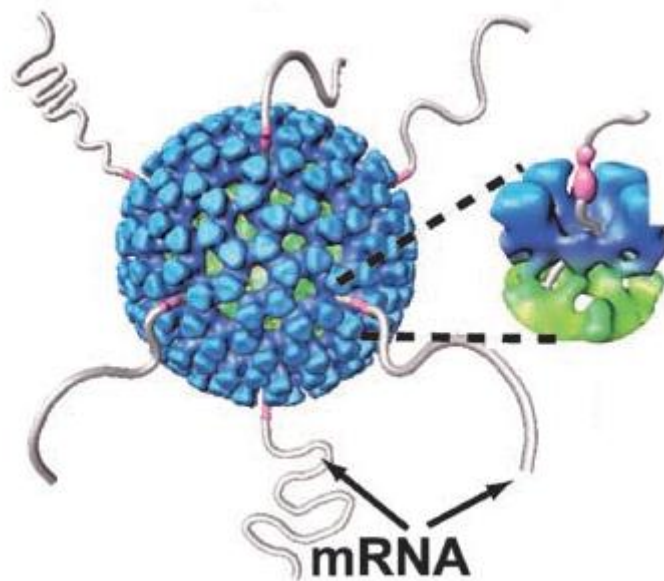


Preparatory investigations for developing a transcript-based rotavirus reverse genetics system

By
Luwanika Mlera, MSc



Thesis submitted for the degree Philosophiae Doctor in Biochemistry at
the North-West University, Potchefstroom Campus

Promoter: Prof. Alberdina A. van Dijk

Co-promoter: Dr. Hester G. O'Neill

August 2012

The cover image depicts a double-layered rotavirus particle with transcripts exiting through type I channels at five-fold vertices. Figure from Prasad *et al.*, 2006 with permission from the publisher.

Wisdom is the right use of knowledge. To know is not to be wise. Many men know a great deal, and are all the greater fools for it. There is no fool so great a fool as a knowing fool. But to know how to use knowledge is to have wisdom.

Charles Spurgeon

Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid."

Albert Einstein

To my daughters Laura Tadiwa and Adriel Maka who thought that when I was not at home I was in the lab, everywhere!

Acknowledgements

I am sincerely grateful to the following people and institutions:

My supervisor **Prof. Alberdina A. van Dijk** for providing me with the opportunity to take on this very ambitious challenge. I appreciate your invaluable and skilful guidance, encouragements and many useful discussions in and out of the 'think-tank'. I am thankful for your being always available in the various valleys and hills of my academic and non-academic life in Potchefstroom. I think that the apron strings are very strong and I will be glad to go with them!

My co-supervisor **Dr. Hester G. O'Neill** for her skilful guidance, the many discussions and encouragements. Thank you for helping me to exploit my potential and teaching me to ask hard questions! I appreciate all your academic and non-academic support.

Dr. Christiaan A. Potgieter for the useful contributions (cells, ideas, plasmids etc), discussions and encouragements.

Dr. Piet van Rijn, thank you for the BTV1 plasmids and making me so envious of the BTV reverse genetics system!

For project and personal finances, I thank the **European Foundation Initiative for Neglected Tropical Diseases (EFINTD)**, the **North-West University**, and the **Poliomyelitis Research Foundation** of South Africa.

Many thanks to my wife **Sheron** for giving up so much to see me pursue this dream. Thanks for enduring my 'love' for the lab. You were a great pillar of support and this space is inadequate to express my gratitude.

My friends: (i) **Khuzwayo Jere** (Dr!) for all the moral support, encouragement and helping to dream of great things; (ii) **Jaco Wentzel** for accepting my bothernomics and the many discussions! Be blessed in what you do next.

Finally, I thank all faculty members and students in the **Biochemistry Division** who made it interesting to be around!

Contents

	Page
Acknowledgements	v
Contents	vi
List of figures	xi
List of tables	xv
Abbreviations	xvii
Summary	xix
Opsomming	xxi
Publications associated with this study	xxiii
Conference presentations	xxiii
Chapter 1: Introduction	1
1.0. Background	1
1.1. Problem identification	2
1.2. Study motivation and rationale	5
1.3. Hypothesis	8
1.4. Aims	8
1.4.1. Specific objectives	8
1.5. Scientific approach and methodology	9
1.5.1. Viruses and propagation in cell culture	9
1.5.2. Whole-genome amplification	10
1.5.3. Determination of whole-genome consensus sequence	10
1.5.4. <i>In vitro</i> transcription and transfection of cells	10
1.5.5. Characterisation of the innate immune response to rotavirus transcripts	11
1.6. Structure of thesis	11
1.7. Publications authorship	13
Chapter 2: Literature review	15
2.0. Introduction	15
2.1. Rotavirus particle structure, genome and genome segment-protein assignment	16

2.1.1. Genome structure, organisation and encoded protein functions	15
2.1.2. Rotavirus particle architecture	21
2.2. Classification of rotaviruses	23
2.3. Rotavirus replication	27
2.3.1. Cell attachment and entry	29
2.3.2. Rotavirus genome transcription, translation and replication	30
2.3.3. Genome packaging and particle morphogenesis	33
2.4. Pathogenesis of rotavirus infection	35
2.5. Immune responses to rotavirus infection	36
2.6. Rotavirus vaccines	38
2.7. Reverse genetics	40
2.7.1. Reverse genetics systems for dsRNA viruses	43
2.8. Rotavirus reverse genetics	46
Chapter 3: Determination of the whole-genome consensus sequence of the prototype rotavirus DS-1 strain	51
3.0. Introduction	51
3.1. Materials and Methods	53
3.1.1. Cells and virus	53
3.1.2. Extraction of dsRNA	53
3.1.3. Oligo-ligation	54
3.1.4. Sequence-independent cDNA synthesis	55
3.1.5. PCR amplification of the DS-1 cDNA	55
3.1.6. Whole-genome 454 pyrosequencing and analyses	56
3.1.7. Modelling of protein and RNA structures	56
3.2. Results	59
3.2.1. Sequence-independent genome amplification and determination of the whole genome consensus sequence	59
3.2.2. General analyses of the rotavirus DS-1 genome segment consensus sequences	63
3.2.3. Analyses of DS-1 genome segments encoding structural proteins VP1– VP4, VP6 and VP7	63
3.2.4. Analyses of DS-1 genome segments encoding non-structural proteins NSP1–NSP6	69

3.3. Discussion	76
-----------------	----

Chapter 4: Characterisation of a South African rotavirus SA11 sample with 454[®] pyrosequencing and molecular clock phylogenetic analyses 81

4.0. Introduction	81
4.1. Materials and Methods	82
4.1.1. Cells, virus samples and propagation	82
4.1.2. 454 [®] pyrosequencing and data analysis	83
4.1.3. Molecular clock phylogenetic analyses	86
4.2. Results	86
4.2.1. Determination of the consensus sequence of the rotavirus SA11 genome	86
4.2.2. Comparison of the rotavirus SA11 consensus sequence to rotavirus SA11 sequences in GenBank	89
4.2.3. Molecular clock analyses and phylogenetic relationships	93
4.3. Discussion	98

Chapter 5: Transfection of cells in culture with *in vitro*-derived rotavirus transcripts 104

5.0. Introduction	104
5.1. Materials and Methods	105
5.1.1. Rotavirus DS-1 genome segment design for <i>in vitro</i> synthesis of exact mRNAs	105
5.1.2. Reconstitution of the synthetic commercial plasmids containing inserts encoding the rotavirus DS-1 genome	106
5.1.3. Transformation of ABLE C cells and plasmid amplification	107
5.1.4. Plasmid extraction	108
5.1.5. Analysis of synthetic whole-genome nucleotide sequences	108
5.1.6. <i>In vitro</i> transcription of the synthetic rotavirus DS-1 genome	110
5.1.7. Additional transcript capping reaction	111
5.1.8. Preparation of transcriptionally active rotavirus SA11 DLPs	112
5.1.9. <i>In vitro</i> transcription of the rotavirus SA11 genome using DLPs	112
5.1.10. Transfection of various cells lines with <i>in vitro</i> -derived rotavirus transcripts	113
5.1.11. Generation and <i>in vitro</i> transcription of defective transcripts	115

5.1.12. Immunocytochemistry	116
5.1.13. Western blot analyses	117
5.1.14. Evaluation of cell death pathways	118
5.2 Results	119
5.2.1. <i>In vitro</i> transcription of all 11 synthetic rotavirus DS-1 genome Segments	119
5.2.2. Preparation of rotavirus SA11 transcripts using rotavirus SA11 DLPs	122
5.2.3. The effect of transfecting rotavirus DS-1 and SA11 transcripts on cell viability	123
5.2.4. Detection of the translation of in vitro-derived rotavirus transcripts after transfection into BSR and COS-7 cells	131
5.3. Discussion	134
Chapter 6: Investigation into the innate immune responses to rotavirus Transcripts	139
6.0. Introduction	139
6.1. Materials and Methods	145
6.1.1. Cells and transcripts	145
6.1.2. Quantitative RT-PCR	145
6.1.3. Western blot analyses	146
6.1.4. Determination of the extent of PKR inhibition	146
6.2. Results	147
6.2.1. Identification of the rotavirus transcript-sensor in HEK 293H cells	147
6.2.2. The interferon response of HEK 293H cells to rotavirus transcripts	149
6.3. Discussion	153
Chapter 7: Concluding remarks and future prospects	160
References	165
Appendices	
Appendix 1	203
Appendix 2	207
Appendix 3	213

Appendix 4	220
Appendix 5	231
Appendix 6	238
Appendix 7	250

List of figures

	Page
Chapter 2	
Figure 2.1. The rotavirus dsRNA genome segments resolved by SDS-PAGE	17
Figure 2.2. Schematic illustration of the organisation of rotavirus dsRNA inside the core particle	20
Figure 2.3. Rotavirus particle architecture	22
Figure 2.4. Schematic representation of the rotavirus replication cycle	28
Figure 2.5. Model showing the rearrangement of VP4 during priming and cell entry	30
Figure 2.6. A schematic illustration of DLP budding and penetration of the ER during the addition of the outer capsid	35
Figure 2.7. Overview of the general innate immune response to rotavirus infection in the cell	37
Figure 2.8. Illustration of reverse genetics strategies for (+) ssRNA viruses	41
Figure 2.9. Reovirus reverse genetics strategies	44
Figure 2.10. A plasmid-based reverse genetics system for manipulation of rotavirus genome segment 4 (VP4)	47
Figure 2.11. A rotavirus reverse genetics system for the recovery of a genome segment 8 recombinant virus with dual selection	49
Chapter 3	
Figure 3.1. Gel electrophoresis of the rotavirus DS-1 genome	59
Figure 3.2. A representation of a contig alignment used in the determination of the consensus sequence	60
Figure 3.3. Models of VP4 protein structures predicted using Chimera UCSF software	67
Figure 3.4. A representation of sequence reads suggesting potential minor population variants in genome segment 4 (VP4)	68
Figure 3.5. Alignment of the deduced NSP1 amino acid consensus	

	sequence (DS-1 NSP1 CS) with rotavirus DS-1 sequences (EF672578 and L18945) and selected rotavirus DS-1-like sequences (GER1H-09, N26-02 and B1711) in GenBank	70
Figure 3.6.	Models of NSP3 (genome segment 7; A) and NSP2 (genome segment 8; B) structures generated using Chimera UCSF software	72
Figure 3.7.	Alignment of the consensus genome segment 10 (NSP4) nucleotide sequence with genome segment 10 sequences from GenBank	73
Figure 3.8.	Alignment of genome segment 11 consensus nucleotide sequence (DS-1-GS 11-CS) with rotavirus DS-1 genome segment 11 sequences from GenBank (M33608 and EF672583)	74
Figure 3.9.	Comparison of the secondary RNA structures of the consensus genome segment 11 and M33608 predicted with RNAfold	75
 Chapter 4		
Figure 4.1.	A photograph of the rotavirus SA11 samples obtained from Diarrhoeal Pathogens Research Unit indicating the only information received for these samples	83
Figure 4.2.	The <i>mVISTA</i> visualisation alignment comparing the nucleotide sequences of SA11-N2 and SA11-N5 (indicated by bracket) to SA11 sequences in GenBank	88
Figure 4.3.	Contig alignments depicting the identification of novel minority coding sequences in rotavirus SA11 in genome segments 4 (VP4), 9 (VP7) and 10 (NSP4)	93
Figure 4.4.	MCC trees constructed with Bayesian MCMC framework in BEAST software, to depict the molecular clock evolutionary relationships between SA11-N2, SA11-N5 and rotavirus SA11 sequences obtained from GenBank	97
 Chapter 5		
Figure 5.1.	Schematic representation of cDNA genome segment engineering to facilitate <i>in vitro</i> transcription by T7 polymerase	106

Figure 5.2.	Plasmid map of pUC57 (ampicillin resistant)	107
Figure 5.3.	Agarose gel (1%) electrophoresis of restriction enzyme digestions of pUC57 plasmids containing synthetic rotavirus DS-1 genome segments	120
Figure 5.4.	5.4. Agarose (1%) formaldehyde gel electrophoresis of <i>in vitro</i> -derived rotavirus DS-1 transcripts	121
Figure 5.5.	Purification of rotavirus SA11 DLPs	122
Figure 5.6.	Agarose (1%) formaldehyde gel electrophoresis of rotavirus SA11 mRNA transcripts (lane 2) obtained from <i>in vitro</i> transcripton using purified rotavirus SA11 DLPs	123
Figure 5.7.	Comparison of the effect of 2-aminopurine (2-AP) and imidazolo-oxindole PKR inhibitor (C16) on BSR cell death following transfection with rotavirus transcripts	127
Figure 5.8.	Evaluation of cell death mechanisms in transfected COS-7 cells	131
Figure 5.9.	Immunological detection of rotavirus protein expression	133
 Chapter 6		
Figure 6.1.	Schematic illustration of the domain structures of the retinoic-acid inducible gene I-like receptors	140
Figure 6.2.	Interferon pathways that induce an antiviral state in response to viral pathogen associated molecular patterns	141
Figure 6.3.	A proposed model of events occurring during early innate immune recognition of rotavirus leading to IFN production	143
Figure 6.4.	Western blot analysis of retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) expression following the transfection of HEK 293H cells with rotavirus DS-1 genome segment 6 (VP6), rotavirus SA11 and BTV-1 segment 3 (VP3) transcripts	148
Figure 6.5.	Western blot analysis of expression of interferon regulatory factors IRF-3 and IRF-7	149
Figure 6.6.	Relative quantities of cytokine-encoding mRNA expression induced in HEK 293H cells by rotavirus DS-1 genome segment 6, rotavirus SA11 and BTV1 S3 transcripts	152
Figure 6.7.	Relative quantities of cytokine-encoding mRNA expression	

induced in HEK 293H cells by rotavirus DS-1 genome
segment 6, rotavirus SA11 and BTV1 S3 transcripts

159

List of tables

	Page
Chapter 2	
Table 2.1. The rotavirus genome segments, encoded proteins and their known functions	18
Table 2.2. Variations between the 5'- and 3'-terminal end sequence of selected rotavirus strains of the different groups	19
Table 2.3. Classification of dsRNA viruses within the <i>Reoviridae</i> family	24
Table 2.4. The whole-genome genotype constellation of selected prototype rotavirus strains	26
Chapter 3	
Table 3.1. Rotavirus DS-1 nucleotide Sequences retrieved from GenBank for comparison with the consensus DS-1 nucleotide sequence obtained by pyrosequencing	58
Table 3.2. Summary of the rotavirus DS-1 whole-genome consensus sequence data, obtained with 454 [®] pyrosequencing, in comparison to DS-1 sequences in GenBank	62
Table 3.3. Nucleotide differences observed between the genome segment 4 consensus sequence and the genome segment 4 sequences in GenBank	65
Chapter 4	
Table 4.1. List of accession numbers of the SA11 rotavirus consensus sequences determined in this study, and sequences retrieved from GenBank	85
Table 4.2. Comparison of the consensus nucleotide and deduced amino acid sequences of SA11-N2 and SA11-N5 to sequences of SA11-H96	90
Chapter 5	
Table 5.1. Restriction enzymes used for restriction digestion analysis of the pUC57 vectors containing the respective 11 different	

	DS-1 rotavirus genome segments	109
Table 5.2.	List of primers used to sequence the rotavirus DS-1 genome segment inserts in the pUC57 vector	110
Table 5.3.	Summary of representative transfections performed in various cell lines using synthetic rotavirus DS-1 whole-genome transcripts	125
Table 5.4	Summary of transfections performed with <i>in vitro</i> DLP-derived rotavirus SA11 transcripts	126
Table 5.5	Cell death following transfection of single genome segment synthetic transcripts derived from rotavirus and bluetongue virus	129

Abbreviations

AHSV: African horse-sickness virus
ATP: adenosine triphosphate
bp: base pairs
BTV: bluetongue virus
CD4: cluster of differentiation 4
CD8: cluster of differentiation 8
CPE: cytopathic effect
CsCl: cesium chloride
CTP: cytosine triphosphate
DNA: deoxyribonucleic acid
DLP: double-layered particle
dsRNA: double-stranded ribonucleic acid
EB: elution buffer
EDTA: ethylene-diamine-tetra-acetic acid
ELISA: enzyme-linked immunosorbent assays
ER: endoplasmic reticulum
FBS: foetal bovine serum
ffu: focus forming units
GTP: guanosine triphosphate
HA: haemagglutinin
HEK: human embryonic kidney
HIV: human immunodeficiency virus
IFN: interferon
IgA: Immunoglobulin A
IgG: Immunoglobulin G
IgY: immunoglobulin Y
kDa: kiloDalton
LPG-2: laboratory of physiology and genetics gene 2
MCRI: Murdoch Children's Research Institute
MDA5: melanoma differentiation associated gene 5
MOI: multiplicity of infection

ml: millilitre
NA: neuraminidase
NTPase: nucleoside triphosphatase
NSP: non-structural protein
ORF: open reading frame
PABP: poly (A) binding proteins
PAGE: polyacrylamide gel electrophoresis
PBS: phosphate-buffered saline
pDC: plasmacytoid dendritic cell
PCR: polymerase chain reaction
pfu: plaque forming units
PKR: dsRNA-dependent kinase
RdRp: RNA-dependent RNA polymerase
RIG-I: retinoic inducible gene I
RNA: ribonucleic acid
RNAi: ribonucleic acid interference
rpm: revolutions per minute
qRT-PCR: quantitative reverse transcription polymerase chain reaction
siRNA: small interfering ribonucleic acid
SA11: simian agent 11
SAGE: Strategic Advisory Group of Experts
ssRNA: single-stranded ribonucleic acid
SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis
siRNA: small interfering RNA
TLP: triple-layered particle
TBST: Tris buffered saline with Tween-20
U: unit
USA: united States of America
UTR: untranslated terminal region
VP: structural viral protein
°C: degrees Celsius

Summary

Reverse genetics systems that are based on either viral transcripts or cDNA genome segments cloned in plasmids have recently been reported for some of the dsRNA viruses of the *Reoviridae* family, namely African horsesickness virus, bluetongue virus and orthoreovirus. For rotaviruses, three reverse genetics systems which only allow the manipulation of a single genome segment have been described. These rotavirus single genome segment reverse genetics systems are not true stand-alone systems because they require a helper virus and a recombinant virus selection step. A true selection-free, plasmid- only or transcript-based reverse genetics system for rotaviruses is lacking.

This study sought to identify and characterise the factors that need to be understood and overcome for the development of a rotavirus reverse genetics system using mRNA derived from the *in vitro* transcription of a consensus nucleotide sequence as well as from double-layered particles. The consensus whole genome sequence of the prototype rotavirus DS-1 and SA11 strains was determined using sequence-independent whole genome amplification and 454[®] pyrosequencing. For the rotavirus DS-1 strain, a novel isoleucine in a minor population variant was found at position 397 in a hydrophobic region of VP4. NSP1 contained seven additional amino acids MKSLVEA at the N-terminal end due to an insertion in the consensus nucleotide sequence of genome segment 5. The first 34 nucleotides at the 5'-terminus and last 30 nucleotides at the 3'-terminal end of genome segment 10 (NSP4) of the DS-1 strain were determined in this study. The consensus genome segment 11 (NSP5/6) sequence was 821 bp in length, 148 bp longer than previously reported. The 454[®] pyrosequence data for a rotavirus SA11 sample with no known passage history revealed a mixed infection with two SA11 strains. One of the strains was a reassortant which contained genome segment 8 (NSP2) from the bovine rotavirus O agent. The other ten consensus genome segments of the two strains could not be differentiated. Novel minor population variants of genome segments 4 (VP4), 9 (VP7) and 10 (NSP4) were identified. Molecular clock phylogenetic analyses of the rotavirus SA11 genomes showed that the two SA11 strains were closely related to the original SA11-H96 strain isolated in 1958.

Plasmids containing inserts of the consensus cDNA of the rotavirus DS-1 strain were purchased and used to generate exact capped transcripts by *in vitro* transcription with a T7 polymerase. Wild-type transcripts of rotavirus SA11 were obtained from *in vitro* transcription using purified rotavirus SA11 double-layered particles. The purified rotavirus DS-1 and SA11 transcripts were transfected into BSR, COS-7 and MA104 cells. Work on MA104 cells was discontinued due their very low transfection efficacy. In BSR and COS-7 cells, rotavirus DS-1 and SA11 transcripts induced cell death. However, no viable rotavirus was recovered following attempts to infect MA104 cells with the BSR and COS-7 transfected cell lysates. The cell death was determined to be due to apoptotic cell death mechanisms. Immunostaining showed that the DS-1 genome segment 6 (VP6) and SA11 transcripts were translated in transfected BSR and COS-7 cells. Based on visual inspection, the translation seemed to be higher in the retinoic acid-inducible gene-1 (RIG-I) deficient BSR cells than in COS-7 cells. This suggested that the transfection of rotavirus transcripts induced an innate immune response which could lead to the development of an antiviral state. Therefore, the innate immune response to rotavirus transcripts was investigated in HEK 293H cells using qRT-PCR and western blot analyses. Results of this investigation showed that RIG-I, but not MDA5 sensed rotavirus transcripts in transfected HEK 293H cells. Furthermore, rotavirus transcripts induced high levels of cellular mRNA encoding the cytokines IFN-1 β , IFN- λ 1, CXCL10 and TNF- α . Other cytokines namely, IFN- α , IL-10, IL-12 p40 and the kinase RIP1 were not significantly induced. Inhibiting the RNA-dependent protein kinase R (PKR) reduced the induction of cytokines IFN-1 β , IFN- λ 1, CXCL10 and TNF- α , but the expression levels were not abrogated. The importance of a consensus sequence and the insights gained in the current study regarding the role of the innate immune response after transfection of rotavirus transcripts into cells in culture, should aid the development of a true rotavirus reverse genetics system.

Key words

Rotavirus; rotavirus DS-1 strain; rotavirus SA11 strain; dsRNA; sequence-independent genome amplification; 454[®] pyrosequencing; consensus genome sequence; *in vitro* transcription; reverse genetics; transfection; innate immune response; antiviral state.

Opsomming

Tru-genetika sisteme wat gebaseer is op transkripsie produkte van virusse of kDNS genoom segmente wat in plasmiede gekloneer is, is onlangs gerapporteer vir sommige ddRNS virusse van die *Reoviridae* familie, naamlik Afrika perdesiekte virus, bloutongvirus en ortoreovirus. Vir rotavirusse is drie tru-genetika sisteme beskryf wat toelaat dat net 'n enkele genoom segment gemanipuleer kan word. Hierdie rotavirus enkel genoom segment tru-genetika sisteme is nie ware alleenstaande sisteme nie, want hulle benodig 'n helper virus en 'n stap om rekombinante virus te selekteer. Daar is nog nie 'n ware seleksie-vrye, slegs plasmied of transkrip gebaseerde tru-genetika sisteem vir rotavirusse nie.

Met hierdie studie is gepoog om die faktore te identifiseer en te karakteriseer wat verstaan en oorkom moet word in die ontwikkeling van 'n rotavirus tru-genetika sisteem wat mRNA gebruik wat afkomstig is van *in vitro* transkripsie van 'n konsensus nukleotied volgorde sowel as van dubbellaag partikels. Die konsensus heel-genoom volgorde van die prototipe rotavirus DS-1 en SA11 stamme is bepaal deur volgorde-onafhanklike heel genoom vermeerdering en 454[®] pirovolgorde bepaling te gebruik. In die rotavirus DS-1 stam is 'n nuwe isoleusien gevind in 'n minderheids populasie variant in posisie 397 in 'n hidrofobe gebied van VP4. NSP1 het sewe addisionele aminosure MKSLVEA in die N-terminale ent weens 'n invoeging in die konsensus nukleotiedvolgorde van genoom segment 5. Die eerste 34 nukleotiede van die 5'-terminus en die laaste 30 nukleotiede van die 3'-terminale ent van genoom segment 10 (NSP4) van die DS-1 stam is in hierdie studie bepaal. Die konsensus genoom segment 11(NSP5/6) volgorde was 821 bp lank, 148 bp langer as wat vantevore gerapporteer is. Die 454[®] pirovolgorde bepalings data van 'n SA11 rotavirus monster sonder enige passasie geskiedenis het 'n gemenge infeksie met twee SA11 stamme onthul. Een van die stamme was 'n hergegroepeerde virus wat genoom segment 8 (NSP2) van die bees rotavirus O agent bevat het. Die ander tien konsensus genoom segmente kon nie onderskei word nie. Nuwe minderheids populasie variante van genoom segmente 4 (VP4), 9 (VP7) en 10 (NSP4) is geïdentifiseer. Molekulêre klok filogenetiese analyses van die SA11 genoom het getoon dat die twee SA11 stamme naby verwant is aan die oorspronklike SA11-H96 stam wat in 1958 geïsoleer is.

Plasmiede wat insetsels van die konsensus kDNS van die rotavirus DS-1 stam bevat, is aangekoop en gebruik om presiese bedekte ("capped") transkripte te genereer deur *in vitro* transkripsie met 'n T-7 polimerase. Wilde-tipe transkripte van rotavirus SA11 is verkry van *in vitro* transkripsie deur gebruik te maak van gesuiwerde SA11 dubbellaag partikels. Die gesuiwerde rotavirus DS-1 en SA11 transkripte is getransfekteer in BSR, COS-7 en MA104 selle. Werk met die MA104 selle is gestaak weens hulle baie lae transfeksie effektiwiteit. In BSR en COS-7 selle het DS-1 en SA11 transkripte seldood geïnduseer. Daar is egter geen lewensvatbare rotavirus herwin na pogings om MA104 selle te infekteer met die BSR en COS-7 getransfekteerde sel-lisate nie. Daar is vasgestel dat die seldood toe te skryf was aan apoptotiese seldood meganismes. Immunokleuring het gewys dat die DS-1 genoom segment 6 (VP6) en SA11 transkripte getransleer is in getransfekteerde BSR en COS-7 selle. Gebasseer op visuele inspeksie, het dit gelyk asof die translase in die retinoësuur-induseerbare geen-1 (RIG-1) defekte BSR selle hoër was as in COS-7 selle. Dit was aanduidend daarvan dat die transfeksie van rotavirus transkripte 'n ingebore immuunrespons geïnduseer het wat kan lei tot die ontwikkeling van 'n teenvirus staat. Daarom is die ingebore immuunrespons op rotavirus transkripte bestudeer in HEK 293H selle deur qRT-PCR en westelike klad analyses. Resultate van hierdie ondersoek het gewys dat rotavirus transkripte deur RIG-1, maar nie MDA5 waargeneem word in getransfekteerde HEK 293H selle. Daarbenewens toon die resultate aan dat die rotavirus transkripte hoë vlakke van sellulêre mRNA geïnduseer het wat kodeer vir die sitokine IFN-1 β , IFN- λ 1, CXCL10 en TNF- α . Ander sitokine, naamlik IFN- α , IL-10, IL-12 p40 en die kinase RIP1 is nie betekenisvol geïnduseer nie. Onderdrukking van die RNS-afhanklike proteïen kinase R (PKR) het die induksie van die sitokine IFN-1 β , IFN- λ 1, CXCL10 en TNF- α verlaag, alhoewel die vlakke van uitdrukking nie heeltemal onderdruk was nie. Die belang van die konsensus genoom opeenvolging en die insae verkry in die huidige studie betreffende die rol van die ingebore immuunrespons na transfeksie van rotavirus transkripte in selkulture behoort te help met die ontwikkeling van 'n ware rotavirus tru-genetika sisteem.

Sleutelwoorde

Rotavirus; rotavirus DS-1 stam; rotavirus SA11 stam; ddRNS; volgorde-onafhanklike genoom vermeerdering; 454[®] pirovolgordebepaling; konsensus genoom volgorde; *in*

vitro transkripsie; tru-genetika; transfeksie; ingebore immuunrespons; teenvirus
toestand

Publications associated with this study

- Jere, K.C., **Mlera, L.**, O'Neill, H.G., and van Dijk A.A (2012). Whole genome sequence analyses of three African bovine rotaviruses reveal that they emerged through multiple reassortment events between rotaviruses from different mammalian species. *Veterinary Microbiology*, 159: 245–250
- Jere, K.C., **Mlera, L.**, Page, N.A., van Dijk A.A., and O'Neill, H.G. (2011) 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. *Infection, Genetics and Evolution*, 11(8): 2072-2082.
- Jere, K.C., **Mlera, L.**, O'Neill, H.G., Potgieter, A.C., Page, N.A., Seheri, M.L., and van Dijk A.A. (2011). Whole genome analyses of African G2, G8, G9 and G12 rotavirus strains using sequence-independent amplification and 454[®] pyrosequencing. *Journal of Medical Virology*, 83: 2018-2042.
- **Luwanika Mlera**, Khuzwayo C. Jere, Alberdina A. van Dijk, and Hester G. O'Neill (2011). Determination of the whole-genome consensus sequence of the prototype DS-1 rotavirus using sequence-independent genome amplification and 454[®] pyrosequencing. *Journal of Virological Methods* 175, 266–271.
- Nyaga, M.M., Jere, K.C., Peenze, I., **Mlera, L.**, Van Dijk, A.A., Seheri, M.L., and Mphahlele, M.J. Sequence analysis of the complete genomes of five African human G9 rotavirus strains. Manuscript in preparation (Submitted to *Infection, Genetics and Evolution*, July 2012).
- **Luwanika Mlera**, Hester G. O'Neill, Khuzwayo C. Jere, and Alberdina A. van Dijk (2011). Evolution of the consensus whole-genome of a South African rotavirus SA11 obtained with 454[®] pyrosequencing: evidence of a mixed infection with two close derivatives of the SA11-H96 strain. *Archives of Virology*, Accepted 18 October 2012, Available online 23 December 2012.

Conference presentations

- **L. Mlera**, A. A. van Dijk and H. G. O'Neill. Consensus genome sequence of SA11 rotavirus determined with sequence-independent genome amplification and 454[®] pyrosequencing. **Virology Africa 2011 Conference**, Cape Town Waterfront, 29 November 2011–2 December 2011 [Poster].
- Jere, K.C., **Mlera, L.**, Page, N.A., van Dijk A.A., and O'Neill, H.G. Evidence that mixed infections promotes generation of novel strains through intragenogroup and intergenogroup genome recombination revealed through whole genome characterization of multiple rotavirus strains from a single stool specimen. **Vaccine for Enteric Diseases**, 13th -16th September 2011, The Novotel Cannes Montfleury, Cannes, France [Poster].
- Jere, K.C., (presenter) **Mlera, L.**, Page, N.A., van Dijk A.A., & O'Neill, H.G. Evidence that mixed infections promotes generation of novel strains through intragenogroup and intergenogroup genome recombination revealed through whole genome characterization of multiple rotavirus strains from a single stool specimen. **The Malawi-Liverpool Wellcome Trust Research 2011 Conference**, 18th -24th September, 2011, Club Makokola, Mangochi, Malawi [Oral].
- **L. Mlera**, K.C. Jere, A.C. Potgieter, A.A. van Dijk and H.G. O'Neill. Molecular characterization of the DS-1 rotavirus strain using 454[®] pyrosequencing technology. **9th International Rotavirus Symposium**, Johannesburg, South Africa, 2-3 August 2010 [Poster].
- **L. Mlera**, K.C. Jere, A.C. Potgieter, A.A. van Dijk and H.G. O'Neill. Molecular characterization of the DS-1 rotavirus strain using 454[®] pyrosequencing technology. **6th African Rotavirus Symposium**, Johannesburg, South Africa, 4 August 2010 [Poster].
- Jere, K.C., **Mlera, L.**, O'Neill, H.G., Potgieter, A.C., Page, N.A., Peenze, I., & van Dijk A.A. Sequence-independent amplification and ultra-deep sequencing of the emerging and prevalent African rotavirus strains. **6th African rotavirus symposium**, 4th August 2010, National Institute for Communicable Diseases, Johannesburg, South Africa [Poster].
- Jere, K.C., **Mlera, L.**, O'Neill, H.G., Potgieter, A.C., Page, N.A., Peenze, I., & van Dijk A.A. Sequence-independent amplification and ultra-deep sequencing of the emerging and prevalent African rotavirus strains. **9th International rotavirus symposium**, 2-3rd August 2010, Johannesburg, South Africa [Poster].