UFS-BOC071 clustered with reference strains from Canada and Spain. The P[6] strains were suspected to be of human origin, since P[6] genotype has a history of being a human genotype, especially in Africa (Seheri et al. 2014, Steel and Ivanoff 2003). The genome segments encoding VP2 and NSP5/6 were also different as these sequences did not cluster close to any reference strains. With genome segment 11 encoding NSP5/6, a human strain from Japan was found to be a close relative with nucleotide percentage identity of 98.32%.

RVB genotypes were based on determining nucleotide identities between study sequences and representative genotypes obtained from a study by Shepherd and co-workers. The RVB genotypes were all associated with porcine RV. Due to low coverages obtained for RVC, only a BLASTn search analysis was performed, to identify close relatives. These were also found to be porcine RVC strains. Picobirnavirus was not further analysed to infer phylogenetic relations, and therefore the zoonotic association is not known.

In this study, RV was detected in piglets between 1 to 5 weeks that were suffering from diarrhoea, with RVA most frequently detected. We can, therefore, conclude that the diarrhoea in pigs was associated with the RV detected. The dogs investigated were not infected with the human enteric viruses investigated in this study. It does not eliminate the possibility of infection with other enteric viruses, especially those associated with pets, like coronavirus and parvovirus or other pathogens, including bacteria and parasites.

Regarding the zoonotic potential of the identified RVs, it is not clear whether there is a possibility or not, as was seen with the genome segment 11 and P[6] strain which is commonly found in humans. No direct transmission of human strains in animals were detected, but, due to the ability of the rotavirus genome to reassort, the presence of the P[6] genotype as well as the close relatives of human origin for genome segment 11, could possibly indicate previous zoonotic events. Therefore, although a definite zoonosis was not identified, it is also not possible to conclude that no zoonosis was present. A whole genome genotyping system and tools are needed for non-group A RV, as the number of porcine non-group A RV studies that could be used to compare with our study, were low. For RVC, the closest strains identified were from countries such as the USA, Japan, China and Vietnam. This was also observed

with RVB, where the study strains were associated with strains from the USA and less frequently with Japanese RVB porcine strains.

Zoonotic infection is a multifactorial problem, which can affect the whole world. This is evident with the current SARS-CoV-2 pandemic. This virus is assumed to have originated from bats (Boni et al. 2020), and is now affecting millions and millions of people across the world. This calls for more epidemiological studies done on animals to better understand interspecies transmission, zoonosis and pathogenicity better.

4.1 References

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Appendix A

A1: Ethical approval

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Animal Research Ethics

26-Apr-2018

Dear Dr Hester O'Neill

Student Project Number: UFS-AED2018/0030

Project Title: Investigation of enteric viruses obtained from domesticated animals in the Bloemfontein region

Department: Microbial Biochemical and Food Biotechnology (Bloemfontein Campus)

You are hereby kindly informed that, at the meeting held on 26-Apr-2018, the Interfaculty Animal Ethics Committee approved the above project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.

2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.

3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Yours Sincerely

Mr. Gerhard Johannes van Zyl
Chair: Animal Research Ethics Committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Animal Research Ethics

21-Jun-2019

Dear Dr Hester O'Neill

Student Project Number: UFS-AED2018/0030

Project Title: Investigation of enteric viruses obtained from domesticated animals in the Bloemfontein region

Department: Microbial Biochemical and Food Biotechnology Department (Bloemfontein Campus)

You are hereby kindly informed that, at the meeting held on 26-Apr-2018, the Interfaculty Animal Ethics Committee approved the amendment for the above mentioned project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.

2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.

3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Note: Amendment Approved

The PI must confirm if a Section 20 permit is required for the transport of the samples.

The PI must enquire If any biosafety issues exist that requires Ethical application from the Biosafety and Environment ethics committee.

Mr. Gerhard Johannes van Zyl
Chair: Animal Research Ethics Committee

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/4

Prof O'Neill
Department of Microbial, Biochemical and Food Biotechnology
University of Free State
Bloemfontein
Email: oneillg@ufs.ac.za

Dear Prof O'Neill,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application sent per email on 9 June 2019, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form dated 05/2019 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
3. All potentially infectious material utilised, collected or generated during the study are to be destroyed at the completion of the study. Records must be kept for five years for auditing purposes;

4. The bovine and swine faecal matter may only be collected in Western Cape province, for which a state veterinary letter has been provided;
5. All samples must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and the National Road Traffic Act, 1996 (Act No. 93 of 1996);
6. Only extracted RNA samples from the rotaviruses obtained may be stored at the Molecular Virology laboratory of the Department of Microbial, Biochemical and Food Biotechnology, University of Free State;
7. Any further use or distribution of samples collected for this study is subject to obtaining a separate Section 20 approval;
8. All waste must be disposed of as biohazardous waste by a registered waste contractor;
9. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval.

Title of research/study: Whole genome consensus sequence determination of bovine and swine rotavirus strains originating from the Western Cape, South Africa

Researcher: Prof Hester Gertruida O'Neill

Institution: Department of Microbial, Biochemical and Food Biotechnology, University of Free State

Our ref Number: 12/11/1/4

Your ref: UFS-AED2018/0030

Expiry date: 2020/12

Kind regards,

Maja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2019 -07- 01

A2. Sample data collection for canine

Sample Identifier	Date of collection	Clinical Status	Geographical region
UFS-BOC002			South
UFS-BOC010	08/08/2018		South
UFS-BOC011	19/05/2019		South
UFS-BOC012	15/05/2018		South
UFS-BOC013			South
UFS-BOC017	18/08/2018		South
UFS-BOC018	13/08/2018		South
UFS-BOC019	17/09/2018		South
UFS-BOC020	18/08/2018		South
UFS-BOC041	13/08/2018	Asymptomatic	North
UFS-BOC042	25/10/2018	Asymptomatic	North
UFS-BOC043	26/10/2018	Asymptomatic	North
UFS-BOC044	06/06/2018	Asymptomatic	North
UFS-BOC045	20/08/2018		North
UFS-BOC046	11/06/2018	Asymptomatic	North
UFS-BOC047	12/06/2018		North
UFS-BOC048	09/08/2018	Asymptomatic	North
UFS-BOC049	30/10/2018	Asymptomatic	North
UFS-BOC050			North
UFS-BOC051	17/10/2018	Asymptomatic	North
UFS-BOC052	18/07/2018	Asymptomatic	North
UFS-BOC053			North
UFS-BOC054	04/06/2018	Asymptomatic	North
UFS-BOC055	17/10/2018	Asymptomatic	North
UFS-BOC056	17/10/2018		North
UFS-BOC057			North
UFS-BOC059			North
UFS-BOC060			North
UFS-BOC081	19/11/2018		West
UFS-BOC082	20/11/2018		West
UFS-BOC083	20/11/2018		West
UFS-BOC084	06/11/2018		West
UFS-BOC085	18/11/2018		West

A2. Sample data collection for canine (continued…)

UFS-BOC086	29/10/2018		West
UFS-BOC087	06/11/2018		West
UFS-BOC101	02/11/2018	Asymptomatic	West
UFS-BOC103	05/11/2018	Asymptomatic	West
UFS-BOC104	17/10/2018	Asymptomatic	West
UFS-BOC105	06/11/2018	Asymptomatic	West
UFS-BOC106	08/11/2018	Asymptomatic	West
UFS-BOC108	02/11/2018	Asymptomatic	West
UFS-BOC109	17/10/2018	Asymptomatic	West
UFS-BOC110	17/10/2018	Asymptomatic	West
UFS-BOC111	01/11/2018		West
UFS-BOC112	30/10/2018	Asymptomatic	West
UFS-BOC113	20/11/2018	Asymptomatic	West
UFS-BOC114	01/11/2018	Asymptomatic	West
UFS-BOC116	30/10/2018	Asymptomatic	West
UFS-BOC117	01/11/2018	Asymptomatic	West
UFS-BOC118	07/11/2018	Asymptomatic	West
UFS-BOC119	08/11/2018	Asymptomatic	West
UFS-BOC120	08/11/2018	Asymptomatic	West
UFS-BOC161	16/05/2018	Asymptomatic	East
UFS-BOC162	16/05/2018	Asymptomatic	East
UFS-BOC163	16/05/2018	Asymptomatic	East
UFS-BOC164	18/05/2018		East
UFS-BOC165	16/05/2018		East
UFS-BOC166	16/05/2018		East
UFS-BOC167	06/05/2018		East
UFS-BOC168	16/05/2018		East
UFS-BOC169	16/05/2018		East
UFS-BOC170	16/05/2018		East
UFS-BOC171	16/05/2018		East
UFS-BOC172	16/05/2018		East
UFS-BOC173	16/05/2018		East
UFS-BOC174	18/05/2018		East
UFS-BOC175	16/05/2018		East

A2. Sample data collection for canine (continued…)

UFS-BOC176	16/05/2018		East
UFS-BOC177	16/05/2018		East
UFS-BOC178	16/05/2018		East
UFS-BOC179	16/05/2018		East
UFS-BOC180	16/05/2018		East
UFS-BOC181	16/05/2018		East
UFS-BOC182	16/05/2018		East
UFS-BOC183	16/05/2018		East
UFS-BOC184	16/05/2018		East
UFS-BOC185	16/05/2018		East
UFS-BOC186	16/05/2018		East
UFS-BOC187	16/05/2018		East
UFS-BOC188	16/05/2018		East
UFS-BOC189	16/05/2018		East
UFS-BOC190	16/05/2018		East
UFS-BOC191	16/05/2018		East
UFS-BOC198	16/05/2018		East
UFS-BOC199	16/05/2018		East
UFS-BOC200	16/05/2018		East
UFS-BOC201	16/05/2018		East
UFS-BOC202	16/05/2018		East
UFS-BOC204	16/05/2018		East
UFS-BOC205	16/05/2018		East
UFS-BOC206	16/05/2018		East
UFS-BOC268	12/11/2018	Symptomatic	West
UFS-BOC269	09/11/2018	Symptomatic	West
UFS-BOC270	15/11/2018	Asymptomatic	West
UFS-BOC271	13/11/2018	Asymptomatic	West
UFS-BOC272	09/11/2018	Asymptomatic	West
UFS-BOC273	12/11/2018	Asymptomatic	West
UFS-BOC274	16/11/2018	Asymptomatic	West
UFS-BOC275	15/11/2018	Asymptomatic	West
UFS-BOC276	16/04/2018	Asymptomatic	West
UFS-BOC277	16/11/2018	Asymptomatic	West

A2. Sample data collection for canine (continued…)

UFS-BOC278	13/11/2018	Asymptomatic	West
UFS-BOC279	15/11/2018	Asymptomatic	West

A3. Sampling data collection for porcine

Sample identifier	Date of collection	Host	Clinical status	Birth-date	Age (days)	Pen ID
UFS-BOC001	10/01/2018	Piglet	Symptomatic	12/12/2017	28	NR
UFS-BOC002	24/12/2018	Piglet	Symptomatic	12/12/2018	12	NR
UFS-BOC003	24/12/2018	Piglet	Asymptomatic	12/12/2018	12	NR
UFS-BOC004	24/12/2018	Piglet	Symptomatic	12/12/2018	12	NR
UFS-BOC005	24/12/2018	Piglet	Symptomatic			NR
UFS-BOC006	24/12/2018	Piglet	Symptomatic			NR
UFS-BOC007	24/12/2018	Piglet	Symptomatic	05/12/2018	19	NR
UFS-BOC008	24/12/2018	Piglet	Symptomatic	10/12/2018	14	NR
UFS-BOC009	24/12/2018	Piglet	Symptomatic	24/11/2018	30	NR
UFS-BOC010	24/12/2018	Piglet	Symptomatic	03/12/2018	21	NR
UFS-BOC011	24/12/2018	Piglet	Symptomatic			NR
UFS-BOC012	24/12/2018	Piglet	Symptomatic			NR
UFS-BOC013	19/02/2019	Piglet	Symptomatic			NR
UFS-BOC014	19/02/2019	Piglet	Symptomatic	12/12/2018	38	NR
UFS-BOC015	19/02/2019	Sow	Asymptomatic			NR
UFS-BOC016	19/02/2019	Piglet	Asymptomatic			NR
UFS-BOC017	19/02/2019	Piglet	Asymptomatic			NR
UFS-BOC018	19/02/2019	Piglet	Symptomatic	29/01/2019	21	NR
UFS-BOC019	19/02/2019	Piglet	Asymptomatic	24/01/2019	26	NR
UFS-BOC020	19/02/2019	Piglet	Asymptomatic	15/02/2019	04	NR
UFS-BOC021	19/02/2019	Piglet	Asymptomatic	31/01/2019	19	NR
UFS-BOC022	19/02/2019	Piglet	Symptomatic	24/01/2019	26	NR
UFS-BOC023	19/02/2019	Piglet	Symptomatic	24/01/2019	26	NR
UFS-BOC024	19/02/2019	Piglet	Asymptomatic			NR
UFS-BOC025	19/02/2019	Piglet	Asymptomatic	24/01/2019	26	NR
UFS-BOC026	19/02/2019	Water				NR
UFS-BOC027	19/02/2019	Piglet	Symptomatic	02/02/2019	17	NR
UFS-BOC028	19/02/2019	Piglet	Asymptomatic	03/02/2019	16	NR

*NR: not recorded

A3. Sample data collection for porcine (continued)

UFS-BOC029	19/02/2019	Piglet	Asymptomatic	26/01/2019	24	NR
UFS-BOC030	19/02/2019	Piglet	Asymptomatic	08/01/2019	42	NR
UFS-BOC031	19/02/2019	Sow	Asymptomatic			NR
UFS-BOC032	19/02/2019	Piglet	Symptomatic			NR
UFS-BOC033	19/02/2019	Piglet	Symptomatic	31/01/2019	19	NR
UFS-BOC034	19/02/2019	Piglet	Asymptomatic	30/01/2019	20	NR
UFS-BOC035	19/02/2019	Piglet	Symptomatic	20/01/2019	30	NR
UFS-BOC036	19/02/2019	Piglet	Symptomatic	23/01/2019	27	NR
UFS-BOC037	19/02/2019	Piglet	Asymptomatic	26/01/2019	24	NR
UFS-BOC042	24/12/2019	Piglet	Asymptomatic	11/12/2019	13	19250
UFS-BOC043	24/12/2019	Piglet	Asymptomatic	11/12/2019	13	19250
UFS-BOC044	24/12/2019	Piglet	Asymptomatic	19/12/2019	05	19322
UFS-BOC045	24/12/2019	Piglet	Asymptomatic	19/12/2019	05	19134
UFS-BOC046	24/12/2019	Piglet	Symptomatic	11/12/2019	13	19250
UFS-BOC047	24/12/2019	Sow	Asymptomatic			19111
UFS-BOC048	24/12/2019	Sow	Asymptomatic			19134
UFS-BOC049	24/12/2019	Sow	Asymptomatic			19322
UFS-BOC050	24/12/2019	Piglet	Symptomatic	04/12/2019	20	19428
UFS-BOC051	24/12/2019	Piglet	Asymptomatic	30/11/2019	24	19102
UFS-BOC052	24/12/2019	Piglet	Asymptomatic	11/12/2019	13	17348
UFS-BOC053	24/12/2019	Piglet	Asymptomatic	19/12/2019	05	19134
UFS-BOC054	24/12/2019	Piglet	Asymptomatic	23/12/2019	01	19111
UFS-BOC055	24/12/2019	Piglet	Asymptomatic	04/12/2019	20	18312
UFS-BOC056	24/12/2019	Piglet	Asymptomatic	04/12/2019	20	19248
UFS-BOC057	24/12/2019	Piglet	Symptomatic	30/11/2019	24	19102
UFS-BOC058	24/12/2019	Piglet	Symptomatic	30/11/2019	24	19102
UFS-BOC059	24/12/2019	Sow	Asymptomatic			19134
UFS-BOC060	24/12/2019	Piglet	Symptomatic	19/12/2019	05	19134
UFS-BOC061	24/12/2019	Piglet	Symptomatic	12/12/2019	12	19141
UFS-BOC062	24/12/2019	Sow	Asymptomatic			19141
UFS-BOC063	24/12/2019	Piglet	Symptomatic	19/12/2019	05	19134
UFS-BOC064	24/12/2019	Piglet	Symptomatic	19/12/2019	05	19134
UFS-BOC065	24/12/2019	Piglet	Asymptomatic	04/12/2019	20	19248
UFS-BOC066	24/12/2019	Piglet	Asymptomatic	11/12/2019	13	19250

*NR: not recorded

A3. Sample data collection for porcine (continued)

UFS-BOC067	24/12/2019	Piglet	Symptomatic	12/12/2019	12	19141
UFS-BOC068	24/12/2019	Piglet	Asymptomatic	14/12/2019	10	18312
UFS-BOC069	24/12/2019	Piglet	Asymptomatic	11/12/2019	13	19250
UFS-BOC070	24/12/2019	Piglet	Symptomatic	11/12/2019	13	19250
UFS-BOC071	24/12/2019	Piglet	Symptomatic	19/12/2019	05	19134
UFS-BOC072	24/12/2019	Sow	Asymptomatic	12/12/2019	12	19121
UFS-BOC073	24/12/2019	Piglet	Asymptomatic	05/12/2019	19	19241
UFS-BOC074	24/12/2019	Sow	Asymptomatic			18312
UFS-BOC075		Water				
UFS-BOC076	20/02/2020	Piglet	Symptomatic	23/01/2020	28	18202
UFS-BOC078	20/02/2020	Piglet	Symptomatic	23/01/2020	28	18202
UFS-BOC079	20/02/2020	Piglet	Symptomatic	23/01/2020	28	18212
UFS-BOC080	20/02/2020	Piglet	Asymptomatic	23/01/2020	28	18212
UFS-BOC081	20/02/2020	Piglet	Symptomatic	24/01/2020	27	18119
UFS-BOC082	20/02/2020	Piglet	Symptomatic	24/01/2020	27	18119
UFS-BOC083	20/02/2020	Piglet	Symptomatic	24/01/2020	27	18119
UFS-BOC084	20/02/2020	Piglet	Symptomatic	01/02/2020	19	19278
UFS-BOC085	20/02/2020	Piglet	Symptomatic	01/02/2020	19	19278
UFS-BOC086	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18135
UFS-BOC087	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18377
UFS-BOC088	20/02/2020	Piglet	Symptomatic	05/02/2020	15	18381
UFS-BOC089	20/02/2020	Piglet	Symptomatic	05/02/2020	15	18377
UFS-BOC090	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18377
UFS-BOC091	20/02/2020	Piglet	Symptomatic	05/02/2020	15	19188
UFS-BOC092	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18377
UFS-BOC093	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18135
UFS-BOC094	20/02/2020	Piglet	Symptomatic	05/02/2020	15	18381
UFS-BOC095	20/02/2020	Sow	Asymptomatic			18377
UFS-BOC096	20/02/2020	Piglet	Symptomatic	05/02/2020	15	18135
UFS-BOC097	20/02/2020	Piglet	Asymptomatic	03/02/2020	15	18135
UFS-BOC098	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	16175
UFS-BOC099	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	19188
UFS-BOC100	20/02/2020	Piglet	Asymptomatic	05/02/2020	15	18381
UFS-BOC101	20/02/2020	Piglet	Symptomatic	05/02/2020	15	18381

A3. Sample data collection for porcine (continued)

UFS-BOC102	20/02/2020	Piglet	Symptomatic	03/02/2020	17	17268
UFS-BOC103	20/02/2020	Piglet	Asymptomatic	05/02/2020	17	18377
UFS-BOC104	20/02/2020	Piglet	Symptomatic	06/02/2020	14	16177
UFS-BOC105	20/02/2020	Piglet	Symptomatic	06/02/2020	14	16177
UFS-BOC106	20/02/2020	Piglet	Symptomatic	06/02/2020	14	16177
UFS-BOC107	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19286
UFS-BOC108	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19183
UFS-BOC109	20/02/2020	Piglet	Asymptomatic	06/02/2020	14	19182
UFS-BOC110	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19182
UFS-BOC111	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19183
UFS-BOC112	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19183
UFS-BOC113	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19268
UFS-BOC114	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19268
UFS-BOC115	20/02/2020	Piglet	Symptomatic	06/02/2020	14	19182
UFS-BOC116	20/02/2020	Piglet	Symptomatic	07/02/2020	13	18361
UFS-BOC117	20/02/2020	Piglet	Asymptomatic	11/02/2020	09	19242
UFS-BOC118	20/02/2020	Piglet	Asymptomatic	11/02/2020	09	19242
UFS-BOC119	20/02/2020	Piglet	Symptomatic	11/02/2020	09	19242
UFS-BOC120	20/02/2020	Piglet	Symptomatic	13/02/2020	09	19161
UFS-BOC121	20/02/2020	Piglet	Asymptomatic	13/02/2020	09	19169
UFS-BOC122	20/02/2020	Piglet	Asymptomatic	13/02/2020	07	19169
UFS-BOC123	20/02/2020	Piglet	Asymptomatic	14/02/2020	06	18235
UFS-BOC124	20/02/2020	Piglet	Symptomatic	21/01/2020	30	18197
UFS-BOC125		Water				

Appendix A4: Genome assembly table

Strain	Paired end reads		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
RVA/Porcine-wt/ZAF/UFS-BOC001/2018/G5P[13]	613280	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	100,0	100,0	99,7	100,0	100,0	100,0	99,9	100,0	100,0	100,0	100,0
		Average coverage	750,2	1047,6	868,5	1508,6	1094,9	1386,0	1138,3	888,7	905,5	1076,9	774,3
		% Identity	92,9	93,2	90,8	89,6	90,4	96,8	94,8	98,1	96,7	95,6	98,3
RVA/Porcine-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	403962	**% ORF**	100,0	100,0	100,0	P[6]: 98,6 P[13]: 87,2	100,0	99,8	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,2	99,7	99,8	P[6]: 98,5 P[13]: 100,0	98,7	97,2	83,4	97,9	96,8	98,0	98,4
		Average coverage	2669,8	3273,1	3513,8	P[6]: 2072,7 P[13]: 1215,3	3948,9	3732,5	3780,7	2780,9	2928,1	2424,6	1657,4
		% Identity	92,8	93,3	90,8	P[6]: 95,2 P[13]: 93,0	90,5	96,7	94,7	98,0	96,8	96,6	98,1
RVC/Porcine-wt/ZAF/UFS-BOC009/2018		**% ORF**	91,4	95,4	98,2	90,3	98,2	72,7	95,6	89,6	84,7	*	86,9
		% Genome segment	95,6	86,5	96,0	94,4	94,5	86,3	95,5	95,5	85,1	*	79,9
		Average coverage	79,8	78,4	103,6	121,1	85,6	77,1	132,0	73,9	87,0	35,2	73,2
		% Identity	95,5	89,7	87,5	87,0	86,6	92,8	89,0	93,2	88,8	*	86,7
RVA/Porcine-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	407178	**% ORF**	100,0	100,0	100,0	P[6]: 98,7 P[13]: 99,9	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	100,0	100,0	100,0	P[6]: 99,2 P[13] 99,9	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		Average coverage	843,3	1073,3	1300,3	P[6]: 343,0 P13]: 699,7	1644,5	1418,4	1820,4	1291,6	1415,8	1582,2	1245,9
		% Identity	92,8	93,0	97,1	P[6]: 94,7 P[13]: 93,0	90,5	98,9	94,9	98,0	96,9	96,4	97,9
RVB/Porcine-wt/ZAF/UFS-BOC050/2019/G14P[5]	19248	**% ORF**	99,4	100,0	100,0	77,0	100,0	100,0	96,0	100,0	100,0	97,1	99,8
		% Genome segment	99,4	99,8	100,0	99,9	100,0	100,0	96,0	100,0	100,0	99,5	99,8
		Average coverage	3484,9	3678,4	4682,1	3825,3	4422,3	2727,8	2865,9	3507,9	2688,0	2964,7	1714,5
		% Identrity	84,5	84,1	83,4	82,6	87,8	88,2	82,4	88,8	84,6	84,7	84,9

Strain	Paired end reads		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
RVA/Porcine-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	419916	**% ORF**	99,8	100,0	100,0	P[13]: 78,2 P[23]: 100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,3	99,6	99,8	P[13]: 99,9 P[23]: 100,0	98,7	98,1	98,5	98,0	95,3	98,0	95,0
		Average coverage	3306,3	3986,5	976,7	P[13]: 94,9 P[23]: 4388,3	4895,9	4512,2	4215,6	2702,6	1919,5	2322,0	1552,2
		% Identity	92,8	93,2	90,8	P[13]: 89,5 P[23]: 95,1	90,5	96,3	94,6	97,8	98,0	96,3	97,9
RVA/Porcine-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	505710	**% ORF**	100,0	100,0	100,0	P[13]: 78,6 P[23]: 100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	97,2	100,0	100,0	P[13]: 100,0 P[23]: 100,0	98,8	98,3	98,8	98,6	96,6	99,5	95,6
		Average coverage	3687,8	4560,4	4781,8	P[13]: 97,8 P[23]: 4879,8	5419,2	4739,9	34416,9	2922,2	2936,7	2590,2	1720,0
		% Identity	92,8	93,3	90,8	P[13]: 89,5 P[23]: 95,1	90,5	96,3	94,6	97,8	96,5	96,3	98,0
RVA/Porcine-wt/ZAF/UFS-BOC064/2019/G5P[23]	437222	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,5	99,7	100,0	100,0	98,0	98,2	98,4	98,0	96,0	98,5	95,6
		Average coverage	3188,1	3908,3	3428,0	4402,6	4866,2	4436,2	4081,1	2831,3	2821,5	2560,9	1760,4
		% Identity	92,8	93,3	90,8	95,1	90,5	96,4	94,6	97,8	96,4	96,3	98,0
RVA/Porcine-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23]	689830	**% ORF**	100,0	100,0	100,0	P[13]: 100,0 P[23]: 98,8	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,6	100,0	99,8	P[13]:98,9 P[23]: 100,0	99,6	99,0	98,4	99,4	99,3	99,5	98,5
		Average coverage	4533,5	5720,6	5720,6	P[13]:5267,5 P[23]: 1691,9	6907,0	6655,1	6235,1	3852,9	4755,5	3910,0	2915,5
		% Identity	92,8	93,2	90,8	P[13]: 89,5 P[23]: 95,1	90,5	96,4	94,5	97,7	96,9	96,3	98.0
RVA/Porcine-wt/ZAF/UFS-BOC076/2020/G5P[23]	603684	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,7	99,9	99,8	100,0	98,9	98,5	98,7	98,5	96,9	98,0	95,6
		Average coverage	3900,2	4363	4385	5073,9	4601,0	4723	4109,6	2857	3543	2930	2451
		% Identity	92,7	93,2	90,7	95,1	90,4	96,4	94,6	97,7	96,5	96,3	98,1
RVA/Porcine-wt/ZAF/UFS-BOC077/2020/G5P[23]	591520	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,7	99,9	99,8	100,0	98,7	99,0	98,8	99,4	95,6	97,9	98,5
		Average coverage	3456,3	3983	3809,0	4175,2	4101	4336	3655,1	2495	3035	2301	1706
		% Identity	92,7	93,2	90,7	95,1	90,4	96,4	94,5	97,7	96,5	96,3	98,1

Strain	Paired end reads		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
RVA/Porcine-wt/ZAF/UFS-BOC078/2020/G5P[23]	313592	**% ORF**	100,0	100	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,7	100,0	100,0	100,0	100,0	99,8	99,8	98,4	99,7	98,5	99,8
		Average coverage	1726,4	1938	2186	1879,9	1987	1987	1718,6	1187	1387	1035	725,2
		% Identity	92,7	93,2	90,7	95,2	90,4	96,7	94,7	97,7	96,5	96,3	97,9
RVA/Porcine-wt/ZAF/UFS-BOC079/2020/G5P[23]	439454	**% ORF**	100,0	100,0	99,2	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,5	99,8	99,7	100,0	98,7	98,1	97,6	97,9	96,0	98,3	95,0
		Average coverage	3437,0	4308	4156	4842,0	5531,0	5325	4730,3	3107	2902	2719	1857
		% Identity	92,8	93,1	90,8	95,1	90,6	96,3	94,6	97,4	96,4	96,2	97,8
RVA/Porcine-wt/ZAF/UFS-BOC081/2020/G5P[23]	349922	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,3	99,7	99,7	100,0	98,6	97,9	98,1	98,0	95,9	97,2	94,7
		Average coverage	2000,2	2438	2648	2868,2	2868	3002	2808,0	1648,0	2192	2160	1704
		% Identity	92,8	93,2	90,7	95,1	90,4	96,6	94,6	97,8	96,5	96,3	98,1
RVA/Porcine-wt/ZAF/UFS-BOC082/2020/G5P[23]	400832	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	97,1	100,0	100,0	100,0	100,0
		% Genome segment	99,4	99,8	99,8	100,0	98,3	97,7	96,9	97,9	96,0	98,3	94,6
		Average coverage	2880,1	3383	3363	37501,0	4035	3987	3274,1	1975	2057	2051	1347
		% Identity	92,8	93,2	90,1	95,1	90,4	96,3	94,6	97,8	96,5	96,3	98,1
RVA/Porcine-wt/ZAF/UFS-BOC083/2020/G5P[23]	420142	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,5	99,8	99,8	100,0	98,7	98,2	98,3	98,0	95,9	96,9	94,9
		Average coverage	2931,8	3364	3673	3731,4	3771	3996	3321,9	1999	2668	2214	1554
		% Identity	92,8	93,2	90,7	95,1	90,4	96,3	94,5	97,8	96,5	96,3	98,1
RVA/Porcine-wt/ZAF/UFS-BOC124/2020/G5P[23]	611202	**% ORF**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		% Genome segment	99,5	99,9	99,8	100,0	98,7	98,2	98,7	98,4	97,0	98,0	95,3
		Average coverage	3572,9	4889	4727	5611,0	6540,0	5876	6043,0	3878	3687	3671	2580,0
		% Identity	92,8	93,2	90,1	95,1	90,5	96,5	94,4	97,9	96,5	96,3	98,0

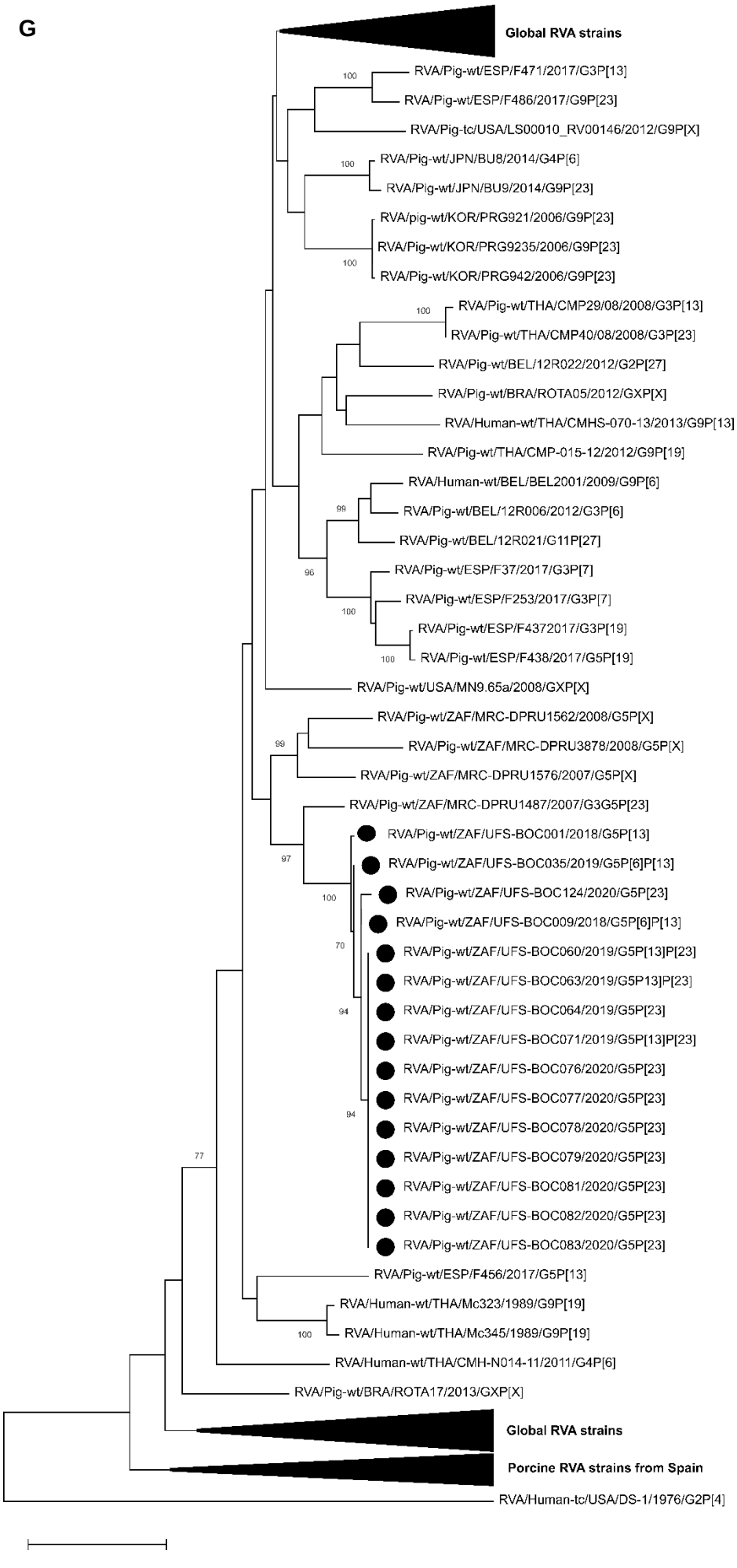
Appendix A4 Rotavirus reference strains used for reference mapping, percentage genome length and percentage length of open reading frames (ORFSs)

RVA: The percentage genome segment length and percentage length of the open reading frame (ORF) of the group A rotavirus South African sequences were based on RVA/Pig-wt/JPN/BU2/2014/G5P[7] (Nagai et al. 2015) except for the following: segment 4, genotype 6 (P[6]), genotype 13 (P[13]) and genotype 23 (P[23]) were based on strain RVA/Pig-wt/CHN/Z84/2007/GXP[6] (Li et al. 2017), strain RVA/Pig-wt/UGA/KYE-14-A048/2014/G3P[13] (Bwogi et al. 2017), and strain RVA/Pig-wt/ZAF/MRC-DPRU/1487/2007/G3G5P[23] (Das et al. 2015), respectively, segment 7, genotype T7 was based on strain RVA/Pig-wt/THA/CMP45/08/2008/G9P[23] (Okitsu et al. 2013), and segment 11, genotype H1 was based on strain RVA/Pig-wt/THA/CMP45/05/2008/GXP[X] (Okitsu et al. 2010).

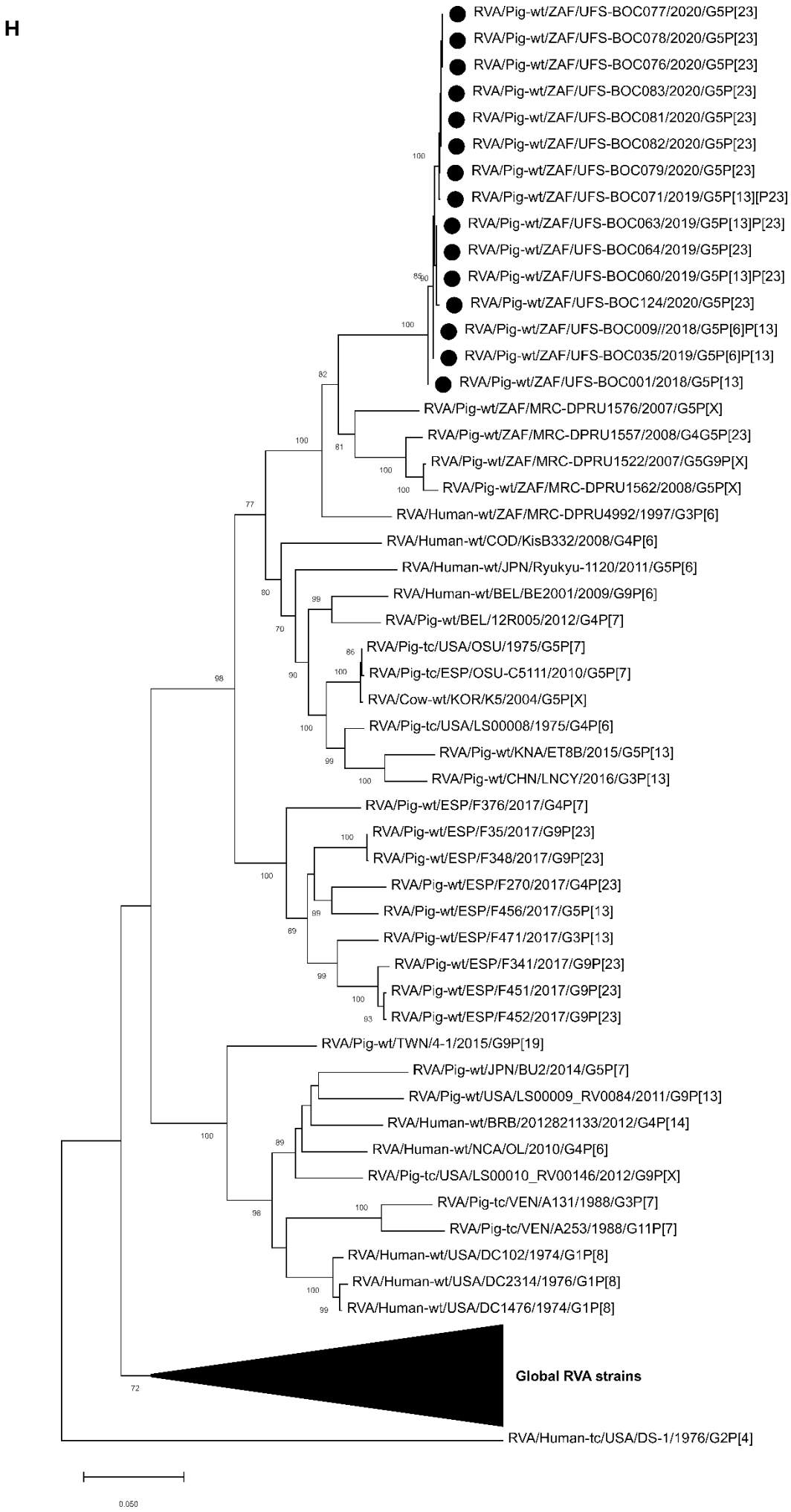
RVB: The percentage genome segment length and percentage length of the open reading frame of the group B rotavirus South African sequences were based on RVB/Pig-wt/USA/IL14/2013/GXP[X] (Herreta-Ibata et al. 2017) except for the following: segment 1 (VP1) was based on RVB/Pig-wt/VNM/14254_5/GXP[X] (Phan et al. 2016), segment 2 (VP2) was based on RVB/Pig-tc/USA/LS00011_Ohio/XXXX/GXP[X] (Strucker et al. 2015), segment 9 (VP7) was based on RVB/Pig-wt/USA/MN-1/2011/GXP[X] (Chen et al. 2017), and segment 5 (NSP1) was based on RVB/Pig-wt/VNM/14176_8/GXP[X] (Phan et al. 2016).

RVC: The percentage genome segment length and percentage length of the open reading frame of the group C rotavirus South African strains were based on strain RV0104 (Chepngeno et al. 2019) except for the following: segment 4 (VP4) and segment 6 (VP6) were based on strain RVC/Pig-wt/USA/RV0143/2011 (Amino et al. 2013), segment 5 (NSP1) was based on strain RVC/Pig-wt/CAN/NA3-16/2015G1P[4] (Lachapelle et al.2017), segment 7 (NSP3) and segment 8 (NSP2) were based on strain RVC/Pig-wt/JPN/87-G2/2009/GXP[X] (Suzuki and Hasebe 2017), and segment 10 (NSP4) was based on strain RVC/Pig-wt/USA/MN29/2012/G6P[5] (Suzuki and Hasebe 2017). *****The average coverage for rotavirus group C of segment 10 (NSP4) was too low to extract consensus sequence and further analyse.

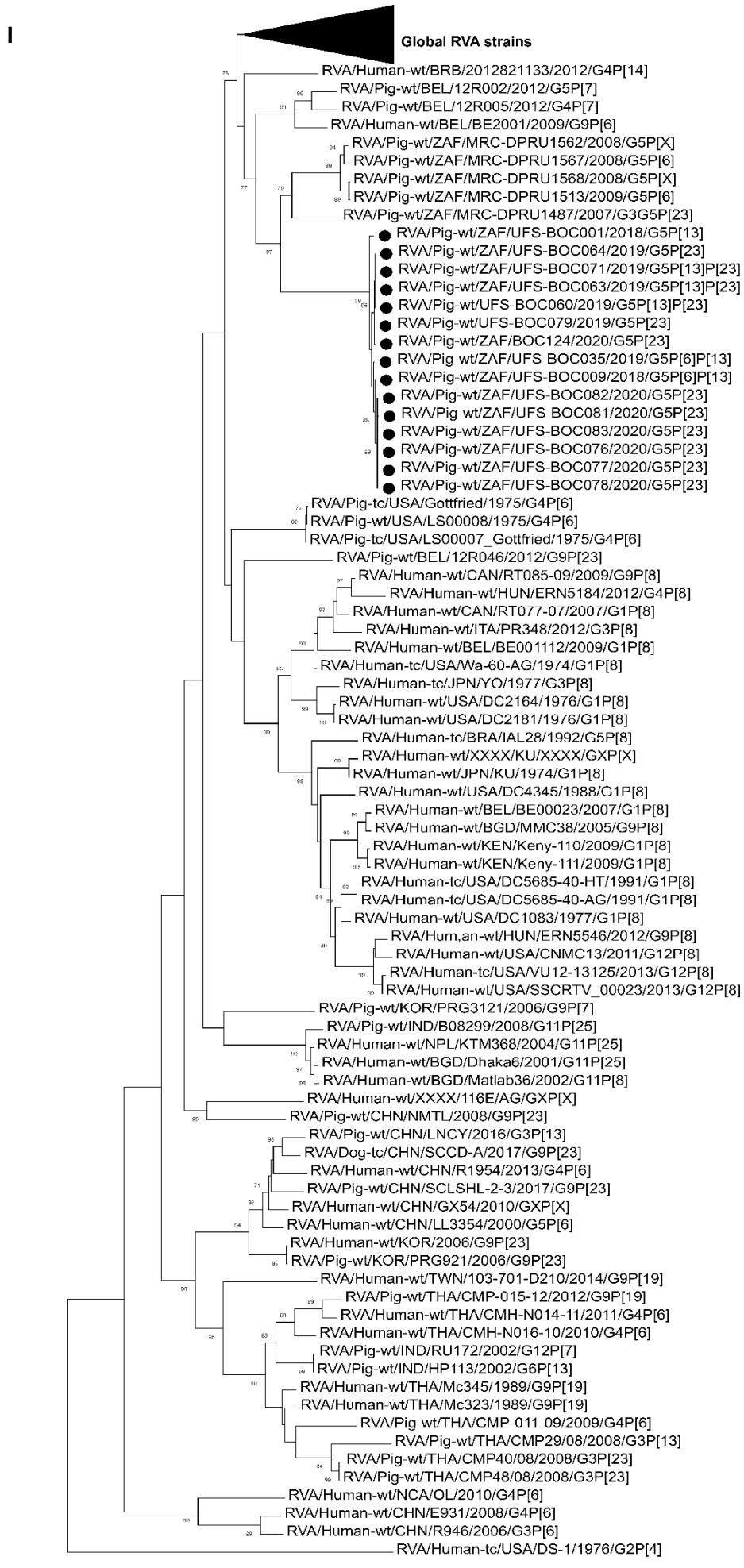
A5: Segment 6 encoding VP6 (I5) protein



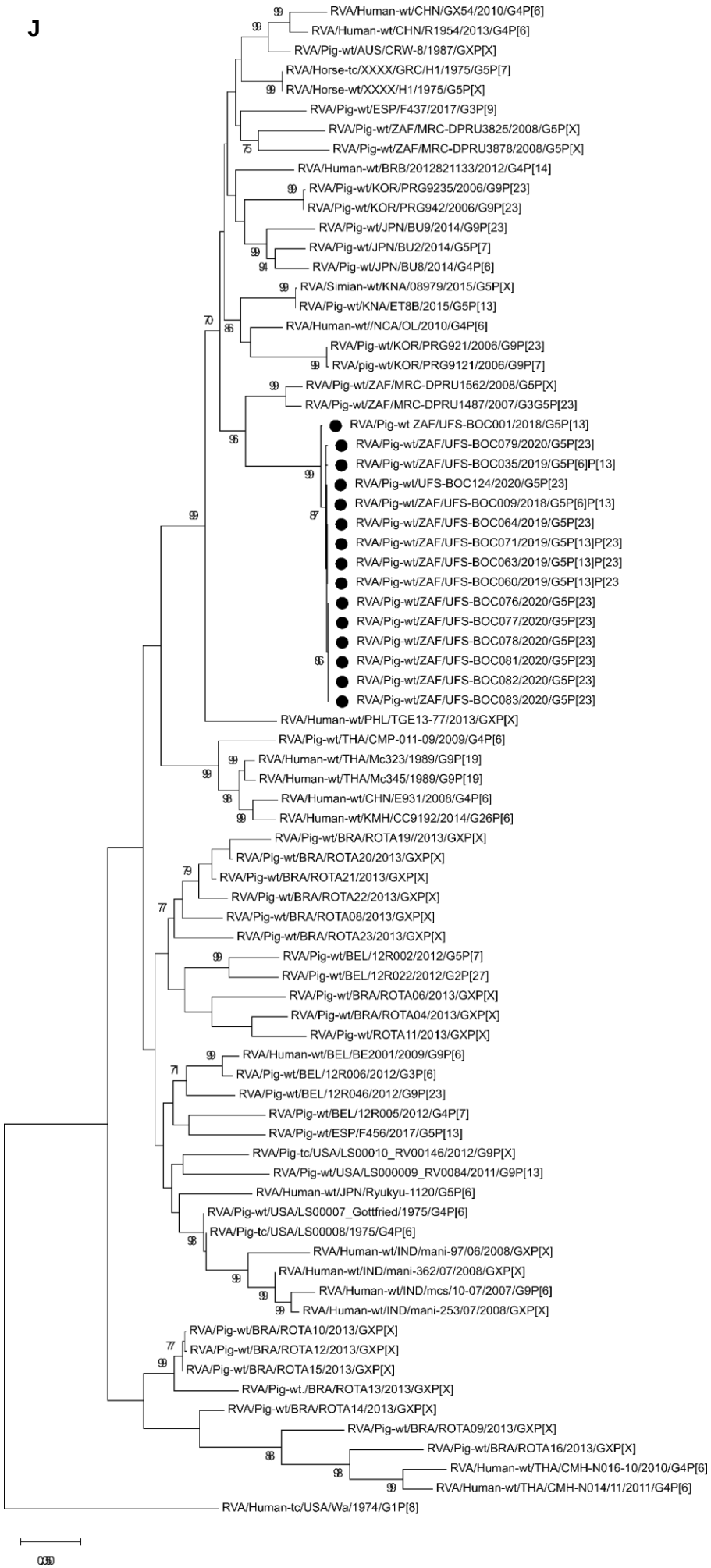
A5: Segment 1 encoding VP1 (R1) protein



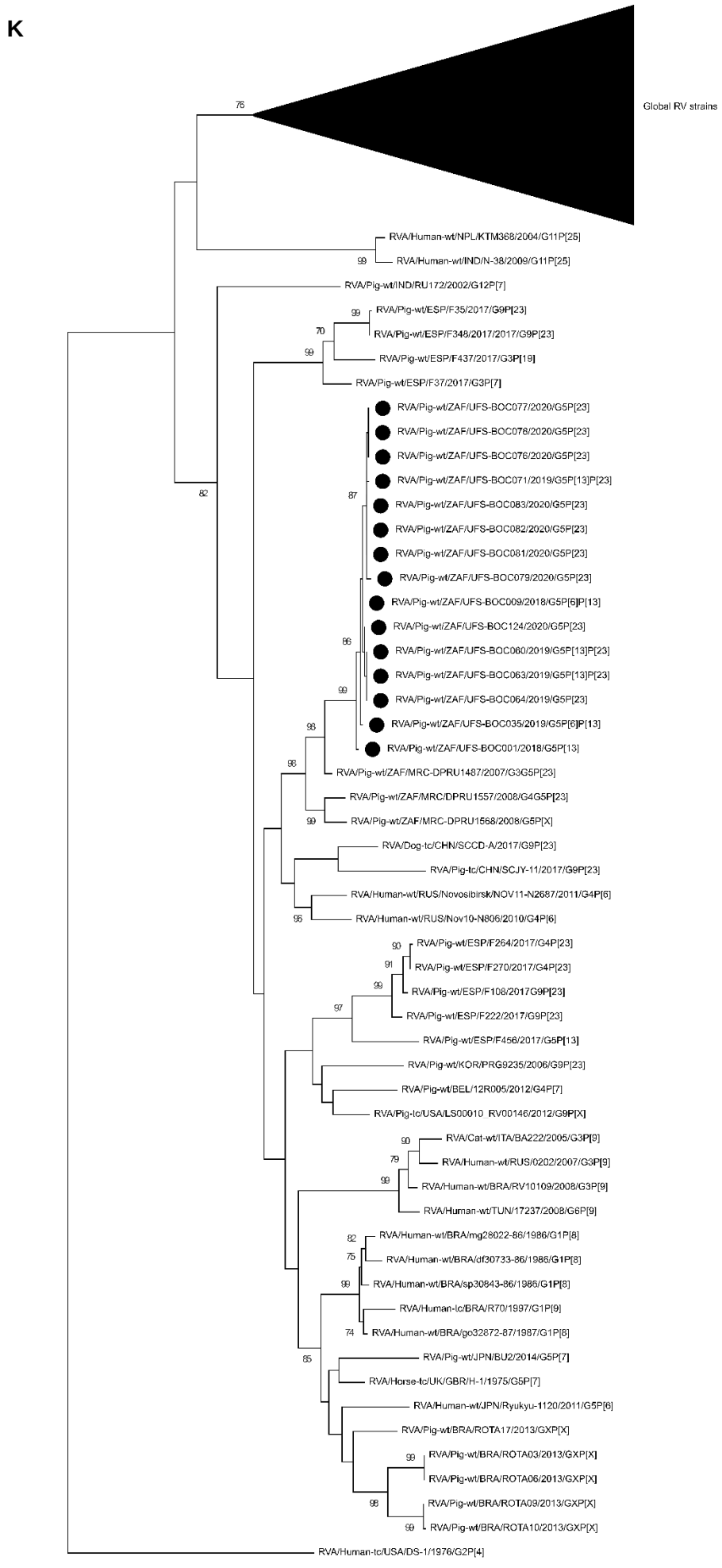
A5: Segment 3 encoding VP3 (M1) Protein



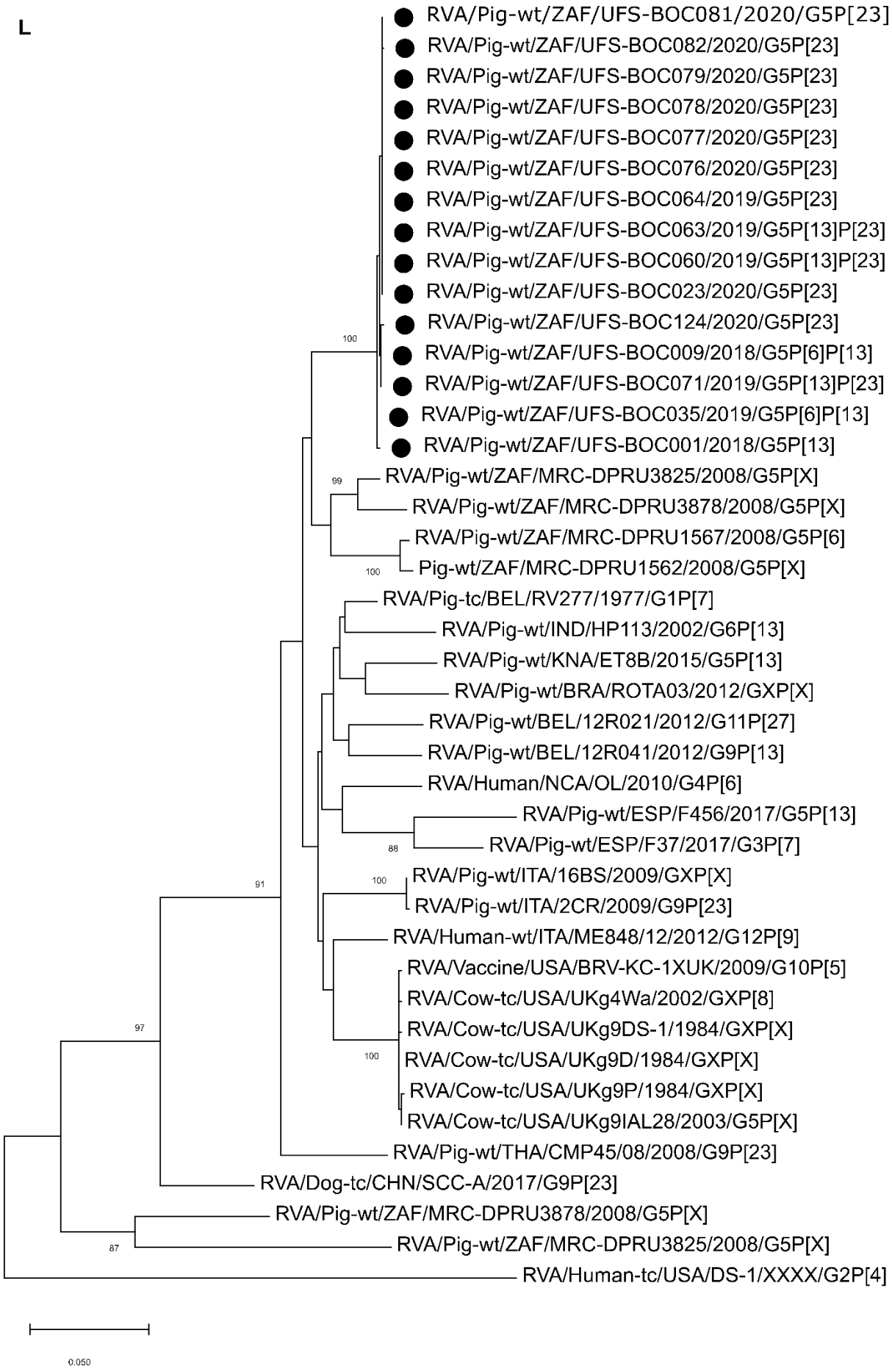
A5: Segment 5 encoding NSP1 (A8) protein



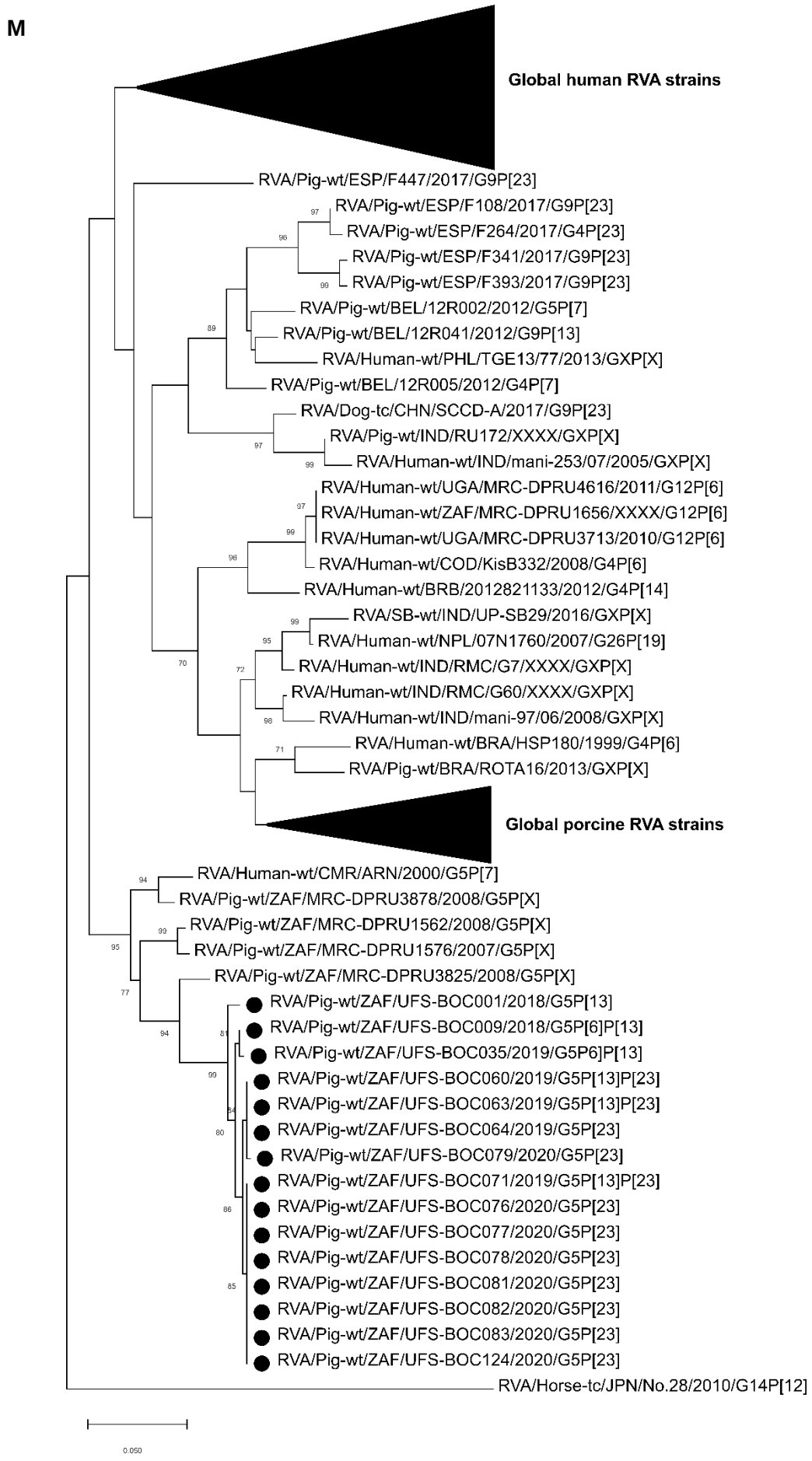
A5: Segment 8 encoding NSP2 (N1) protein



A5: Segment 7 encoding NSP3 (T7) protein



A5: Segment 10 encoding NSP4 (E1) protein



A6. Segment 4 encoding P[13] nucleotide identities

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	83,16
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	83,55
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23]	99,53
RVA/Pig-wt/MOZ/MZ-MPT-199/2016/G9P[13]	83,55
RVA/Pig-wt/MOZ/MZ-MPT-198/2016/G9P[13]	83,55
RVA/Pig-wt/MOZ/MZ-MPT-195/2016/G9P[13]	83,55
RVA/Pig-wt/MOZ/MZ-MPT-194/2016/G9P[13]	83,55
RVA/Pig-wt/MOZ/MZ-MPT-139/2016/G9P[13]	83,55
RVA/Pig-wt/MOZ/MZ-MPT-192/2016/G9P[13]	83,55
RVA/Pig-wt/CAN/F7P4-A8/2006/GXP[13]	90,13

A6. Segemnt 4 encoding P[6] nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,57
RVA/Human-wt/CHN/R1954/2013/G4P[6]	94,95
RVA/Human-wt/VNM/NT0042/2007/G4P[6]	94,68
RVA/Human-wt/VNM/NT0077/2007/G4P[6]	94,51
RVA/Human-wt/VNM/NT0621/2008/G4P[6]	94,47
RVA/Pig-wt/CHN/Z84/2007/GXP[6]	95,25

A6. Segment 4 encoding P[23]

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC060/G5P[13]P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC063/G5P[13]P[23]	100,00
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	95,28
RVA/Cow-wt/IND/IA-967RVB/2012/GXP[X]	93,62
RVA/Pig-wt/BEL/12R047/2012/G9P[23]	91,96
RVA/Pig-wt/BEL/12R046/2012/G9P[23]	91,96
RVA/Pig-wt/CHN/ZZ-12/2012/GXP[23]	91,33

A6. Segment 9 encoding VP7 nucleotide identities

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,68
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,68
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,15
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,58
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,15
RVA/Pig-wt/ZAF/MRC-DPRU1567/2008/G5P[6]	95,84
RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5P[X]	95,96
RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5P[X]	94,31
RVA/Pig-wt/ZAF/MRC-DPRU1522/2007/G5G9P[X]	94,20

A6. Segment 6 encoding VP6 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,58
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,83
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,33
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,33
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,24
RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	96,81
RVA/Pig-wt/USA/MN9.65a/2008/GXP[X]	94,63
RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5P[X]	94,63
RVA/Human-wt/HUN/BP1547/2005/G4P[6]	94,63
RVA/Pig-wt/CHN/ZZ-12/2012/GXP[23]	94,63

A6. Segment 1 encoding VP1 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,72
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,72
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,60
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,60
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,60
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,45
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,39
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,39
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,39
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,45
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,42
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,42
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,42
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,57
RVA/Pig-wt/ZAF/MRC-DPRU1522/2007/G5G9P[X]	92,92
RVA/Pig-wt/ZAF/MRC-DPRU1557/2008/G4G5P[23]	92,79
RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5P[X]	92,67
RVA/Pig-wt/ZAF/MRC-DPRU1562/2008/G5P[X]	92,58
RVA/Pig-wt/ZAF/MRC-DPRU1567/2008/G5P[6]	92,58

A6. Segment 2 encoding VP2 nucleotide identity

Strain	**%**
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,51
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,63
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,36
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,36
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,36
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,40
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,25
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,29
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,33
RVA/Pig-tc/USA/LS00006_OSU/1975/G5P[X]	93,19
RVA/Pig-tc/ESP/OSU-C5111/2010/G5P[7]	93,15
RVA/Cow-wt/KOR/KJ56-1/2004/GXP[X]	93,15
RVA/Pig-tc/USA/OSU/1975/G5P[7]	93,15
RVA/Pig-wt/KOR/K71/2006/GXP[X]	93,12

A6. Segment 3 encoding VP3 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,32
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,40
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,36
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,36
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,36
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,36
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,40
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,20
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,36
RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	90,68
RVA/Pig-wt/ZAF/MRC-DPRU1562/2008/G5P[X]	90,68
RVA/Pig-wt/ZAF/MRC-DPRU1567/2008/G5P[X]	90,48
RVA/Pig-wt/ZAF/MRC-DPRU1568/2008/G5P[X]	90,44
RVA/Pig-wt/ZAF/MRC-DPRU1513/2009/G5P[X]	90,36

A6. Segment 5 encoding NSP1 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	100,00
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	99,52
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,86
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,93
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,93
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,93
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,93
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,86
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,86
RVA/Pig-tc/JPN/CRW-8/1987/GXP[X]	88,96
RVA/Horse-wt/XXXX/H1/XXXX/GXP[X]	89,23

A6. Segment 8 encoding NSP2 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,58
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,58
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,37
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,37
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,37
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,27
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,27
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,27
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,27
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,16
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,37
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,37
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,37
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,48
RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	98,11
RVA/Pig-wt/ZAF/MRC-DPRU1557/2008/G4G5P[23]	96,02
RVA/Pig-wt/ZAF/MRC-DPRU1568/2008/G5P[X]	95,81
RVA/Hu-wt/RUS/Novosibirsk/Nov11-N2687/2011/G4P[6]	94,65
RVA/Human-wt/RUS/Nov10-N806/2010/G4P[6]	94,34

A6. Segment 7 encoding NSP3 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,52
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,38
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,45
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,45
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,45
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,45
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,38
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,52
RVA/Pig-tc/JPN/CRW-8/1987/GXP[X]	89,19
RVA/Horse-wt/XXXX/H1/XXXX/GXP[X]	89,44

A6. Segment 10 encoding NSP4 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	98,86
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	98,67
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	98,48
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	98,30
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	98,48
RVA/Pig-wt/ZAF/MRC-DPRU3825/2008/G5PX_NCBI1	95,64
RVA/Pig-wt/ZAF/MRC-DPRU1562/2008/G5PX_NCBI2	93,56
RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5PX_NCBI3	93,37
RVA/Human-wt/CMR/6784/2000/G5P[7]	93,37
RVA/Pig-wt/ZAF/MRC-DPRU3878/2008/G5P[X]	92,80

A6. Segment 11 encodingNSP5/6 nucleotide identity

Strain	%
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13]	100,00
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13]	99,66
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13]	99,83
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23]	99,49
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23]	99,49
RVA/Pig-wt/ZAF/UFS/BOC064/2019/G5P[23]	99,49
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13P[23]	99,49
RVA/Pig-wt/ZAF/UFS-BOC076/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC077/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC078/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC079/2020/G5P[23]	99,49
RVA/Pig-wt/ZAF/UFS-BOC081/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC082/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC083/2020/G5P[23]	99,66
RVA/Pig-wt/ZAF/UFS-BOC124/2020/G5P[23]	99,49
RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	98,32
RVA/Pig-wt/BRA/ROTA25/2013/GXP[X]	98,15
RVA/Human-wt/HUN/BP271/2000/G4P[6]	98,15
RVA/Human-wt/IND/mani-362/07/2006/GXP[X]	97,81

A7. Segment 9 encoding VP7 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/USA/MT-139/2015/GXP[X]_NCBI closest strain	82,06
RVB/Rat-wt/USA/IDIR/XXXX/G1P[8]	37,92
RVB/Human-wt/SEN/MRC-DPRU4680/2010/G2P[X]	44,87
RVB/Cow-wt/JPN/J-2002/2002/G3P[X]	45,45
RVA/Pig-wt/JPN/PB-70-H5/2007/G4PX]	54,35
RVB/Pig-wt/IND/AN142127/2013/G5P[X]	63,21
RVB/Pig-wt/JPN/PB=F18/2008/G6P[X]	45,88
RVB/Pig-wt/JPN/PB-S15/2002/G7P[X]	44,20
RVB/Pig-wt/USA/MN09-65/2009/G8P[X]	44,50
RVA/Pig-wt/JPN/PB-68-E4/2007/G9P[X]	42,81
RVB/Pig-wt/USA/MO09-34/2009/G10P[X]	45,45
RVB/Pig-wt/JPN/PB-S5/2002/G11P[X]	66,82
RVB/Pig-wt/USA/MN09-68/2009/G12P[X]	71,20
RVB/Pig-wt/JPN/PB-23-44/2005/G13P[X]	69,98
RVB/Pig-wt/USA/MN09-59/2009/G14P[X]	79,80
RVB/Pig-wt/JPN/PB-S49-2/2003/G15P[X]	71,91
RVB/Pig-wt/USA/IA09-67/2009/G16P[X]	70,19
RVB/Pig-wt/USA/OK09-51/2009/G17P[X]	72,03
RVB/Pig-wt/USA/MN09-63/2009/G18P[X]	68,93
RVB/Pig-wt/IND/AN142129/2013/G19P[X]	62,45
RVB/Pig-wt/IND/AN142127/2013/G20P[X]	63,21
RVB/Pig-wt/IND/AN142530/2013/G21P[X]	50,55
RVB/Pig-wt/USA/MN-98/2014/G22P[X]	62,00
RVB/Pig-wt/USA/MN-126/2014_G23P[X]	53,25
RVB/Pig-wt/USA/MN-127/2014/G24P[X]	59,26
RVB/Pig-wt/USA/OK-63/2013/G25P[X]	65,96
RVB/Pig-wt/JPN/PB-68-G4/2007/G26PX]	65,85

A7. Segment 4 encoding P[5] nucleotide idenity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/USA/IL14/2013_NCBI_closest_strain	78,89
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]	6,67
RVB/Human-wt/SEN/MRC-DPRU4680/2010/G2P[2]	44,53
RVB/Cow-wt/JPN/J-2002/2002/GXP[3]	49,96
RVB/Pig-wt/USA/IL15B/2013/G16P[4]	51,44
RVB/Pig-wt/USA/IL14/2013/G16P[5]	78,89

A7. Segment 6 encoding VP6 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/USA/IA09-67/2009/G16P[X] NCBI_closest strain	86,80
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_I1	66,08
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_I2	67,17
RVB/Cow-wt/IND/RUBV226/2004/G5P[3]_I3	56,40
RVB/Pig-wt/VNM/14250_10/2012/G7P[X]_I4	56,14
RVB/Pig-wt/Japan/PB-93-I5/2008/GXP[X]_I5	55,09
RVB/Pig-wt/USA/WI09-73/2009/GXP[X]_I6	69,12
RVB/Pig-wt/USA/MN09-69/2009/GXP[X]_I7	68,29
RVB/Pig-wt/USA/MN09-72/2009/GXP[X]_I8	70,29
RVB/Pig-wt/USA/MN09-6/2009/G8P[X]_I9	69,19
RVB/Pig-wt/Japan/PB-S13-5/GXP[X]_I10	70,29
RVB/Pig-wt/Japan/PB-S24-11/GXP[X]_I11	83,75
RVB/Pig-wt/USA/IL09-1/2009/G18[X]_I12	74,32
RVB/Pig-tc/USA/LS00011_Ohio/XXXX/GXP[X]_I13	81,03

A7. Segment 1 encoding VP1 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/VNM/14151_62/XXXX/GXP[X]_NCBI_closest strain	80,61
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_R1	57,82
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_R2	60,03
RVB/Goat-wt/USA/Minnesota-1/2016/G3P[3]_R3	54,03
RVB/Pig-wt/USA/IL14/2013/G16P[4]/P[5]_R4	73,14
RVB/Cow-wt/IND/RUBV282/2005/G5P[3]_R5	55,92

A7. Segment 2 encoding VP2 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/ESP/P2B/2017/GXP[4]_NCBI_closest strain	81,11
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_C1	64,08
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_C2	67,24
RVB/Goat-wt/USA/Minnesota-1/2016/G3P[3]_C3	54,47
RVB/Pig-wt/USA/IL14/2013/G16P[4]/P[5]_C4	78,38
RVB/Cow-wt/IND/RUBV282/2005/G3P[3]	53,98

A7. Segment 3 encoding segment 3 nucleotide idenity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/USA/KS2/2012/G16P[X]_NCBI_closest strain	83,46
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_M1	51,73
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_M2	70,25
RVB/Goat-wt/USA/Minnesota-1/2016/G3P[3]_M3	63,85
RVB/Pig-wt/USA/IL14/2013/G16P[4]/P[5]_M4	83,16

A7. Segment 5 encoding NSP1 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/JPN/PB-71-H5/2007/G1P[1]_NCBI_closest strain	83,32
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_A1	20,20
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_A2	33,13
RVB/Goat-wt/CHN/KB63/1986/GXP[X]_A3	42,66
RVB/Cow-wt/JPN/G-2006/G3P[X]_A4	45,67
RVB/Cow-wt/IND/RUBV282/2005/G3P[3]_A5	45,04
RVB/Pig-wt/JAP/PB-93-I5/2008/GXPX]_A6	60,21
RVB/Pig-wt/JAP/PB-93-I5/2008/GXP[X]_A7	63,42
RVB/Pig-tc/USA/LS00011_Ohio/XXXX/GXP[X]_A8	76,99

A7.Segment 8 encoding NSP2 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/VNM/14151-62/XXXX/G1P[1]_NCBI_closest strain	87,03
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_N1	66,80
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXPX]_N2	66,47
RVB/turkey-wt/USA/Minnesota-1/2016/GXP[X]_N3	49,23
RVB/Cow-wt/IND/RUBV282/2005/G3P[3]_N4	58,41
RVB/Pig-wt/JPN/PB-93-I5/2008/GXP[X]_N5	57,44
RVB/Pig-wt/JPN/PB-70-H5/2007/GXP[X]_N6	57,70
RVB/Human-wt/XXX/IS2/XXXX/GXP[X]_N7	20,32
RVB/Pig-wt/JPN/PB-S22-3/2002/G14P[X]_N8	75,55
RVB/Pig-wt/JPN/PB-S23-1/2002/G20P[X]_N9	78,03
RVB/Pig-wt/USA/IL16/2013/GXP[X]_N10	83,79

A7. Segment 7 encoding NSP3 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100
RVB/Pig-wt/ESP/B304/2017/G12P[X]_NCBI_closest strain	78,75696
RVB/Rat-wt/USA/IDIR/XXXX/G1P[1]_T1	8,870682
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_T2	41,33317
LC185676.1_Bovine_group_B_rotavirus_gene_for_NSP3_complete_cds_T3	31,57367
RVB/Pig-wt/USA/IL14/2013/G16P[4]/P[5]_T4	78,12385
RVB/Pig-wt/USA/KS2/2012/G16P[X]_T5	55,31859
RVB/Pig-tc/USA/LS00011_Ohio/XXXX/GXP[X]_T6	51,57113

A7. Segment 10 encoding NSP4 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/CHN/VIRES/2017/GXP[X]_NCBI_closest strain	80,31
RVB/Rat-wt/USA/IDIR/XXXX/GXP[X]_E1	35,07
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_E2	41,39
RVB/Cow-wt/JPN/G-2006/2006/G3P[3]	22,42
RVB/Pig-wt/USA/IL14/2013/G16P[4]/P5]_E4	77,19

A7. Segment 11 encoding NSP5/6 nucleotide identity

Strain	%
RVB/Pig-wt/ZAF/UFS-BOC050/2019/G14P[5]	100,00
RVB/Pig-wt/JPN/PB-68-C17/2007/GXP[X]_NCBI closest strain	80,97
RVB/Rat-wt/USA/IDIR/XXXX/GXP[X]_H1	50,51
RVB/Human-wt/SEN/MRC-DPRU4680/2010/GXP[X]_H2	51,18
RVB/Cow-wt/JPN/G-2006/2006/G3P[3]_H3	30,60
RVB/Pig-wt/JPN/PB-93-I5/2008/GXP[X]_H4	30,70
RVB/Cow-wt/JPN/RUBV282/2005/G5P[X]_H5	22,10
RVB/Pig-wt/JPN/PB-68-G4/2007/G26P[X]_H6	62,26
RVB/Pig-wt/USA/IL16/2013/GXP[X]_H7	75,16

Appendix A8: P[13] nucleotide alignment


680
700
720
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] TATTAATCAT GGACTTCCAC CAATGCAGAA TACAAGAAAC GTAGTACCAG TTGCATTATC 720
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] TATTAATCAT GGACTTCCAC CAATGCAGAA TACAAGAAAC GTAGTACCAG TTGCATTATC 720
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] TATTAATCAT GGACTTCCAC CAATGCAGAA TACAAGAAAC GTAGTACCAG TTGCATTATC 720
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] TATTAATCAT GGACTTCCAC CAATGCAGAA TACAAGAAAC GTAGTACCAG TTGCATTATC 720
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] TATTAATCAC GGTCTTCCAC CAATGCAGAA TACTAGGAAC GTAGTATCAG TTGCATTATC 720
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] TATTAATCAC GGTCTTCCAC CAATGCAGAA TACTAGGAAC GTAGTATCAG TTGCATTATC 719
740
760
780
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] AGCTAGAGAT ATCATAATAC AAAGAGCAAG TGCTAACGAA GATACTATTA TATCAAAGAC 780
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] AGCTAGAGAT ATCATAATAC AAAGAGCAAG TGCTAACGAA GATACTATTA TATCAAAGAC 780
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] AGCTAGAGAT ATCATAATAC AAAGAGCAAG TGCTAACGAA GATACTATTA TATCAAAGAC 780
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] AGCTAGAGAT ATCATAATAC AAAGAGCAAG TGCTAACGAA GATATTATTA TATCAAAGAC 780
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] GGCTAGAGAT GTGATAACGC GAAAAGTAAG TGTTAATGAA GACATCGTGG TATCTAAAAC 780
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] GGCTAGAGAT GTGATAACGC GAAAAGTAAG TGTTAATGAA GACATCGTGG TATCTAAAAC 779
800
820
840
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] TTCTTTATGG AAAGAAATGC AGTACAATAG AGACATTAAA ATTAGATTTA AATTCGCTAA 840
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] TTCTTTATGG AAAGAAATGC AGTACAATAG AGACATTAAA ATTAGATTTA AATTCGCTAA 840
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] TTCTTTATGG AAAGAAATGC AGTACAATAG AGACATTAAA ATTAGATTTA AATTCGCTAA 840
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] TTCTTTATGG AAAGAAATGC AGTACAATAG AGACATTAAA ATTAGATTTA AATTCGCTAA 840
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] ATCCTTATGG AAAGAAATGC AATATAATAG AGATATCACA ATTAGATTTA AATTCGCTAA 840
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] ATCCTTATGG AAAGAAATGC AATATAATAG AGATATCACA ATTAGATTTA AATTCGCTAA 839
860
880
900
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] TCAAATAATC AAATCCGGAG GATTGGGTTA TAAATGGTCA GAAATATCTT TTAAACCAGC 900
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] TCAAATAATC AAATCCGGAG GATTGGGTTA TAAATGGTCA GAAATATCTT TTAAACCAGC 900
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] TCAAATAATC AAATCCGGAG GATTGGGTTA TAAATGGTCA GAAATATCTT TTAAACCAGC 900
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] TCAAATAATC AAATCCGGAG GATTGGGTTA TAAATGGTCA GAAATATCTT TTAAACCAGC 900
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] TCAAATAATC AAATCTGGAG GACTAGGCTA TAAATGGGCA GAGATTTCTT TCAGACCAGC 900
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920
940
960
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] AAATTATCAA TACACATATA CTAGAGATGG AGAAGAAATT ACAGCTCATA CTACGTGCTC 960
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RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] AAATTATCAA TACACATATA CTAGAGATGG AGAAGAAATT ACAGCTCATA CTACGTGCTC 960
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RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] GAATTATCAA TATACATATA CTAGAGATGG AGAAGAAGTC ACAGCACATA CTACATGTTC 960
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] GAATTATCAA TATACATATA CTAGAGATGG AGAAGAAGTC ACAGCACATA CTACATGTTC 959
980
1,000
1,020
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1,060
1,080
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RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] ATCGAGATAC GAAGTTATTA AAGAAAACTC ATATGTATAT ATAGATTATT GGGATGATTC 1080
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RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] ATCGAGATAC GAAGTTATTA AAGAAAACTC ATATGTATAT ATAGATTATT GGGATGATTC 1080
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1,120
1,140
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RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] ACAAGCATTC AGAAATATGG TATATGTAAG ATCATTAGCA GCTGATCTAA ACTCAGTTAC 1140
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RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] ACAAGCATTC AGAAACATGG TATATGTCAG GTCACTGGCG GCTGATTTAA ATTCAATAAC 1139
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1,180
1,200
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] TTGTAGTGGT GGTAGTTATA GTTTTGCATT ACCCTTAGGA AATTTTCCAG TTATGTCAGG 1200
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] TTGTAGTGGT GGTAGTTATA GTTTTGCATT ACCCTTAGGA AATTTTCCAG TTATGTCAGG 1200
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] TTGTAGTGGT GGTAGTTATA GTTTTGCATT ACCCTTAGGA AATTTTCCAG TTATGTCAGG 1200
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] TTGTAGTGGT GGTAGTTACA GTTTTGCATT ACCCTTAGGA AATTTTCCAG TTATGTCAGG 1200
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] TTGCAGCGGT GGTAGCTATA ATTTCGCATT GCCTTTAGGA AATTTTCCGG TTATGTCAGG 1200
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] TTGCAGCGGT GGTAGCTATA ATTTCGCATT GCCTTTAGGA AATTTTCCGG TTATGTCAGG 1199
1,220
1,240
1,260
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] AGGCGCCGTA TCATTACATC CTTCAGGAGT GACGTTGTCA ACACAGTTTA CAGATTTTGT 1260
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] AGGCGCCGTA TCATTACATC CTTCAGGAGT GACGTTGTCA ACACAGTTTA CAGATTTTGT 1260
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] AGGCGCCGTA TCATTACATC CTTCAGGAGT GACGTTGTCA ACACAGTTTA CAGATTTTGT 1260
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RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] AGGTGCTATA TCACTACATC CATCCGGAGT GACATTATCA ACTCAGTTTA CGGATTATGT 1260
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] AGGTGCTATA TCACTACATC CATCCGGAGT GACATTATCA ACTCAGTTTA CGGATTATGT 1259
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1,320
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1,340
1,360
1,380
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 1380
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 1380
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 1380
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1380
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1380
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1379
1,400
1,420
1,440
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 1440
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RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 1440
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RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1440
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1439
1,460
1,480
1,500
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 1500
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RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 1500
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1500
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1500
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1,520
1,540
1,560
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RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1560
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1560
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1,580
1,600
1,620
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 1620
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RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 1620
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1620
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1620
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1,660
1,680
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RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 1680
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RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1679
1,700
1,720
1,740
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 1740
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 1740
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 1740
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1740
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1740
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
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1,780
1,800
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RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 1800
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RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 1800
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 1800
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1,840
1,860
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RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
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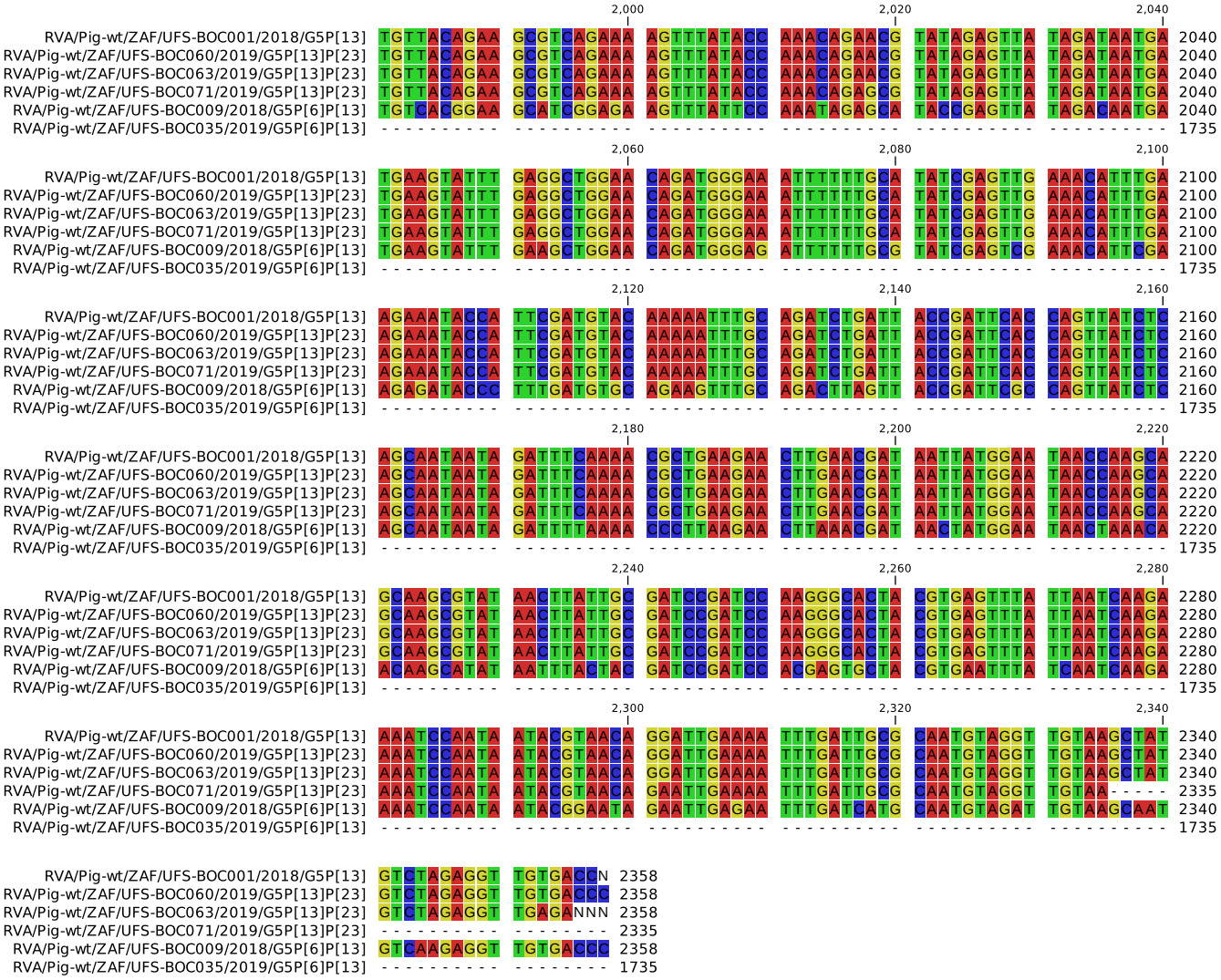
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2,020
2,040
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RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
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2,080
2,100
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RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2100
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2100
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RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2100
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
2,120
2,140
2,160
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 2160
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2160
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2160
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 2160
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2160
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
2,180
2,200
2,220
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 2220
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2220
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2220
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 2220
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2220
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
2,240
2,260
2,280
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 2280
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2280
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2280
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RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2280
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
2,300
2,320
2,340
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 2340
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2340
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2340
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 2335
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2340
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735
RVA/Pig-wt/ZAF/UFS-BOC001/2018/G5P[13] 2358
RVA/Pig-wt/ZAF/UFS-BOC060/2019/G5P[13]P[23] 2358
RVA/Pig-wt/ZAF/UFS-BOC063/2019/G5P[13]P[23] 2358
RVA/Pig-wt/ZAF/UFS-BOC071/2019/G5P[13]P[23] 2335
RVA/Pig-wt/ZAF/UFS-BOC009/2018/G5P[6]P[13] 2358
RVA/Pig-wt/ZAF/UFS-BOC035/2019/G5P[6]P[13] 1735

Table 1.1 Rotavirus structure

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Figure 1.1 Sapovirus structure

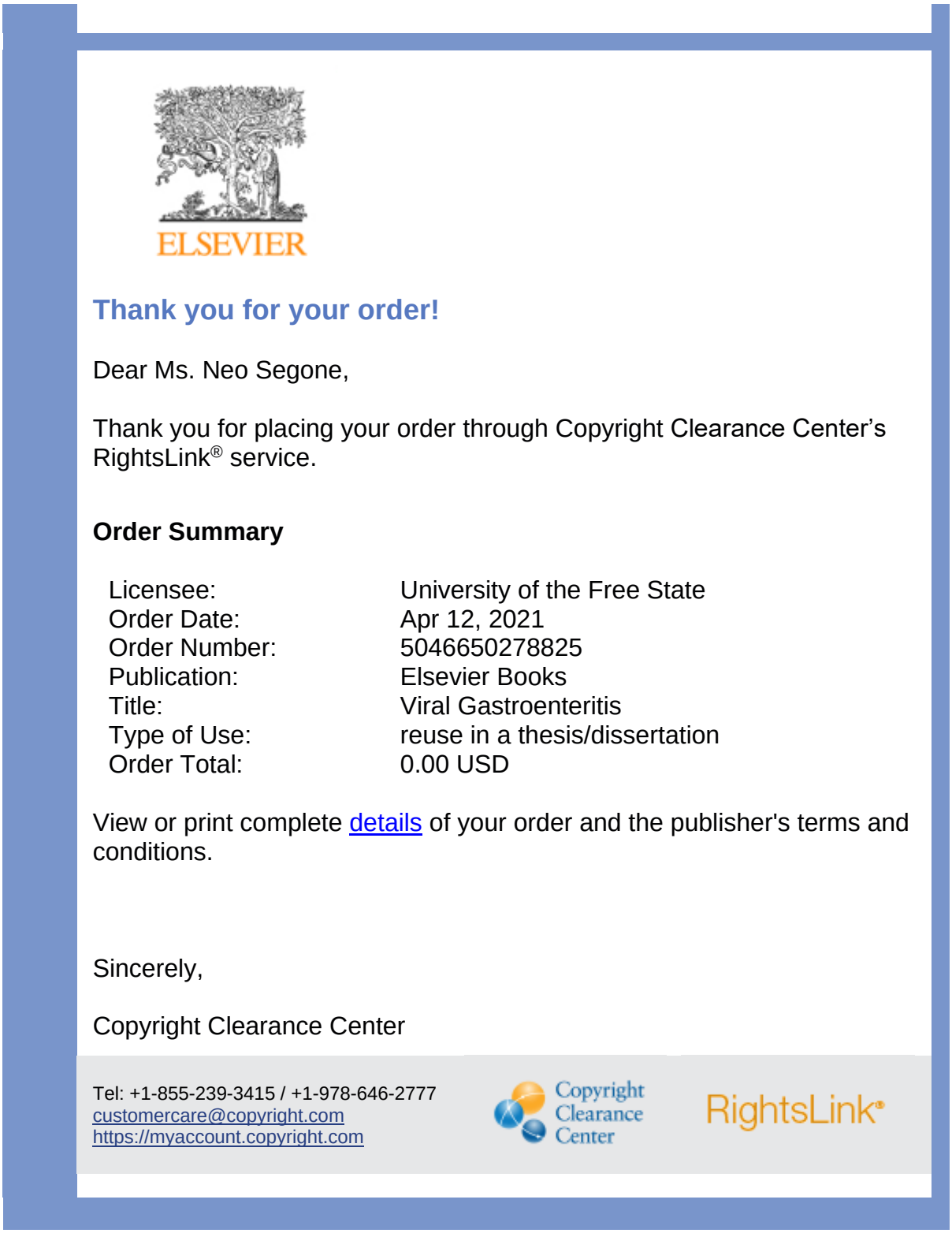

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Table 1.1 Astrovirus structure

https://www.pnas.org/content/108/31/12681

Table 1.1 Adenovirus structure

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3386624/

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Figure 1.1

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Figure 1.3


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Publication: Expert Review of Anti-infective Therapy
Publisher: Taylor & Francis
Date: Nov 2, 2015
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Figure 1.5

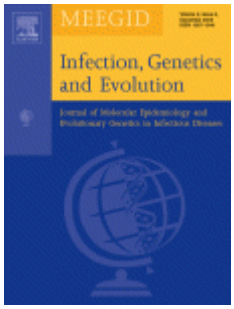

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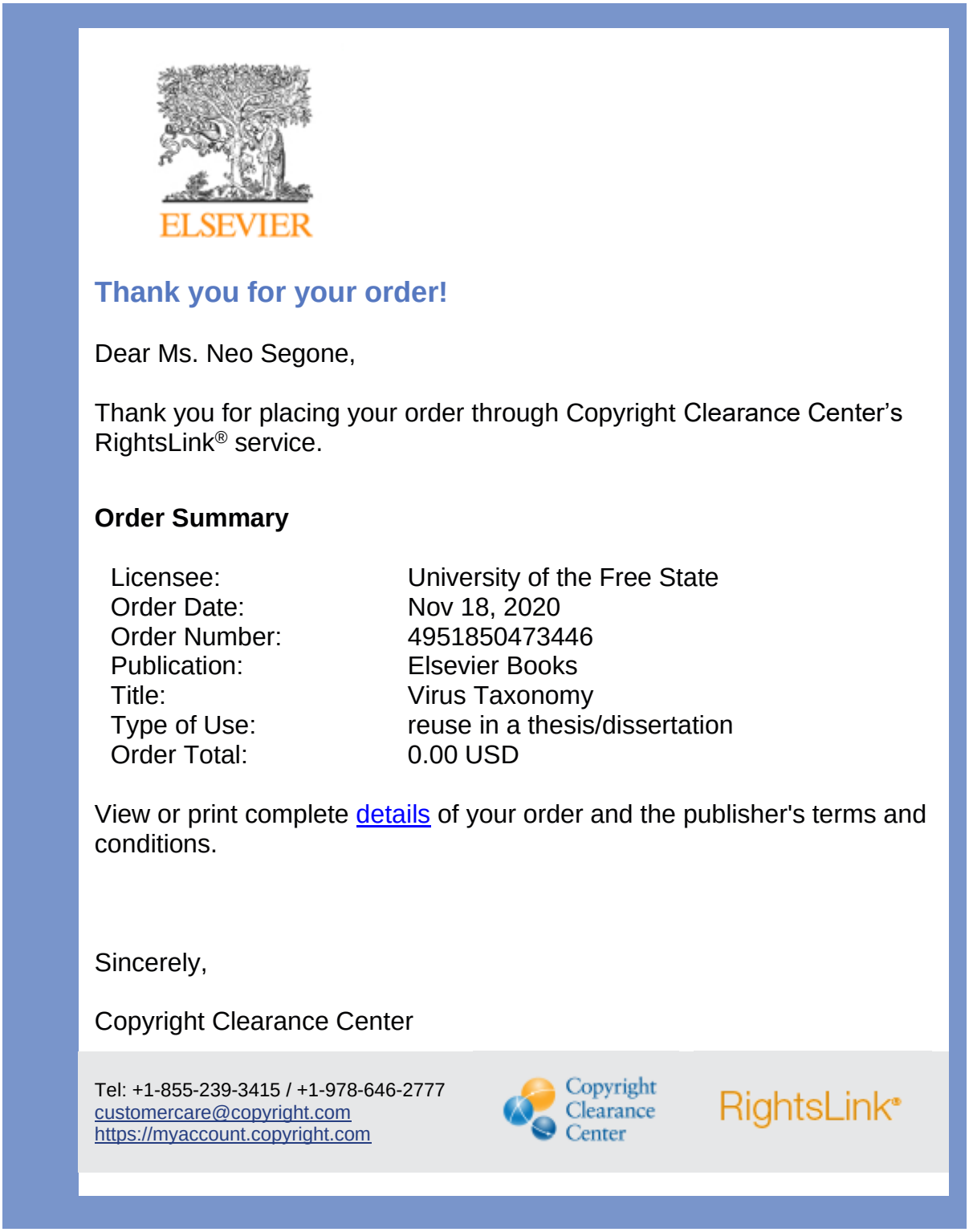
Figure 1.8



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