CHAPTER ONE
INTRODUCTION, AIMS OF THE STUDY, MATERIALS AND METHODS

1.1. Background and problem identification

Rotaviruses are the major aetiological agents of severe dehydrating diarrhoea and are associated with approximately 453 000 childhood mortalities annually across the globe (Tate et al., 2011). Approximately 98% of these cases are reported in developing countries (Grimwood and Buttery, 2007), of which around 232 000 deaths are estimated to occur in under-five-year-old children in sub-Saharan countries (Tate et al., 2011). In South Africa, more than 14 000 child deaths are linked to rotavirus infections yearly (Black et al., 2003). Improved sanitary measures have drastically reduced diarrhoea-associated deaths caused by other non-viral enteric pathogens in both developed and developing countries. However, the incidence rates of rotavirus-associated gastroenteritis has remained even (Glass et al., 2006b). This underscores the need for effective rotavirus vaccines as a prevention measure.

Rotavirus particles contain an eleven-segmented double-stranded RNA (dsRNA) genome, and comprise a genus in the Reoviridae virus family. The genome encodes six structural proteins (VP) and six non-structural proteins (NSP). The VPs are assembled into three concentric layers of capsid proteins around the dsRNA genome. The core consists of VP1, VP2 and VP3. The inner capsid is made up of VP6, and the outer capsid is composed of VP4 and VP7 proteins. Most of the NSPs are involved in the replication processes of rotaviruses (Reviewed in Estes and Kapikian, 2007).

Rotaviruses infect a broad spectrum of species and are classified in various ways, see section 2.1 and 2.5. A dual typing system, which is based on the serotypes of the surface proteins (VP4 and VP7) or genotypes of the VP4 and VP7 encoding genome segments, is commonly used to distinguish rotavirus strains. VP7-specific serotypes or genotypes are termed G types (glycoprotein) and VP4-specific serotypes or genotypes are termed P types (protease-sensitive). To date, 27 different G- and 35 P-types have been described (Matthijnssens et al., 2011). Strains with G1, G2, G3, G4, G9 and G12 in combination with P[4], P[6] or P[8] are commonly characterised in humans. Strains with G3, G4, G5, G9 and G11 genotypes in association with P[6], P[7], P[13], P[19], P[23], P[26] and P[27] are common in pigs, whereas strains with G6,
G8 and G10 in association with P[1], P[5], P[11], P[15], and P[21] are frequently detected in bovines (Martella et al., 2010). Identification of rotavirus strains containing animal-like rotavirus genome segments in humans generally leads to conclusive evidence of animal-to-human interspecies transmission (Matthijnssens et al., 2009, Tsugawa and Hoshino, 2008, Matthijnssens et al., 2006). However, proper deciphering of the origin of the zoonotic strains currently circulating in humans has been hampered by the limited number of animal rotavirus strains that have been fully characterised genetically worldwide. For instance, no African bovine strain has been completely characterised to date despite some studies conducted in Africa suggesting a common origin between human and bovine rotavirus strains (Esona et al., 2009, Matthijnssens et al., 2008) or bovine-to-human interspecies transmission (Ghosh et al., 2011).

The information derived from the dual typing system is not sufficient to understand the viral tropism, complete evolutionary mechanisms followed by specific rotavirus strains, the associated virulence factors of rotaviruses and many more. A rotavirus strain classification system that includes all 11 genome segments was proposed by the Rotavirus Classification Working Group (RCWG) to address such shortfalls (Matthijnssens et al., 2008). Although the full genome classification scheme is extremely useful in evolutionary and epidemiological studies of rotaviruses, the amount of time and reagents used to amplify and characterise each genome segment individually limits its application in many laboratories. Therefore, improving and/or developing procedures that can swiftly characterise the entire rotavirus genome, such as the sequence-independent genome amplification approach (Potgieter et al., 2009), will speed up the implementation of the full genome-based rotavirus classification system.

In addition to interspecies transmission, the ability of rotavirus genome segments to mutate and reassort contributes towards rotavirus strain diversity (Iturriza-Gómez et al., 2003). The exchange of the dsRNA genome segments between parent rotaviruses infecting the same host cell can result in novel progeny with divergent phenotype characteristics (Gouwe and Brantly, 1995). This phenomenon has been exploited in vitro, and has led to the production of reassortant rotaviruses (Vesikari et al., 2006b) and rotavirus-like particle (RV-VLP) experimental vaccines (Crawford et al., 1999).

Two live-attenuated rotavirus vaccines, Rotarix® (a human G1P[8] monovalent vaccine) and RotaTeq® (a bovine-human G1, G2, G3, G4, and P[8] pentavalent reassortant vaccine), have been licensed and are recommended by the World Health Organisation (WHO) for routine immunisation in different countries (World Health Organization, 2009). Although high efficacy
against severe dehydration diarrhoea with these vaccines has been reported, protection against natural rotavirus infection has not yet been achieved (Ruiz-Palacios et al., 2006, Vesikari et al., 2006c). Rotarix® and RotaTeq® are administered orally to babies and were both shown to reduce the severity of natural infection in infants. However, the presence of rotavirus maternal antibodies in breast milk may reduce the vaccine-take by binding to the vaccine antigens (Moon et al., 2010). This may lead to reduction of the vaccine efficacy.

A previously licensed rotavirus vaccine, RotaShield®, was withdrawn due to its association with intussusception (Murphy et al., 2001). To avoid this risk, the first dose of both Rotarix® and RotaTeq® has to be administered before the age of 12 weeks. However, lower risk of intussusception one week post-vaccination with both Rotarix® and RotaTeq® (Buttery et al., 2011, Greenberg, 2011, Patel et al., 2011), Kawasaki syndrome associated with Rotarix® (Center for Disease Control, 2008) and persistent rotavirus vaccine excretion and chronic diarrhoea in a patient with severe combined immune deficiency (SCID) vaccinated with RotaTeq® (Patel et al., 2010) have been reported. Although Baylis et al., (2011) found that the high amount of porcine circovirus type 1 (PCV1) DNA present in Rotarix®, which was also reported previously by Victoria et al., (2010), does not correspond to the presence of biologically active virus particles, how PCV1 may affect the infants who are receiving the vaccine doses in the long term is not known.

Differences in prevalent circulating rotavirus genotypes in developed and developing countries (Santos and Hoshino, 2005) and the emergence of new rotavirus strains (Le et al., 2008) could potentially impede the effectiveness of the live-attenuated rotavirus vaccines (Glass et al., 2006b). Although G1P[8] genotypes constitute almost 70% of the reported strains in developed countries (Gray et al., 2008), only 30% and 23% of the reported rotavirus strains from South America and Africa, respectively, are G1P[8] (Santos and Hoshino, 2005). In addition, G2, G8 and G9 have been detected with increasing frequencies in the past two decades in developing countries (Mwenda et al., 2010, Todd et al., 2010). Although significant protection against severe rotavirus gastroenteritis caused by G1, G2, G3, G4, and G9 rotavirus types has been shown (Vesikari et al., 2007), some studies seem to suggest that Rotarix® does not protect very well against G2 rotavirus strains (Kirkwood et al., 2011, Gurgel et al., 2007). However, this observation may also be caused by natural oscillations of rotavirus genotypes (Carvalho-Costa et al., 2011). In addition, formulation of the available vaccines did not include the G8 genotypes that are prevalent in Africa (Santos and Hoshino, 2005), G9 and G12 genotypes that emerged post 1990s (Vesikari et al., 2006a, Vesikari et al., 2004). Future rotavirus vaccine
development efforts should, therefore, also focus on the selection of prevalent strains in African and Asian countries in order to include their genotypes and offer preparedness against all rotavirus strains worldwide.

Experimental RV-VLP vaccines that consists of the three concentric rotavirus protein layers but devoid of the genetic material have induced both serum and faecal immunoglobulins through intranasal and intramuscular administration in mice (El-Attar et al., 2009, Bertolotti-Ciarlet et al., 2003, Johansen et al., 2003). Both homologous and heterologous protection against natural infection have been achieved by experimental RV-VLP vaccines in mice (Crawford et al., 1999). This was not the case with the licensed live-attenuated vaccines in animal studies (Glass et al., 2006a).

RV-VLPs are constructed through molecular manipulation of the genome segments of rotaviruses that encode the structural proteins (Estes and Cohen, 1989). Coding regions of VP2, VP4, VP6 and VP7 have been successfully cloned and expressed in insect cells using the baculovirus vector expression system (BVES). Expressed proteins have demonstrated similar structural and antigenic properties to native rotaviruses, and also induced immunity in animal models, as mentioned above (Kim et al., 2002, El-Attar et al., 2001, Conner et al., 1996, Crawford et al., 1994). Despite the progress made, the stability of the recombinantly produced VP4 has been problematic for RV-VLP experimental vaccine development (Parez et al., 2006). To address this, Istrate et al. (2008) utilised an RF VP8*-2/6/7-RV-VLP immunogen, first described by Parez et al. (2006), whereby the amino terminal end of the truncated VP2 was fused with VP8* (amino acid residues 1 – 241 of the VP4). VP8*-2/6/7-RV-VLPs induced specific-rotavirus serum IgA, serum IgG and faecal IgA after a single intramuscular dose. However, studies by El-attar et al. (2009), where gnotobiotic pigs were used, failed to repeat these results using rotavirus chimaeric particles in insect cells consisting of VP8* from a porcine strain fused with VP2 from a bovine strain.

RV-VLPs are a promising future strategy for developing next generation rotavirus vaccines as they are safe due to their inability to replicate once administered, therefore, circumventing the intussusception problem occasionally associated with the live-attenuated vaccine candidates. Since RV-VLP vaccines have demonstrated promising results through parenteral administration in animals, the inhibitory effect of maternal antibodies on the infectivity of live oral vaccines (Moon et al., 2010) could be addressed by using this route. Furthermore, it should be possible to generate chimaeric multivalent RV-VLP vaccines through genetic engineering by including epitopes from both local circulating and emerging rotavirus strains. A similar approach is used
to produce chimaeric influenza VLPs within 1.5 months by replacing the neuraminidase (NA) and haemagglutinin (HA) genes in a quadruple recombinant construct expressing the four influenza structural proteins with genes expressing NA and HA proteins associated with strain that have recently emerged (Latham and Galarza, 2001). Therefore, studies that can further focus on strengthening the stability of both VP4 and VP7 on RV-VLPs, and also optimisation of the recombinant rotavirus protein expression systems are imperative towards the development of the RV-VLP vaccines.

1.2. Study motivation and rationale

The majority of deaths associated with rotavirus infections occur in developing countries. Oral rehydration salts are administered to severely dehydrated children as supportive therapy to relieve dehydration. There is no treatment for rotavirus-associated gastroenteritis (Grimwood and Bines, 2007), hence, much attention has focussed on immunisation as an intervention strategy. The available live-attenuated rotavirus vaccine candidates have shown through a number of clinical trials good efficacies against severe diarrhoea and hospitalisation (Ruiz-Palacios et al., 2006, Vesikari et al., 2006c). A significant decline in diarrhoea-related deaths after vaccination with rotavirus vaccines suggests potential benefit from immunisation with rotavirus vaccines in some countries (Richardson et al., 2010, El Khoury et al., 2011). Such data led to recommendation by WHO to incorporate both Rotarix® and RotaTeq® into national immunisation programs of several countries. Although these vaccines are currently in use based on a better benefit-to-cost ratio, some shortfalls related to their usage that requires careful consideration and attention is well documented. For instance, a) unlike in developed countries, the vaccine efficacy levels achieved by both Rotarix® and RotaTeq® are fairly low in developing countries (Armah et al., 2012, Madhi et al., 2010, Zaman et al., 2010); b) variations have been reported in the rotavirus strains circulating in developed compared to developing countries. These vaccines were, however, not developed to primarily target some of the prevalent rotavirus strains frequently detected in the developing countries for instance, G8 and G9 rotaviruses (Bahl et al., 2005, Santos et al., 2005, Santos and Hoshino, 2005, Cunliffe et al., 1998). As such, the extent of cross-protection rendered by the licensed rotavirus vaccines to some of the strains prevalent in developing countries is not known; and c) whether cross-protection will be achieved against the emerging strains such as G12 rotaviruses or strains that will emerge in future is not known.
In order to improve the efficacy of the available licensed rotavirus vaccines or formulate alternative candidates, complete knowledge of the rotavirus strains circulating in developed and developing countries is vital. However, the molecular rotavirus characterisation techniques currently in use are associated with numerous shortfalls. The genotyping assays currently in use utilises sequence-dependent oligonucleotides that targets the conserved regions within the nucleotide sequences of the genome segments (Iturriza-Gomara et al., 2004, Gouvea et al., 1990). Therefore, most of the available nucleotide sequence data is partial for many strains. In addition, the nucleotide sequence data for some genome segments is not available for most strains as the whole genomes of a limited number of rotavirus strains have been fully characterised (Matthijnssens et al., 2011). Furthermore, genotype-specific oligonucleotides can not detect emerging strains that have mutations in their primer binding regions. Improving and/or combining the recent advances in molecular characterisation of dsRNA viruses, for instance, sequence-independent whole genome amplification (Potgieter et al., 2009), next generation sequencing technology (Margulies et al., 2005) and the online genotyping tool for group A rotaviruses, RotaC (Maes et al., 2009), may render possible solutions to such problems. Combining these techniques may enable easier generation of the genetic data of the whole genomes of rotavirus strains that can be useful for epidemiological, evolutionary and further understanding of the functions associated with specific rotavirus genome segments. This proposed approach can also be useful for post-vaccine introduction surveillance studies to inform the possibility of the emergence of novel strains that may result through reassortment between vaccine and wild-type rotavirus strains. The availability of the nucleotide sequences of the whole genomes of the circulating strains can be useful for designing of the next generation vaccine candidates such as chimaeric RV-VLPs. Chimaeric RV-VLPs can be prepared by incorporating multivalent genotypes of the emerging strains or strains speculated not to be well protected by some rotavirus vaccines (Kirkwood et al., 2002, Gurgel et al., 2007). In time, RV-VLP vaccines may follow the success of the virus-like particle (VLP) vaccines formulated against hepatitis B and human papilloma viruses, currently in use, that have demonstrated encouraging results towards preventing viral infections in humans (Azarkeivan et al., 2009, Paavonen et al., 2007).

Furthermore, next generation sequencing technologies with higher depth of coverage than Sanger sequencing allows identification of a complete consensus nucleotide sequence despite the presence of quasiespecies in the virus population after sequence-independent cDNA amplification of the dsRNA virus genome (Potgieter et al., 2009). Therefore, construction of RV-VLPs by utilising deduced amino acids derived from consensus nucleotide sequences
generated directly from dsRNA of local and emerging strains extracted directly from clinical specimens has a better potential in mimicking the native rotaviruses compared to RV-VLPs generated from nucleotide sequences derived from tissue culture adapted strains through Sanger sequencing technology. Therefore, complete characterisation of the genomes and preparing chimaeric RV-VLP vaccine candidates from the prevalent strains in the developing countries (Santos and Hoshino, 2005) as well as emerging rotavirus strains (Le et al., 2008) may contribute towards reducing the high rotavirus mortality cases since the burden of rotavirus disease is greatest in developing countries.

1.3. Hypotheses

This study was based two hypotheses:

1.3.1. Combining whole genome sequence-independent cDNA synthesis and amplification that uses a single set of primers, 454® pyrosequencing and the online genotyping tool, RotaC, may allow swift characterisation of the whole genome of rotavirus strains and whole genome characterisation of multiple rotavirus strains present in a single sample.

1.3.2. Use of codon-optimised nucleotide sequences of rotavirus genome segments encoding structural proteins derived from dsRNA extracted directly from clinical specimens to generate recombinant baculoviruses may allow generation RV-VLPs from any rotavirus strain without the need for tissue culture adaptation.

1.4. Aims and specific objectives of study

1.4.1. Main aims

1.4.1.1 To characterise the full genomes of human G2, G8, G9 and G12 rotaviruses associated with P[4], P[6] and P[8] genotypes and bovine G6P[5] and G8P[1] rotavirus strains in stool samples collected in Africa by using sequence-independent cDNA synthesis and amplification, 454® pyrosequencing and the automated online genotyping tool for group A rotaviruses, RotaC.

1.4.1.2 To prepare chimaeric RV-VLPs using African field rotavirus strains.
1.4.2. Specific objectives

1.4.2.1 To select human rotavirus strains with P[4], P[6] and P[8] P-types associated with G9 and G12 rotavirus strains that emerged in the past two decades, and G2 and G8 that are detected frequently in Africa.

1.4.2.2 To select bovine rotavirus strains with G5 and G8 G-types commonly characterised in cattle worldwide.

1.4.2.3 To determine and characterise the sequences of the whole genomes of the selected human and bovine rotavirus strains.

1.4.2.4 To determine the origin and identify the molecular evolution followed by the selected African human and bovine rotavirus strains.

1.4.2.4 To engineer recombinant baculoviruses containing genes coding for outer capsid VP4 and VP7 proteins of the selected human rotavirus strains, followed by production of RV-VLPs through co-infection with baculoviruses expressing VP2 and VP6 proteins prepared from strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6].

1.4.2.6 To verify baculovirus expression of recombinant rotavirus VP2, VP4, VP6 and VP7 using sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot.

1.4.2.7 To confirm whether chimaeric RV-VLPs assemble using transmission electron microscopy (TEM).

1.5. Materials, methods and the study design

1.5.1. Selection of rotavirus strains and ethical approval

The study formed part of a South Africa-Cuba science collaborative project between the National Institute for Communicable Diseases (NICD), North-West University (NWU), Stellenbosch University, University of Cape Town (UCT) and the University of Limpopo, Medical University of Southern Africa Campus (UL, Medunsa). The human and bovine rotavirus strains that were characterised in this study were solicited from the NICD and UL, Medunsa. Ethical approval was granted from the Research Ethics committees of the NICD (protocol number M060449) and Medunsa (protocol number MR58-2003) prior to collection of the stool samples. Material transfer agreements (MTA) were signed between the sample
providers (Medunsa and NICD) and the North-West University (NWU), Appendix A. The
whole genome characterisation of rotavirus strains and engineering of the chimaeric RV-LPs in
the NWU laboratory did not require ethical approval from the NWU ethics committee as the
study did not involve any human or animal subjects.

1.5.2. Research material used in this study

1.5.2.1. Materials

Rotavirus strains, baculoviruses, plasmids, bacteria and insect cells used in this study are
presented in Table 1.1. Description and references to the materials can be found in each chapter
indicated in Table 1.1.

1.5.2.2. Experimental methods

A summary of the methods used in this thesis is listed in Table 1.2. Description and references
to the materials can be found in each chapter indicated in Table 1.2.

1.6. The structure of this thesis

This thesis is presented in seven chapters that include three original peer-reviewed articles.

Following this introductory chapter;

• **Chapter two:** Presents an extensive literature review of the burden of rotavirus disease, the
epidemiology of rotavirus, the classification of rotaviruses, the interaction between
rotaviruses and their hosts, the currently licensed rotavirus vaccines and the alternative
experimental rotavirus vaccines developed against rotaviruses.

• **Chapter three:** An original article, published in *Journal of Medical Virology*, 83:2018–
2042 (2011), which elucidates the genetic origin of five human African rotavirus strains
classified in this study. The whole genomes of five rotavirus strains were classified
according to the full genome rotavirus classification scheme recently introduced by the
RCWG.

• **Chapter four:** An original article, published in *Infection, Genetics and Evolution*,
11:2072–2082 (2011), which demonstrates the robustness of combining dsRNA rotavirus
sequence-independent genome amplification, 454® pyrosequencing and the automated
Table 1.1. Rotavirus strains, baculoviruses, bacteria, plasmids and insect cell lines used in this study.

<table>
<thead>
<tr>
<th>Material</th>
<th>Use</th>
<th>Chapter number</th>
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<td><strong>Human rotavirus strains</strong></td>
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<tr>
<td>RVA/Human-wt/MWI/1473/2001/G8P[4]</td>
<td>Whole genome characterisation; source for the nucleotide sequences of genome segments 4 and 9 for RV-VLP production</td>
<td>3 6</td>
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<tr>
<td>RVA/Human-wt/ZAF/GR10924/1999/G9P[6]</td>
<td>Whole genome characterisation; source for the nucleotide sequences of genome segments 2 and 6 for RV-VLP production</td>
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<tr>
<td>RVA/Human-wt/ZAF/2371WC/2008/G9P[8]</td>
<td>Whole genome characterisation; source for the nucleotide sequences of genome segments 4 for RV-VLP production</td>
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<td><strong>Bovine rotavirus strains</strong></td>
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<td><strong>Bacteria</strong></td>
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<td><em>Escherichia coli</em></td>
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<td>AcBACACC</td>
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<td><strong>Plasmid (existing)</strong></td>
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<td>pFBq</td>
<td>Cloning host</td>
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<td><strong>Plasmid (commercially constructed)</strong></td>
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<td>pUC57G2; pUC57G8; pUC57G12</td>
<td>Host for optimised VP7 encoding nucleotide sequence</td>
<td>6</td>
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<td>pUC57P4; pUC57P8</td>
<td>Host for optimised VP4 encoding nucleotide sequence</td>
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<tr>
<td>pFBqVP2; pFBqVP4</td>
<td>Engineering of baculoviruses expressing VP7 and VP4 separately</td>
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<td>pFBqVP4/VP7</td>
<td>Engineering of baculoviruses expressing VP7 and VP4 simultaneously</td>
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<td><strong>Baculoviruses (existing)</strong></td>
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<td>pFBqVP2/VP6</td>
<td>Expression of VP2 and VP6</td>
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<td><strong>Insect cells</strong></td>
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<td><em>Spodoptera frugiperda</em> (SF9)</td>
<td>Preparation of the recombinant baculoviruses, infection and plaque titrations of the baculoviruses and RV-VLP production</td>
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<td><em>Trichoplusia ni</em> (High Five)</td>
<td>Production of RV-VLP</td>
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</table>
Table 1.2. Methods used in this study. The detailed description and references for each method can be found in each indicated chapter.

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<td>DNA transfection</td>
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<td>Purification of cDNA amplicon</td>
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<td>Quantification of cDNA</td>
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<td>Sucrose gradient centrifugation</td>
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<td>Transmission electron microscopy (TEM)</td>
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<td>Western blot techniques</td>
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<td>454® pyrosequencing with the GS FLX Titanium technology</td>
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online genotyping tool for group A rotaviruses, RotaC, in understanding the evolutionary relationship of multiple rotavirus strains that co-infected a single child.

- **Chapter five:** An original article, published online in *Veterinary Microbiology* (Available online, 6 April 2012, [http://dx.doi.org/10.1016/j.vetmic.2012.03.040](http://dx.doi.org/10.1016/j.vetmic.2012.03.040)), which reports the first whole genome characterisation of three bovine rotavirus strains from Africa. The origin of the G8P[1] and G6P[5] bovine rotavirus strains were investigated and the genomic relatedness to zoonotic bovine strains isolated from humans was also assessed.
• **Chapter six**: Describes the preparation of chimaeric RV-VLPs using insect-cell-optimised open reading frames (ORFs) encoding structural proteins of the wild-type African rotavirus strains characterised directly from stool samples. The RV-VLPs were designed to contain VP2 and VP6 core proteins prepared from strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6], whereas their outer capsid proteins (VP4 and VP7) contained a mixture of genotypes representing emerging and selected prevalent rotavirus genotypes in Africa.

• **Chapter seven**: General discussion, recommendations and conclusions.

References are provided at the end of each chapter. The references used in Chapters one, two, six and seven are listed according to the requirement stipulated in the manual for post-graduate studies of the NWU. The references used in Chapters three, four and five are provided as specified by each journal in which the articles were published.

### 1.7 Authorship

The article presented in Chapter three entitled ‘Whole genome analyses of African G2, G8, G9 and G12 rotavirus strains using sequence-independent amplification and 454® pyrosequencing’, Khuzwayo C. Jere was involved in the design of the study, laboratory assays, data analysis and manuscript writing. Luwanika Mlera was involved in data analysis and manuscript writing. A. Christiaan Potgieter isolated the dsRNA, synthesised the cDNA and sequenced the whole genome of strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6]. Nicola A. Page and Mapaseka L. Seheri were involved in the collection of stool specimens and manuscript editing. Hester G. O’Neill and Alberdina A. van Dijk were involved in the study design, data analysis, manuscript writing and supervision.

The article presented in Chapter four entitled ‘Whole genome analysis of multiple rotavirus strains from a single stool specimen using sequence-independent amplification and 454® pyrosequencing reveals evidence of intergenotype genome segment recombination’, Khuzwayo C. Jere was involved in the design of the study, laboratory assays, data analysis and manuscript writing. Luwanika Mlera was involved in data analysis and manuscript writing. Nicola A. Page was involved in the collection of study specimen and manuscript writing. Hester G. O’Neill and Alberdina A. van Dijk were involved in the study design, data analysis and manuscript writing.

The article presented in Chapter five entitled ‘Whole genome sequence analyses of three African bovine rotaviruses reveal that they emerged through multiple reassortment events...
between rotaviruses from different mammalian species’, Khuzwayo C. Jere was involved in the study design, laboratory assays, data analysis and manuscript writing. Luwanika Mlera was involved in manuscript writing. Ina Peenze was involved in the collection of stool specimens and manuscript editing. Hester G. O’Neill and Alberdina A. van Dijk were involved in the manuscript writing and supervision.

All authors’ signed the declarations on the next page:

As a co-author, I hereby approve and give consent that the mentioned articles can be used for the Ph.D. thesis of Mr K.C. Jere. I declare that my role in the study, as indicated above, is a representation of my actual contributions.

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