Assessment of selected environmental water samples in Kenya for the presence of clinically important enteric viruses

by

NICHOLAS MUKARIA KIULIA

Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae

MSc (Medical Virology)

Department of Medical Virology

Faculty of Health Sciences

University of Pretoria

Pretoria

October 2013

© University of Pretoria

DECLARATION

I, Nicholas Mukaria Kiulia, declare that this work was not copied or repeated from any other studies either from national or international publications. Procedures were carried out in accordance with the ethical rules as prescribed by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

Signature:.......................................

Date: 2014-07-04 ....

© University of Pretoria

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisor Prof Maureen B. Taylor for her advice, guidance and encouragement for the entire period of this project and its write up.

I thank Drs Janet Mans, Nicola Page, Nicksy Gumede-Moeletsi, Walda van Zyl, Marianne Wolfaardt and Ms Lerato Seakamela for their support, dedication, patience and their guidance in the technical aspects of this project.

I wish to thank Drs Jason Mwenda and Atunga Nyachieo for their mentorship.

Finally, many thanks to my wife Maureen Obara for her support and encouragement and my family, friends and fellow students for their interest and encouragement.

The research for this dissertation was funded in part by:

i) National Research Foundation/National Council for Science and Technology, Kenya: South Africa/Kenya Research Programme:

This work is based, in part, on the research supported by the National Research Foundation. Any opinion, finding and conclusion or recommendation expressed in this material is that of the author and the NRF/NACOSTI does not accept any liability in this regard.

ii) Merck & Co inc, USA, as well as partial support from the Institute of Primate Research, Kenya

© University of Pretoria

ASSESSMENT OF SELECTED ENVIRONMENTAL WATER SAMPLES IN KENYA FOR THE PRESENCE OF CLINICALLY IMPORTANT ENTERIC VIRUSES

by

NICHOLAS MUKARIA KIULIA

SUPERVISOR: PROF MB TAYLOR
DEPARTMENT: MEDICAL VIROLOGY
DEGREE: MAGISTER SCIENTIAE (MEDICAL VIROLOGY)

SUMMARY

Worldwide it is estimated that 1.1 billion people drink unsafe water and 1.7 million deaths are caused by drinking of unsafe water and poor sanitation. Enteric viruses are transmitted by the faecal-oral route and their spread has in part been attributed to the consumption of contaminated food and drinking water. Viruses are a major cause of waterborne disease but the health impact of waterborne viral infections is underestimated. Waterborne illness associated with viruses are more common than those caused by bacteria making it important to estimate the prevalence and diversity of enteric viruses in environmental water sources in order to assess the potential public health risks posed by the contaminated water sources. In Kenya, despite these risks data regarding the occurrence of the viruses in environmental water sources is limited, and in addition, data on the prevalence and molecular epidemiology of enteroviruses (EVs) and noroviruses (NoVs) in the clinical and environmental settings is lacking. Therefore, in this investigation the microbiological quality, the prevalence and molecular epidemiology of selected clinically relevant enteric viruses namely, EVs, NoVs and

© University of Pretoria

rotaviruses (RVs) in Kenyan urban and rural high risk water sources, namely the Mboone, Mutoine and Nairobi rivers and water from the Dadaab refugee camp, were investigated. Microbial indicator organisms, namely *Escherichia. coli* and total coliforms were detected at levels >200 cfu/mℓ in 100% (40/40) of the samples thereby exceeding the World Health Organization (WHO) recommended guidelines for drinking water (<1 most probable number/100 mℓ), suggesting that there is gross faecal contamination in these Kenyan water sources. The RVs were detected in 85% (34/40) of the samples and it was the most predominant virus detected being identified in 83% of the Mboone river samples, 100% of the Mutoine river samples, 83% of the Nairobi river samples and 50% of the household water from the Dadaab refugee camp. The G types detected were G1 and G9 and mixed G1+G9 being detected in the Nairobi river. The P types detected were, P[4], P[6] and P[8], with mixed P types P[4]+P[6], P[4]+P[8], P[6]+P[8] and P[4]+P[6]+P[8] being detected in the three surface water sources. Noroviruses were detected in 63% (25/40) of the selected water samples, with 60% (24/40) of the samples positive for NoV GII and 20% (8/40) for NoV GI. A number of genotypes were identified, namely, NoV GI.1, NoV GI.3 and NoV GI.9 and NoV GII.4, GII.6, GII.12, GII.16 and GII.17 with NoV GII.17 predominating. Enteroviruses were detected in 58% (23/40) of the samples by direct real-time reverse transcriptase-polymerase chain analysis of the recovered virus concentrates and were identified in the three rivers (Mboone, Mutoine and Nairobi rivers) accounting for 50%, 83% and 58% of water samples, respectively, but not in the borehole or household water from Dadaab. No polioviruses (PVs) were identified using the recommended WHO methods designed for identification of PVs and differentiation between vaccine-derived PVs and wild-type PVs in clinical specimens. In conclusion, this is the first comprehensive report on the molecular epidemiology of NoVs in Kenyan water sources. From this study it is evident that further nationwide studies are necessary to fully establish the prevalence, distribution and clinical relevance of enteric viruses in Kenyan water sources.

© University of Pretoria

PRESENTATIONS AND PUBLICATIONS

Presentations

Kiulia NM, Mans J, Page NA, Gumede-Moeletsi N, Mwenda JM, Taylor MB. Enteric viruses in selected surface waters in urban and rural Kenya [Presentation]. 19th International Scientific Conference on "Basic and Clinical Research for Improved Health hosted by the Institute of Primate Research and Kenya Agricultural Research Institute-Trypanosomiasis Research Centre 11-13 September 2013, Southern Sun, Mayfair, Nairobi, Kenya.

© University of Pretoria

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENTS	ii
SUMMARY	iii
PRESENTATIONS AND PUBLICATIONS	v
TABLE OF CONTENTS	vi
ABBREVIATIONS AND SYMBOLS	x
LIST OF FIGURES	xiii
LIST OF TABLES	xv
CHAPTER 1: LITERATURE REVIEW	1
1.1 GENERAL INTRODUCTION	1
1.2 ROTAVIRUS	2
1.2.1 History	2
1.2.2 Virology	3
1.2.3 Classification	3
1.2.4 Pathogenesis and immunity	5
1.2.5 Clinical features	6
1.2.6 Laboratory diagnosis	6
1.2.7 Management, treatment and prevention	7
1.2.8 Epidemiology	8
1.3 NOROVIRUS	10
1.3.1 History	10
1.3.2 Virology	10
1.3.3 Classification	11
1.3.4 Pathogenesis and immunity	14
1.3.5 Clinical features	15
1.3.6 Laboratory diagnosis	16
1.3.7 Management, treatment and prevention	16

© University of Pretoria

1.3.8 Epidemiology 17

1.4 ENTEROVIRUS 20

1.4.1 History 20

1.4.2 Virology 20

1.4.3 Classification 21

1.4.4 Pathogenesis and immunity 21

1.4.5 Clinical features 22

1.4.6 Laboratory diagnosis 23

1.4.7 Management, treatment and prevention 23

1.4.8 Epidemiology 25

1.4.8.1 Non polio enteroviruses 25

1.4.8.2 Polioviruses 26

1.4.8.2.1 Global polio eradication initiatives 26

1.4.8.2.2 Polio eradication efforts in Kenya 26

1.4.8.2.3 Environmental surveillance of polioviruses 27

1.5 RECOVERY AND DETECTION OF ENTERIC VIRUSES IN WATER 28

1.5.1 Recovery of enteric viruses in water 28

1.5.1.1 Ultracentrifugation 28

1.5.1.2 Entrapment by ultrafiltration 29

1.5.1.3 The glass wool adsorption-elution technique 29

1.5.2 Detection and molecular characterisation of enteric viruses in water 30

1.5.2.1 Viral isolation in cell cultures 30

1.5.2.2 Electron microscopy and immunological-based assays 30

1.5.2.3 Molecular-based assays 30

1.6 MICROBIAL INDICATORS OF FAECAL POLLUTION 31

1.7 MOTIVATION FOR THIS INVESTIGATION 32

1.8 HYPOTHESIS 33

1.8.1 Null hypothesis (HO) 33

1.8.2 Alternative hypothesis (HA) 33

1.9 AIM OF INVESTIGATION 33

© University of Pretoria

CHAPTER 2: MATERIALS AND METHODS 34
2.1 ETHICAL APPROVAL 34
2.2 AREA OF STUDY AND SITE SELECTION 34
2.3 SAMPLE COLLECTION 35
2.4 SAMPLE PROCESSING AND VIRUS RECOVERY 35
2.5 MICROBIAL INDICATOR ORGANISMS 36
2.6 VIRUS ISOLATION 37
2.6.1 Cell cultures 37
2.6.1.1 Human hepatoma cell line (PLC/PRF/5) 37
2.6.1.2 Buffalo green monkey kidney (BGM) 37
2.6.1.3 L20B 37
2.6.1.4 Vero African Green Monkey cell line 38
2.6.2 Cell culture propagation and maintenance 38
2.6.3 Viral isolation and detection 38
2.7 VIRUS DETECTION 39
2.7.1 Nucleic acid extraction 39
2.7.1.1 Automated nucleic acid extraction 39
2.7.1.2 Manual nucleic acid extraction 39
2.7.2 Virus amplification 39
2.7.2.1 Mengoviruses 39
2.7.2.2 Enteroviruses 40
2.7.2.3 Rotaviruses 40
2.7.2.4 Noroviruses 40
2.7.3 Quality control 41
2.8 VIRUS CHARACTERISATION 41
2.8.1 Rotavirus genotyping 41
2.8.2 Poliovirus genotyping 42
2.8.3 Norovirus genotyping 43
2.8.3.1 Virus amplification 43
2.8.3.2 Cloning and sequencing 46
2.8.3.3 Phylogenetic analysis 48

© University of Pretoria

2.8.3.4 Nucleotide sequence accession numbers 50
2.8.4 Statistical analysis 50

CHAPTER 3: RESULTS 51
3.1 Water samples 51
3.2 Microbiological analysis 52
3.3 Viral isolation in cell culture 53
3.4 Viral detection 53
3.5 Viral characterisation 56
3.5.1 Rotavirus 56
3.5.2 Poliovirus 57
3.5.3 Norovirus 57
3.5.3.1 Nucleotide sequence analysis of region C of the NoV capsid gene 57

CHAPTER 4: DISCUSSION AND CONCLUSIONS 65
CHAPTER 5: REFERENCES 72

APPENDIX A: 112
A.1 Kiulia NM, Mans J, Page NA, Gumede-Moeletsi N, Mwenda JM, Taylor MB. Enteric viruses in selected surface waters in urban and rural Kenya

APPENDIX B: 114
ETHICAL APPROVAL

© University of Pretoria

ABBREVIATIONS AND SYMBOLS

AFP	acute flaccid paralysis
BGM	Buffalo green monkey kidney
BLAST	Basic Local Alignment Search Tool
BLEIA	automated bioluminescent enzyme immunoassay
bOPV	bivalent oral polio vaccine
bp	base pair
CDC	Centers for Disease Control and Prevention
CSF	Cerebrospinal fluid
cfu	colony forming units
CI	confidence interval
CD	Compact Dry
CV	Coxsackievirus
CNS	central nervous system
Ct	cycle threshold
CPE	cytopathic effect
°C	degrees celcius
DNA	deoxyribonucleic acid
ds	double stranded
E	Echovirus
EAC	external amplification control
EIA	enzyme immune assay
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EPA	Environmental Protection Agency
EPI	expanded programme of immunisation
EV	Enterovirus
FCV	feline calicivirus
GAVI	Global Alliance for Vaccines and Immunization
GPEI	Global Poliomyelitis Eradication Initiative
GPLN	Global Polio Laboratory Network
G	Genogroup
g	Gram
h	Hour
IEM	Immunoelectron microscopy
IPV	inactivated polio vaccine
ITD	intratypic differentiation
HEV	human enterovirus

© University of Pretoria

HIV	human immunodeficiency virus
IF	immunofluorescence
HAdV	human adenovirus
HAstV	human astrovirus
HAV	hepatitis A virus
HBGA	histo-blood group antigen
HCℓ	hydrochloric acid
HuCV	human calicivirus
IAC	internal amplification control
IEM	Immunoelectron microscopy
Ig	immunoglobulin
IPR	Institute of Primate Research
IPTG	isopropyl β D-1-thiogalactopyranoside
IRC	Institutional Review Committee
kb	Kilobase
LA	latex agglutination
LB	lysogeny broth
ℓ	Litre
Magenta-Gal	5-bromo-6-chloro-3-indoxyl-/3-D-galactopyranoside
MEGA	Molecular Evolutionary Genetics Analysis
mo	Month
μℓ	Microliter
μM	Micromolar
mg	Milligram
mℓ	Milliliter
min	Minute
mm	Millimeter
MNV	murine norovirus
mOPV	monovalent oral polio vaccine
NaCl	sodium chloride
NaOH	sodium hydroxide
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NoV	Norovirus
NPEVs	non-polio enteroviruses
NSP	non-structural protein
nt	Nucleotide
NTR	Non-translated region
NV	Norwalk virus
OPV	oral polio vaccine

© University of Pretoria

ORF	open reading frame
PBS	phosphate-buffered saline
PV	Poliovirus
P domain	protruding domain
PCR	polymerase chain reaction
PEG	polyethylene glycol
QA	quality assurance
QC	quality control
RHDV	rabbit haemorrhagic disease virus
RdRp	RNA dependent RNA polymerase
RNA	ribonucleic acid
ARSN	Africa Rotavirus Surveillance Network
RT	reverse transcription
rt	real time
RV	Rotavirus
SA	South Africa
SaV	Sapovirus
sec	Second
ss	single-stranded
SV	simian virus
TLR	Toll-like receptor
tOPV	trivalent oral polio vaccine
UK	United Kingdom
UNICEF	United Nations Children's Fund
URTIs	upper respiratory tract infections
USA	United States of America
VAPP	Vaccine-associated paralytic poliomyelitis
VDPV	vaccine-derived poliovirus
VLP	virus-like particle
VP	viral protein
WHO	World Health Organization
WPV	wild-type poliovirus
X-gluc	5-bromo-4-chloro-3-indoxyl-/3-D-glucuronic acid
y	Year

© University of Pretoria

LIST OF FIGURES

FIGURE		Page
Figure 1.1	Electron micrograph of rotavirus showing the short spiked wheels.	3
Figure 1.2	Three-dimensional structure of the rotavirus particle showing gene coding assignments and virion locations of RV proteins.	4
Figure 1.3	Electron micrograph of norovirus.	11
Figure 1.4	The norovirus genome showing the three open reading frames and the hypervariable P2 domain of the capsid protein.	11
Figure 1.5	Phylogenetic analysis showing the genera of *Caliciviridae* and their prototype.	12
Figure 1.6	Phylogenetic tree of noroviruses depicting the five genogroups (GI-V) and 32 genotypes based on sequence diversity in the complete capsid protein VP1.. Human strains cluster within GI, GII and GIV.	13
Figure 1.7	Phylogenetic analysis showing the diversity of norovirus genogroup, genotype and subtypes.	14
Figure 1.8	Electron micrograph of polioviruses.	20
Figure 2.1	Diagrammatic map showing different geographic region where water samples were collected. : A: Mboone river, Maua, Meru County; B: Mutoine and Nairobi river, Nairobi County; C: Dadaab refugee camp.	35
Figure 3.1	Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoVs GI strains detected in Kenyan river water samples (● Nairobi river ■ Mutoine river).	60
Figure 3.2	Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII strains detected in Kenyan river water samples (●Nairobi river ■ Mutoine river ▲Mboone river ▼Dadaab Household water).	61

© University of Pretoria

Figure 3.3 Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.17 strains detected in Kenyan river water samples (●Nairobi river ■ Mutoine river ▲Mboone river). 62

Figure 3.4 Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.4 strains detected in Kenyan river water samples (● Nairobi river ■ Mutoine river). 63

Figure 3.5 Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.6 strains detected in Kenyan river water samples (■ Mutoine river and ▼Dadaab Household water one). 63

Figure 3.6 Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.12 and GII.16 strains detected in Kenyan river water samples (▲Mboone river ■ Mutoine river). 64

© University of Pretoria

LIST OF TABLES

TABLE		Page
Table 2.1	Primers used for genotyping of VP7 (G) types of rotaviruses.	44
Table 2.2	Primers used for genotyping of VP4 (P) types of rotaviruses.	45
Table 2.3	Real-time RT- PCR primers and probes used for the characterisation of polioviruses.	46
Table 2.4	Primer used for genotyping of noroviruses.	48
Table 2.5	GenBank accession numbers of the NoV GI isolates used as reference strains for phylogenetic analysis.	49
Table 2.6	GenBank accession numbers of the NoV GII isolates used as reference strains for phylogenetic analysis.	50
Table 3.1	Summary of the water samples collected during the sampling period February 2012 to January 2013.	51
Table 3.2	Summary of the presumptive *E. coli* and total coliforms detected during the sampling period February 2012 to January 2013.	52
Table 3.3	Summary of the enterovirus positive results after cell culture amplification.	54
Table 3.4	Summary of results for the enteric viruses detected in water sources from rural, urban and refugee settings from Februrary 2012 to January 2013.	55
Table 3.5	Enteric viruses detected in water sources from rural, urban and refugee settings from February 2012 to January 2013.	56
Table 3.6	Summary of rotavirus G and P genotypes detected in rural, urban and refugee settings.	57
Table 3.7	Summary of poliovirus typing results.	58
Table 3.8	Summary of the norovirus genotypes detected in water samples.	59

© University of Pretoria

CHAPTER ONE

LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

It is estimated that worldwide 1.1 billion people drink unsafe water (Ashbolt, 2004) and annually 1.7 million deaths are caused by drinking of unsafe water and poor sanitation (Ashbolt, 2004; Grabow, 2007). Viruses are a major cause of waterborne disease but the health impact of waterborne viral infections is underestimated (Grabow, 2007; Mena, 2007). Human enteric viruses, which primarily infect and replicate in the gastrointestinal tract, have been associated with waterborne transmission (Carter, 2005; Grabow, 2007; Koroglu *et al.,* 2011). More than 100 types of enteric viruses may be present in faecally contaminated water (Koopmans and Duizer, 2004; Fong and Lipp, 2005) and their presence in water sources is of public health concern due to their low infectious dose of 10 to 100 infectious particles (Wyn-Jones and Sellwood, 2001; Fong and Lipp, 2005). Worldwide enteric viruses such as rotaviruses (RV), noroviruses (NoV), sapoviruses (SaV), human astroviruses (HAstV), human adenoviruses (HAdVs) and hepatitis A virus (HAV) are important agents of gastroenteritis and hepatitis, respectively (Bern and Glass, 1994; Parashar and Glass, 2003; Hollinger and Emerson, 2007). Diarrhoea remains one of the major causes of childhood morbidity and mortality in developing countries. Over a billion diarrhoeal cases occur each year among children younger than five years of age resulting in approximately 2.5 million deaths annually (O'Ryan *et al.,* 2005). A contributing factor to the high incidence of diarrhoeal cases in developing countries is ingestion of contaminated food and water resulting from poor sanitation and limited access to clean water (Hot *et al.,* 2003, Aryal *et al.,* 2012). Rotaviruses are the most important cause of gastroenteritis in infants and children (Tate *et al.,* 2012), with NoVs being the second most prevalent cause of diarrhoea in infants and children (Patel *et al.,* 2009) and the most important cause of acute gastroenteritis in all age groups (Glass *et al.,* 2009). Enteroviruses, one of the enteric virus group, have been associated with diverse syndromes from febrile illness to aseptic meningitis, poliomyelitis and myocarditis (Caro *et al.,* 2002; Pallansch and Roos, 2007). The predominant mode of transmission of enteric viruses is via the faecal-oral route, directly

© University of Pretoria

from person-to-person or indirectly through the ingestion of faecally-contaminated food and water (Cáceres *et al.,* 1998; Boone and Gerba, 2007; Wikswo and Hall, 2012). These viruses are discharged into sewage and have the potential to pollute surface (Pintó and Saiz, 2007), ground (Gerba, 2007) and drinking water (Carter, 2005). Polluted water can in turn contaminate fresh produce if used for irrigation purposes (Steele and Odumeru, 2004). The enteric viruses, which are shed in large numbers in the faeces of infected individuals, are stable in the environment and may survive wastewater treatment (Baggi and Peduzzi, 2000; Carter, 2005; Kahler *et al.,* 2011). Indicator micro-organisms have traditionally been used to assess the microbial quality of water (Ashbolt *et al.,* 2001) but enteric viruses have been detected in water which conform to quality limits for indicator organisms (van Heerden *et al.,* 2005; Pusch *et al.,* 2005). Enteric viruses are considered by the United States of America (US) Environmental Protection Agency (EPA) as the most meaningful, reliable and effective index for environmental monitoring (Vantarakis and Papapetropoulou, 1998; Lee and Kim, 2002; Miles *et al.,* 2009). In addition, in developed countries, the presence of enteric viruses in different water sources has been used to estimate the level of water contamination (Gratacap-Cavallier *et al.,* 2000; Kittigul *et al.,* 2005). Since wastewater treatment plants collect and treat community effluent, evaluation of viruses occurring in sewage samples (Katayama *et al.,* 2008) and wastewater sources (Mueller *et al.,* 2009) are a useful form of passive surveillance to give an indication of which viruses are circulating in both symptomatic and asymptomatic individuals in the surrounding communities.

1.2 ROTAVIRUS

1.2.1 History

Rotaviruses were discovered in duodenal biopsies from hospitalised infants and young children with acute gastroenteritis by Bishop and colleagues in 1973 (Bishop *et al.,* 1973). Thereafter, RVs were also identified in faecal specimens by electron microscopy (EM) (Flewett, 1976). Subsequently, laboratories all over the world soon began to detect the virus in stools of a large proportion of paediatric patients with gastroenteritis. Efficient and practical tests were developed to detect RV in clinical specimens thus facilitating the study of this virus (Estes and Kapikian, 2007).

© University of Pretoria

1.2.2 Virology

Rotaviruses are double-stranded (ds) segmented ribonucleic acid (RNA), non-enveloped viruses which are 55 to 75 nm in diameter (Bishop *et al.,* 1973; Estes and Kapikian, 2007; Attoui *et al.,* 2012). They have a distinct morphologic appearance by electron microscopy (EM) which resembles a small wheel with a hub short spikes and a narrow rim (Figure 1.1). This distinctive morphology characteristic led to the name "rotavirus" from the latin "rota" for a wheel and distinguishes them from the orbiviruses and orthoreoviruses (Bishop *et al.,* 1973; Attoui *et al.,* 2012).

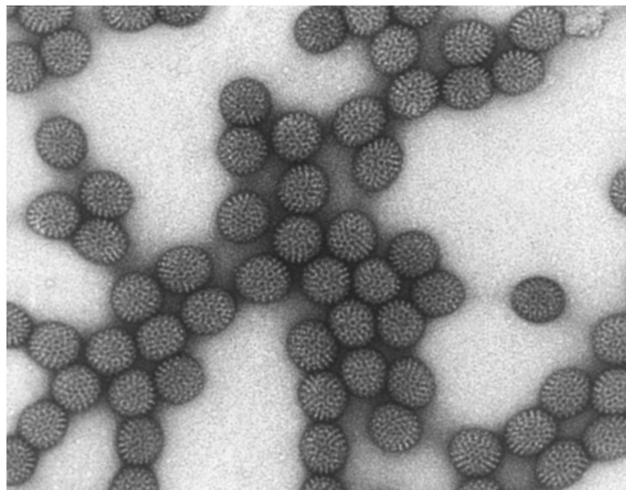

Figure 1.1: Electron micrograph of rotavirus showing the short spiked wheels
(Palmer E: http://phil.cdc.gov/phil/details.asp?pid=197)

The viral capsid is triple-layered with the inner layer enclosing the virus genome which comprises 11 segments of ds RNA, each coding for either structural viral protein (VP) or non-structural proteins (NSP) (Figure 1.2) (Estes and Kapikian, 2007; Attoui *et al.,* 2012). The segmented genome of RV readily reassorts during co-infection and it plays a key role in virus evolution (Estes and Kapikian, 2007).

1.2.3 Classification

Rotaviruses belong to genus *Rotavirus* in the family *Reoviridae,* subfamily *Sedoreovirinae,* which includes other genera such as *Orbivirus, Seadornavirus, Phytoreovirus, Cardoreovirus* and *Mimoreovirus* (Attoui *et al.,* 2012). Three genera of

© University of Pretoria

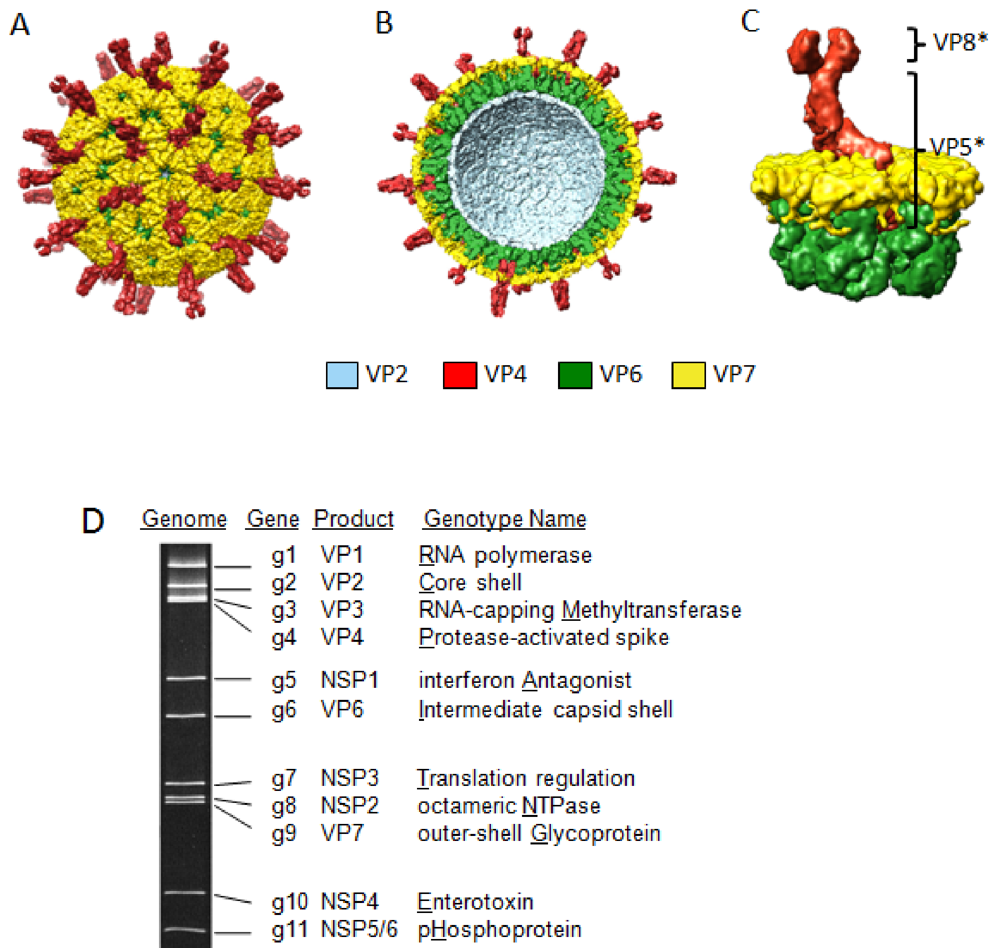

Figure 1.2: Three-dimensional structure of the rotavirus particle showing gene coding assignments and virion locations of RV proteins.
(Patton JP. http://www.discoverymedicine.com/John-T-Patton/2012/01/26/rotavirus-diversity-and-evolution-in-the-post-vaccine-world/)

family *Reoviridae,* namely *Rotavirus, Orthoreovirus, Orbivirus* infect humans and animals (Attoui *et al.,* 2012). Initially RVs were classified serologically into serogroups and serotypes. A serogroup included viruses that share cross-reacting antigens that can be detected by serological methods such as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence (IF). Based on these techniques, RVs were classified into at least seven serogroups A to G (Estes and Kapikian, 2007). Groups A, B and C RVs have been detected in both humans and animals, whereas groups D, E, F and G are associated with animals (Estes and Kapikian, 2007). Rotavirus can further be classified into genotypes by molecular-based analysis of the VP4, VP6 and VP7 genes (Estes and Kapikian, 2007; World Health Organization [WHO], 2009a; Attoui *et al.,* 2012). Recently a new nucleotide sequence-based complete genome classification was introduced by the Rotavirus Classification Working Group for group A rotavirus, which

© University of Pretoria

assigns specific genotypes to each of the 11 genome segments of a particular RV strain based on nucleotide sequence data and according to established nucleotide percent cut-off values (Matthijnssens *et al.,* 2011). Using the complete genome classification criteria, 51 new genotypes have been ratified as of April 2011, 27 different G and 35 P genotypes have been described in both humans and animals (Matthijnssens *et al.,* 2011; Attoui *et al.,* 2012).

1.2.4 Pathogenesis and immunity

Rotaviruses infect the mature absorptive villous epithelium of the upper two thirds of the small intestine. After replication in the upper small intestine, infectious particles are released into the intestinal lumen and undergo further replication in the distal areas of the small intestine (Estes and Kapikian, 2007). Infection is generally confined to the intestinal mucosa. Although, RVs can be found in the lamina propria and regional lymphatics, replication in these sites and systemic spread usually do not occur in immunocompetent persons (Estes and Kapikian, 2007). Despite the superficial nature of mucosal infection RVs induce both local intestinal and systemic immune response.

After a primary infection neutralising antibodies directed primarily against the VP7 glycoprotein of the infecting strain (homotypic response) are induced in infants and young children (Estes and Kapikian, 2007). Repeated RV infections elicit both a homotypic and heterotypic (against strains with different G serotypes) antibody response. Protection against RV diarrhoea correlates with serum antibody titres following natural infection and infected children are more protected against re-infection with similar rather than different G serotypes (Estes and Kapikian, 2007). A protective role of transplacentally transferred maternal antibody among infants <3 months of age has also been speculated, since RV disease is uncommon in this age group. However serum neutralising antibody responses among vaccine recipients have sometimes correlated poorly with protection from disease. Therefore, the exact role of serum antibody in protection against disease remains unclear (Offit, 1996; Jiang *et al.,* 2002).

© University of Pretoria

1.2.5 Clinical features

The incubation period for RV is usually five to seven days but it may be as short as 48 hours (WHO, 2002a; Estes and Kapikian, 2007). The onset of symptoms is sudden and frequent with the initial symptomatic abdominal colic. This is followed by the onset of watery diarrhoea, fever, malaise, abdominal discomfort and dehydration or a combination of some of these symptoms that occurs primarily in infants and young children (Bernstein, 2009). Clinically, RV infection causes a spectrum of symptoms, which ranges from asymptomatic infection in the new-born babies to severe dehydrating diarrhoea in infants and young children (Estes and Kapikian, 2007). For severe cases the duration of hospitalisation ranges from two to 14 days with a mean of four days (Parashar *et al.,* 1998, 2003; WHO, 2002a). The highest attack rate is usually among infants and young children six to 24 months old and the next highest in infants less than six months old (Tate *et al.,* 2012). Rotavirus infection in adults has also been reported (Cardemil *et al.,* 2012; Gunn *et al.,* 2012; Shen *et al.,* 2013). Rotavirus infection in adults typically manifests with symptoms such as nausea, malaise, headache, abdominal cramping, diarrhoea, and fever (Anderson and Weber, 2004). Outbreaks of RV infection have also been reported in closed adult communities like geriatric wards, nursing homes, cardiology wards and elderly care facilities (Cubitt and Holzel, 1980; Holzel *et al.,* 1980; Cardemil *et al.,* 2012; Gunn *et al.,* 2012).

1.2.6 Laboratory diagnosis

The accurate diagnosis of RV infection is important not only for rapid identification of the patient with severe RV diarrhoea, but also for the identification of any potential sources of infection (Dennehy *et al.,* 1999). The routine laboratory diagnosis of RV gastroenteritis requires the detection of virus or viral antigen (Estes and Kapikian, 2007). The most rapid method of RV diagnosis is by identification of group A RV antigen in stool specimen by immunological-based assays such as latex agglutination (LA) immunochromatographic assays and enzyme immunoassays (EIA) (Estes and Kapikian, 2007; WHO, 2009a). Electron microscopy can also be used as this "catchall" method will detect non-group A RVs that are not detected by conventional serologic assays as they do not share the common group antigen.

© University of Pretoria

Although RV can be isolated in many types of continuous cell lines including the Buffalo green monkey kidney cells (BGM), African green monkey kidney cells (BSC-1, MA-104 and Vero), human colon adenocarcinoma cell lines (CaCo-2 and HT-29) and Rhesus monkey kidney cells (LLC-MK_2) (Birch *et al.,* 1983; Ward *et al.,* 1984; Aboudy *et al.,* 1989; Arnold *et al.,* 2009), this is not used for the routine diagnosis of RV diarrhoea. Molecular-based techniques such as polyacrylamide gel electrophoresis (PAGE) and reverse transcriptase-polymerase chain reaction (RT-PCR) are methods used to detect group A and non-group A RVs (Estes and Kapikian, 2007; WHO, 2009a) but are also not used in routine diagnosis.

1.2.7 Management, treatment and prevention

Treatment of RV infection is supportive and primarily aimed at the replacement of fluids and electrolytes lost due to vomiting or diarrhoea as no antiviral therapies are currently available (Diggle, 2007; Pammi and Haque, 2011). Oral rehydration is more readily available and has gained widespread use worldwide as a lifesaving treatment (Samadi *et al.,* 1998; WHO, 2002a; Munos *et al.,* 2010). In patients with severe dehydration and shock, intravenous rehydration is indicated for efficient replacement of fluid loss (Estes and Kapikian, 2007). Improvement in therapy has also resulted in a marked decrease in mortality from all forms of RV infantile diarrhoea. Because RVs are highly infectious and are spread by the faecal-oral route, careful attention to hand washing disinfection and disposal of contaminated materials may limit the spread especially in nurseries and hospitals where nosocomical infections occur frequently (Sherchan *et al.,* 2011; Bruijning-Verhagen *et al.,* 2012). The RV vaccines have been developed and have been introduced in the expanded programme of immunisation (EPI) in many countries including some countries in Africa (Mwenda *et al.,* 2010; Msimang *et al.,* 2013). These vaccines are very effective in preventing RV gastroenteritis (WHO, 2009b). The WHO recommends routine vaccination of infants with either of two available vaccines: RotaTeq® (RV5), licensed in 2006, which is given in three doses at ages two months, four months, and six months; or Rotarix® (RV1) which was licensed in 2008, and is given in two doses at ages two months and four months (WHO, 2009b).

© University of Pretoria

1.2.8 Epidemiology

The primary mode of transmission of RV is via faecal-oral route but respiratory transmission has also been speculated (Estes and Kapikian, 2007). Because the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces, (Dennehy, 2000; Estes and Kapikian, 2007). Animal to human transmission does not appear to be common although human RV strains that possess a high degree of genetic homology with animal strains have been identified (Komoto *et al.,* 2013; Nakagomi *et al.,* 2013; Ndze *et al.,* 2013).

Worldwide, RVs are known to be the single most significant cause of severe gastroenteritis causing diarrhoea in young children younger than five years of age in both developed and developing countries (Estes and Kapikian, 2007; Parashar *et al.,* 2009; Tate *et al.,* 2012). Rotaviruses also affect older children and adults but are known to cause severe diarrhoea disease in children below two years of age (Estes and Kapikian, 2007; Parashar *et al.,* 2009). Recently the WHO global RV surveillance network estimated an annual global mortality of 453 000 with approximately 85% of these deaths occurring in sub-Saharan Africa (Tate *et al.,* 2012) and other developing countries of Asia and South America (Tate *et al.,* 2012). It is estimated that 130 million children develop RV-related diarrhoea each year, 18 million of whom experience moderate to severe dehydration (Hoshino and Kapikian, 2000; Tate *et al.,* 2012).

Because the disease cannot be eliminated through improvement in water and sanitation (Centers for Disease Control and Prevention [CDC], 2006), the development of a safe and effective rotavirus vaccine has been a priority, particularly but not exclusively in developing countries where the burden of disease is highest (WHO, 2009b). The accelerated introduction of a RV vaccine in EPI programme has been designated a global priority by the WHO, the Global Alliance for Vaccines and Immunization (GAVI), and many international groups because of the high burden of disease in developing countries (Mwenda *et al.,* 2010). Rotavirus vaccines are currently pre-qualified by the WHO and their introduction into the national immunisation programmes has recently been recommended (WHO, 2009b). These vaccines have been introduced in many countries of the world (WHO, 2009b). As a consequence the

© University of Pretoria

incidence and severity of RV infections has declined significantly in countries that have added RV vaccine to their routine childhood immunisation programmes (Desai et al., 2011; Dudareva-Vizule *et al.,* 2012; Choi *et al.,* 2013; Hemming *et al.,* 2013; Msimang *et al.,* 2013; Tate *et al.,* 2013; Vesikari *et al.,* 2013).

Rotavirus infections display a distinct seasonal pattern in temperate climates with epidemic peaks occurring predominantly in the cooler winter months of the year. The monthly peaks may vary from regions and from year to year (Parashar *et al.,* 1998, 2009; Tate *et al.,* 2012). The seasonal nature of RV infections is not universal and in countries with tropical climates, RV disease has been reported to occur all year round, with seasonal peaks occurring during the dry cool months of the year (Armah *et al.,* 1994; Kiulia *et al.,* 2008). These different seasonal patterns observed in tropical and temperate settings may lead to differing patterns of transmission and perhaps to different reservoirs of infection. In addition, it may also have an influence on the age of infection seen in the different climatic settings. Rotavirus infection is predominantly seen in older children (> 12 month) in temperate countries compared to the younger age (< 6 months) in tropical countries (Armah *et al.,* 1994). In Kenya RV diarrhoea occurs throughout the year (Urasawa *et al.,* 1987; Kiulia *et al.,* 2008), and seasonal peaks are observed during the dry seasons (January to March and June to September (Makino *et al.,* 1983; Mutanda *et al.,* 1984) than in wet seasons. This is in contrast to other African countries like southern Africa (South Africa, Madagascar, Zambia and Zimbabwe) where RV occurs during the autumn and winter and which overlap with the dry seasons and in northern Africa (Egypt and Morocco) where it occurs in autumn and winter seasons but not in dry seasons (Tazi-Lakhsassi *et al.,* 1988; Pazzaglia *et al.,* 1993).

Over the last 30 years studies have addressed RV epidemiology in Kenya in both urban and rural settings (Mwenda *et al.,* 2003; Kiulia *et al.,* 2006, 2008, 2009; Nokes *et al.,* 2010). In general, these studies have focused on the occurrence of RV-associated acute diarrhoea in clinics, outpatient visits and hospitalisations. These studies show an average prevalence rate of 6% to 56% (Kiulia *et al.,* 2008). All four epidemiologically important RV G types (G1-G4) have been detected in Kenya (Mutanda *et al.,* 1990; Gatheru *et al.,* 1993; Nakata *et al.,* 1999; Kiulia *et al.,* 2006, 2008; Mwenda *et al.,* 2010). Recent studies have shown that uncommon strains (G9, G8 and G12) may account for a significant proportion of RV strains detected in Kenyan children who

© University of Pretoria

present with diarrhoea (Cunliffe *et al.,* 2001; Steele and Ivanoff 2003; Kiulia *et al.,* 2008; Mwenda *et al.,* 2010) and also in environmental water sources (Kiulia *et al.,* 2010) thus, highlighting the importance of this virus. Although water was previously not considered to be an important route of RV transmission (Grabow, 2007) there is an increase in RV-associated waterborne outbreaks (Gratacap-Cavallier *et al.,* 2000; Rutjes *et al.,* 2009). In a pilot study (2007 to 2008) clinically relevant RVs were identified in rural and urban Kenya water sources (Kiulia *et al.,* 2010).

1.3 NOROVIRUS

1.3.1. History

An epidemic of acute non-bacterial gastroenteritis in humans was first described in 1929 as a ''winter vomiting disease''. Since none of the known bacterial causes of gastroenteritis could be isolated, a viral aetiology was suspected by Zahorsky, 1929 as quoted by Patel *et al.,* (2009). In the 1940s and 1950s, the association of the disease with a virus was demonstrated through a series of human challenge studies (Green, 2007). 1968, an outbreak of acute non-bacterial gastroenteritis occurred in an elementary school in Norwalk, Ohio, that caused illness in 50% of 232 students and teachers was reported by Adler and Zickl, (1969) as quoted by Atmar and Estes, (2009). Thus, the identified virus was named Norwalk virus and the genus was first called Norwalk-like viruses, which in 2002 was changed to NoV by the International Committee on Taxonomy of Viruses (Clarke *et al.,* 2012).

1.3.2 Virology

Noroviruses are non-enveloped, icosahedral viruses with a single-stranded RNA genome 7.5 to 7.7 kilobase (kb) in length encoding a single major structural protein (VP1). The virus, when visualised by EM, is 26 to 34 nm in diameter; small, round, with an amorphous surface and ragged outer edge (Figure 1.3). It can also appear to have cup-shaped or cup-like structures like other caliciviruses (Green, 2007).

The VP1 protein consists of three open reading frames (ORF) (Figure 1.4), which has a hypervariable domain (P2 domain) as the most exposed part of the virion. The ORF1

© University of Pretoria

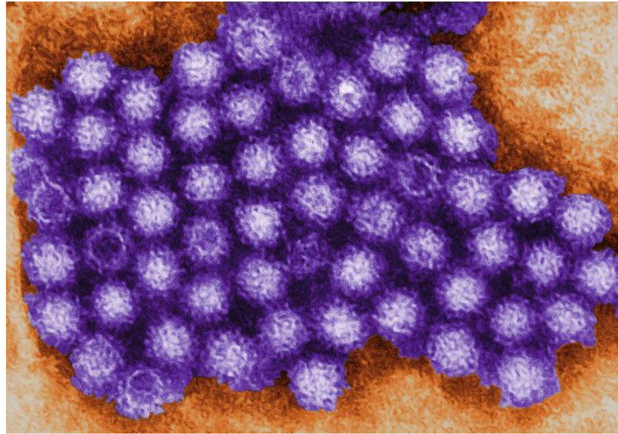

Figure 1.3: Electron micrograph of norovirus

(Humphrey CD: http://phil.cdc.gov/phil/details.asp?pid=10708)

encodes for a non-structural polyprotein, which is processed into several proteins, including a helicase, protease and RNA-dependent RNA polymerase. Open reading frames 2 encodes the major capsid protein and ORF3 a minor capsid protein.

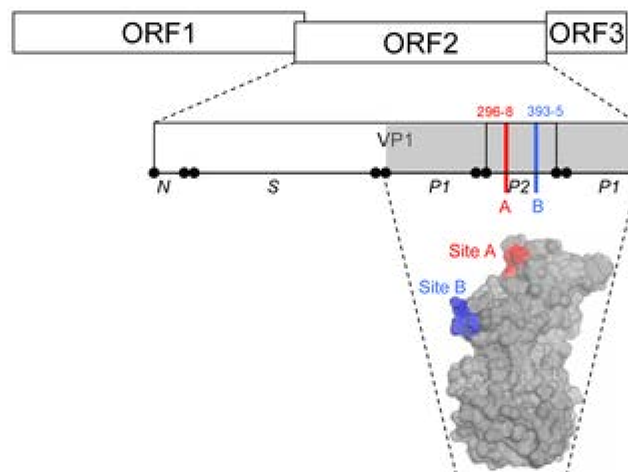

Figure 1.4: The norovirus genome showing the three open reading frames and the hypervariable P2 domain of the capsid protein (Zakikhany *et al.,* 2012)

1.3.3 Classification

Noroviruses are classified in the genus *Norovirus* of the family *Caliciviridae* (Clarke *et al.,* 2012). The other genera in the calicivirus family are *Vesivirus, Lagovirus, Sapovirus* and *Nebovirus* (Figure 1.5) (Clarke *et al.,* 2012). Norovirus and SaV infect

© University of Pretoria

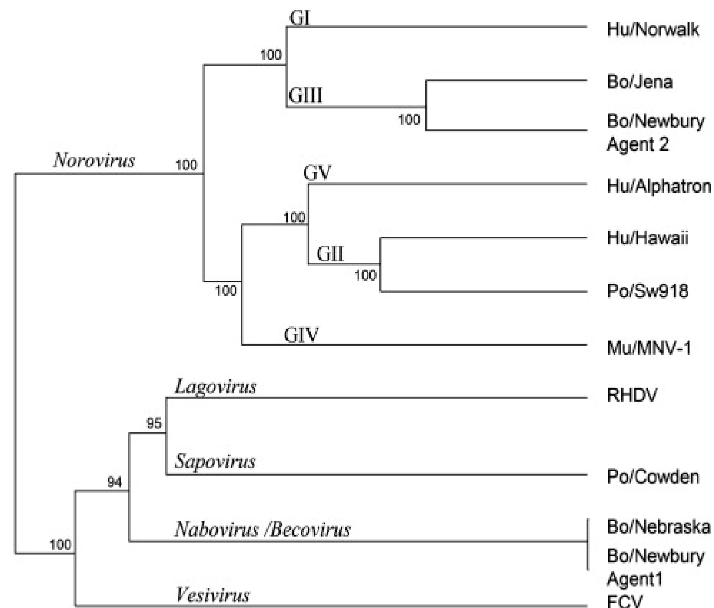

Figure 1.5: Phylogenetic analysis showing the genera of *Caliciviridae* and their prototype (Scipioni *et al.,* 2008)

humans. Lagovirus and Vesivirus infect animals and include rabbit haemorrhagic disease virus (RHDV) and feline calicivirus (FCV) (Clarke *et al.,* 2012). Nebovirus has only been detected in calves (Clarke *et al.,* 2012). There are other proposed genera that might be members of calicivirus family such as *Recovirus* (for rhesus enteric calicivirus) with Tulane virus as the prototype and Valovirus with St-Valerien as the prototype (Clarke *et al.,* 2012). In general NoVs tend to be species specific and, to date, no animal strains have been identified in humans (Atmar, 2010).

The diversity among NoVs is well documented (Atmar and Estes, 2009; Glass *et al.,* 2009). Initially, the classification of NoVs was based on cross-challenge studies in volunteers (Lewis *et al.,* 1995) and analysis of antisera cross-reactivity by immunoelectron microscopy (IEM) (Lewis *et al.,* 1995). Currently the NoVs are classified on the basis of nucleotide sequence diversity in the VP1-encoding ORF2 gene, which divides the majority of human NoVs into five genogroups (GI to GV) (Patel *et al.,* 2009; Clarke *et al.,* 2012). Human infection has been associated with genogroups I, II and IV while genogroups III and V infect cows and mice, respectively (Figure 1.6) (Zheng *et al.,* 2006; Scipioni *et al.,* 2008; Clarke *et al.,* 2012).

© University of Pretoria

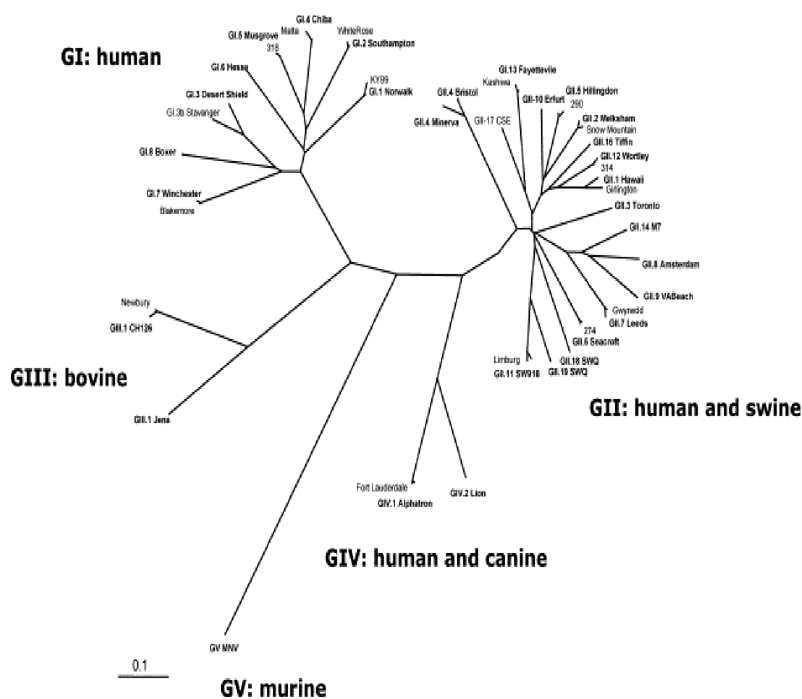

Figure 1.6: Phylogenetic tree of noroviruses depicting the five genogroups (GI-V) and 32 genotypes based on sequence diversity in the complete capsid protein VP1. Human strains cluster within GI, GII and GIV (Patel *et al.,* 2009)

Porcine strains are found in GII and recently a lion (Martella *et al.,* 2007) and a dog (Martella *et al.,* 2008) strain have been identified in genogroup IV, suggesting the potential for zoonotic transmission.

The difference in amino acid sequence of the major capsid protein is as much as 43% between isolates within the same genogroup and up to 61% between isolates from different genogroups. The strains may be further classified into genetic clusters within each genogroup (Figure 1.6) (Zheng *et al.,* 2006; Patel *et al.,* 2009).

On the basis of >85% sequence similarity in the complete VP1 genome, NoVs can be classified further into genotypes, with at least eight genotypes belonging to GI and 21 genotypes belonging to GII (Figure 1.7) (Glass e*t al.,* 2009). Recently a new nomenclature system for classification of NoVs GII.4 has been proposed by using both ORF1 and VP1 sequences, as recombination is common and recognising recombinant viruses may be important (Kroneman *et al.,* 2013). Since 2001, the NoVs GII.4 are the

© University of Pretoria

major aetiological agent of outbreaks of gastroenteritis around the world (Siebenga *et al.,* 2009; Zakikhany *et al.,* 2012; Kim *et al.,* 2013; Maritschnik *et al.,* 2013, Silva *et al.,* 2013). Recent studies have demonstrated that these viruses evolve over time through serial changes in the VP1 sequence, which allow evasion of immunity in the human population (Siebenga *et al.,* 2009).

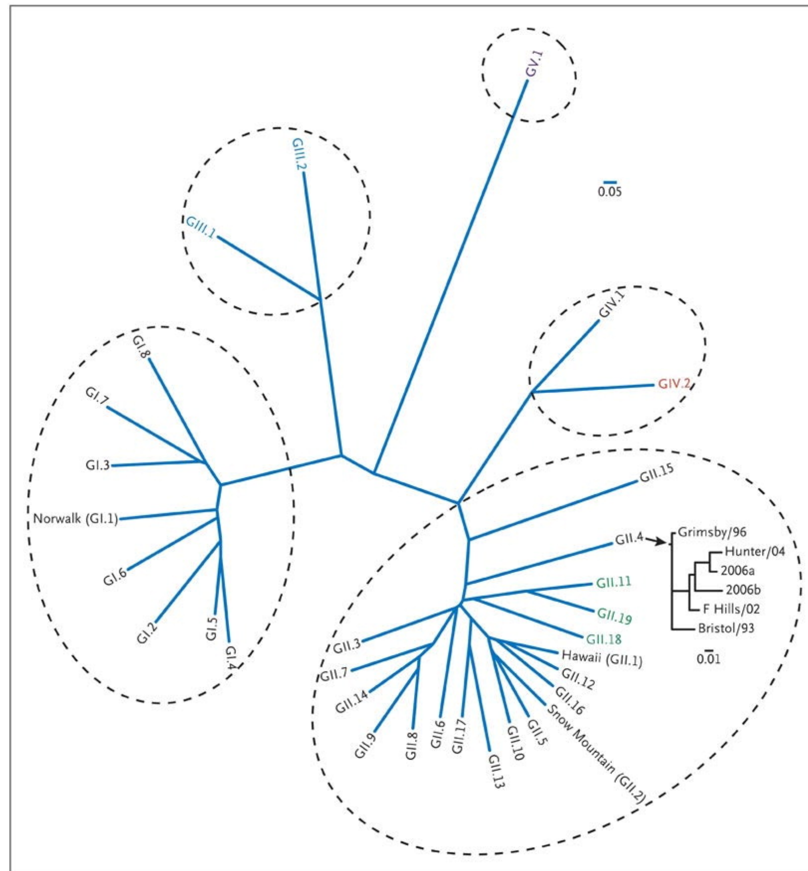

Figure 1.7: Phylogenetic analysis showing the diversity of norovirus genogroup, genotype and subtypes (Glass *et al.,* 2009).

1.3.4 Pathogenesis and immunity

The current understanding of the pathogenesis, susceptibility and immune response of NoV infections came from data on more than 1000 volunteers who participated in challenge studies (Glass *et al.,* 2009). Specimens (proximal jejunal biopsy) collected from ill volunteers showed a broadening and blunting of the intestinal villi, crypt-cell hyperplasia, cytoplasmic vacuolization, and infiltration of polymorphonuclear and mononuclear cells into the lamina propria (Green, 2007). There were no histologic

© University of Pretoria

changes that were seen in the gastric fundus or in antrum or colonic mucosa. The extent of involvement of the small intestine remains unknown. The duration of immunity to NoV gastroenteritis is controversial and studies suggest from six months to two years (Simmons *et al.,* 2013). Exposure to NoV usually occurs in childhood, with antibody prevalence rising to greater than 50% by the fifth decade of an individual's life. It appears that individuals develop short-term immunity following infection and immunity is strain specific. Therefore, due to the antigenic diversity, immunity to NoVs appears to be short-lived (Green, 2007). Short-lived immunity may explain in part the high attack rates in all age groups in an outbreak (Simmons *et al.,* 2013).

1.3.5 Clinical features

The incubation period of NoV infection ranges from 10 to 51 hours, and the infectious dose is very low (Glass *et al.,* 2009). It is estimated the infectious dose to be between 18 and 1000 viral particles (Teunis *et al.,* 2008). Many people shed virus prior to onset of illness, and peak faecal virus shedding may occur after gastroenteritis symptoms have resolved (Atmar and Estes, 2009). Norovirus associated gastroenteritis is characterised by the sudden onset of vomiting, watery diarrhoea or both symptoms (Patel *et al.,* 2009). Additional symptoms such as nausea, abdominal cramping and pain, malaise, anorexia, fever, chills, headache and myalgias can also be experienced (Desselberger and Gray, 2003; Chen and Chiu, 2012). Vomiting has been reported more frequently with GII.4 strains in nursing home outbreaks than with outbreaks caused by other strains (Friesema *et al*., 2009). Unusual presentations of NoV infection have been described in special populations. A cluster of necrotising enterocolitis was noted in a neonatal intensive care unit and NoV infection was confirmed in half of the affected patients (Turcios-Ruiz *et al*., 2008). Noroviruses have also been implicated in necrotising enterocolitis in premature infants (Bagci *et al.,* 2010) and benign infantile seizures (Chen *et al.,* 2009; Bartolini *et al.,* 2011). Other cases of NoV infection has been described in association with exacerbations of inflammatory bowel disease (Khan *et al*., 2009). Although the association of NoV infection with these unusual presentations requires further confirmation, the impact of NoVs in immunocompromised and in immunocompetent patients has been reported (Glass *et al.,* 2009; Bok and Green, 2012). While these case reports provide only anecdotal evidence that NoV infection may have varied clinical outcomes. Therefore, it has been suggested that NoVs should be

© University of Pretoria

considered as potential aetiological agents, not only in gastroenteritis but also other diseases (Glass *et al.,* 2009).

1.3.6 Laboratory Diagnosis

The diagnosis of NoV infection has evolved over the past several decades. In the 1970s and 1980s, the primary means of diagnosis was by EM (Atmar and Estes, 2001). The specific diagnosis of NoV is routinely made by use of immunological-based assays (Patel *et al.,* 2009). An automated bioluminescent enzyme immunoassay (BLEIA) has been described and is potentially useful for the rapid diagnosis of NoV in epidemic and sporadic gastroenteritis (Sakamaki *et al.,* 2012). Immunological-based assays using either an ELISA or immunochromatographic format are commercially available (Patel *et al.,* 2009). The principal disadvantage of these tests kits has been their poor sensitivity, being as low as 32% in the identification of sporadic cases of NoV infection. The sensitivity is also affected by NoV genotype, as some less common genotypes are not identified by these assays (Sakamaki *et al.,* 2012). The RT-PCR assays first became available in the 1990s and are currently the most sensitive methods for detection of NoVs in clinical samples but are not used in routine diagnosis (Patel *et al.,* 2009; Zheng *et al.,* 2010). These molecular assays are very sensitive and can detect as few as 10 virus particles (Marshall and Bruggink, 2006; Miura *et al.,* 2013). These assays target conserved areas in the genome, including the polymerase gene (region A), the ORF1/ORF2 junction (region B), and areas in the VP1 gene (regions C and D). The specificity of the assays is confirmed by probe hybridisation or sequencing of the amplicons. The sequence data using the latter approach can be used for genotyping and in molecular epidemiologic studies (Yee *et al.,* 2007; Le Guyader *et al.,* 2008). The real-time (rt) RT-PCR assays also allow more rapid evaluation of clinical and environmental samples (Butot *et al.,* 2010; Miura *et al.,* 2013).

1.3.7 Management, treatment and prevention

In most persons NoV illness is self-limited and mild, and either no treatment is needed or it is supportive, e.g. oral rehydration, antiemetics, and analgesics agents. For patients who become more severely volume depleted, intravenous fluid and electrolyte replacement may be required in the emergency centre or in the hospital (Green, 2007).

© University of Pretoria

Currently, there are no vaccines or antiviral therapies approved for the treatment of NoV infections, and such efforts have been significantly hampered by the lack of a simple cell culture or small-animal model for human NoVs (Green, 2007). Ribavirin and interferons inhibit viral replication in a Norwalk viral replicon system, but no clinical data are available to evaluate their utility in active infection (Chang and George, 2007). Immune globulin has also been proposed as a treatment either given orally or intravenously, but only limited anecdotal information has suggested a potential beneficial effect of such therapy (Florescu *et al.,* 2008; Glass *et al.,* 2009).

Preventive methods are also currently non-specific in nature. These methods include appropriate hand hygiene, environmental decontamination, and furlough of symptomatic food handlers and healthcare workers during the period of illness and for up to two to three days after symptom resolution (Parashar *et al.,* 2001; Harris, *et al.,* 2010). Measures to identify contaminated foodstuffs are also used to prevent NoV transmission. For example, current regulations call for screening of shellfish-growing waters or shellfish for faecal coliform bacteria to identify exposure to sewage pollution (Le Guyader *et al.,* 2009). However, this approach has failed to identify viral contamination of shellfish associated with a number of NoV outbreaks. With the development of methods to directly detect viral contamination of foodstuffs implicated in foodborne NoV outbreaks, it should be possible to adapt these assays for screening high risk foods (Le Guyader *et al.,* 2003, 2004, Bosch *et al.,* 2011). Other approaches to inactivate NoVs contaminating food, including high-pressure processing and irradiation, are under evaluation (Atmar*,* 2010; Lou *et al.,* 2011).

1.3.8 Epidemiology

Norovirus is the leading cause of acute viral gastroenteritis in humans worldwide, affecting people of all ages (Green, 2007; Glass *et al.,* 2009; Atmar, 2010; Tam *et al.,* 2012). It has been reported to be second only to RVs in causing severe childhood gastroenteritis (Glass *et al.,* 2009). Similarly, NoV infection can be particularly severe in the elderly, even resulting in death (Harris *et al.,* 2008; Glass *et al.,* 2009). The main route of transmission of NoVs is faecal-oral, although airborne transmission also occurs resulting in high secondary attack rates (Green, 2007; Atmar and Estes, 2009). Contamination of food, water and direct person-to-person spread have all been

© University of Pretoria

implicated in outbreaks of NoV gastroenteritis (Mathijs *et al.,* 2012). Gastrointestinal disease outbreaks associated with NoV occur frequently in closed settings, for example in schools, military camps and also in cruise ships (Carling *et al.,* 2009; Vivancos *et al.,* 2010; Wikswo *et al.,* 2011; Yap *et al.,* 2012), but have the greatest impact and occur with high frequency in health care settings, particularly hospitals and nursing homes (Götz *et al.,* 2002; Trivedi *et al.,* 2012).

Noroviruses are most frequently recognised as causes of outbreaks of acute gastroenteritis, but sporadic illness also occurs commonly. These viruses are responsible for 47% to 96% of outbreaks of acute gastroenteritis and for 5% to 36% of sporadic cases of acute gastroenteritis reported from countries around the world (Atmar and Estes, 2006; Patel *et al.,* 2009). In many cases, food is a frequent vehicle for virus transmission (Huang *et al.,* 2013). Contamination of the food with faecal material can occur at any step during its production, for example, contamination of shellfish usually occurs prior to harvesting, contamination of berries e.g., raspberries may occur prior to harvesting for instance due to irrigation with faecally-contaminated water, during harvesting contamination by infected field workers or during processing prior to distribution contamination in the factory by infected food handlers (Okabayashi *et al.,* 2008). Other foods implicated in NoV disease outbreaks, including pumpkin salad and sandwiches, have been contaminated at the site of preparation by infected food handlers (Götz *et al.,* 2002) and also cases of asymptomatic food handlers have been reported (Okabayashi *et al.,* 2008; Nikolay *et al.,* 2011; Yu *et al.,* 2011).

Noroviruses cause infection throughout the year, but in temperate climates there is distinct winter seasonality (Lopman *et al.,* 2009). Infection with NoV GII occurs more commonly that those with G1 (Patel *et al.,* 2009), though G1 has also been showed to cause gastroenteritis outbreaks in Sweden as a result of water contamination (Nenonen *et al.,* 2012). The GII NoVs have been the most prevalent strains causing infection worldwide (Patel *et al.,* 2009; CDC, 2011a). Among these, the GII.4 genotype was most prevalent in the majority of the years, and large increases in the number of outbreaks have coincided with the emergence of novel variants (Desai *et al.,* 2012; CDC, 2013a). Their very low infectious dose (<10 viral particles) combined with high levels of shedding and long persistence in the environment make NoVs extremely infectious (Teunis *et al.,* 2008; Patel *et al.,* 2009). Although generally NoV related

© University of Pretoria

illness is regarded as mild more severe outcomes are increasingly described among elderly and immunocompromised patients (Bok and Green, 2012; Trivedi *et al.,* 2012). The combination of large and difficult to control outbreaks and severe illness in some patients leads to major problems in healthcare settings, such as hospitals and nursing homes (Trivedi *et al.,* 2012). Over the last ten years many surveillance studies on NoVs around the world have shown that the spread of NoVs is a global problem and in developing countries limited studies has been done (Mans *et al.,* 2010, 2014; Bitler *et al.,* 2013). Waterborne outbreaks of NoV-associated gastroenteritis are well documented (Matthews *et al.,* 2012). The recent systematic review on the global epidemiological trends in NoV outbreaks highlights the risk factors associated with attack rate and NoV genogroup (Matthews *et al.,* 2012).

In Africa, various studies have reported the occurrence of NoVs in both clinical (Mans *et al.,* 2010, 2014; Hassine-Zaafrane *et al.,* 2013; Huynen *et al.,* 2013; Trainor *et al.,* 2013) and environmental samples (Kiulia *et al.,* 2010; Mans *et al.,* 2013; Murray *et al.,* 2013). Recently a study in Kenya has reported the detection of NoVs in immunocompromised children (Mans *et al.,* 2014). In Morocco, NoVs have been detected in shellfish collected along the Mediterranean Sea and Atlantic Coast of Morocco (Benabbes *et al.,* 2013). Very few studies in Africa have reported the occurrence of NoV in environmental water sources. These studies come from Kenya (Kiulia *et al.,* 2010), South Africa (Mans *et al.,* 2013; Murray *et al.,* 2013) and Ghana (Gibson *et al.,* 2011). In the Kenyan study a high (90%) NoV detection rate was reported for selected urban water sources and NoVs were detected in 8% to 25% of rural water sources (Kiulia *et al.,* 2010), but this study did not report on the NoVs genotypes circulating in these water sources. Therefore, there is a need to determine the molecular epidemiology of NoV genotypes in Kenyan water sources to clearly understand the epidemiology of these which will provide data that can assist in policy making

© University of Pretoria

1.4 ENTEROVIRUSES

1.4.1 History

Poliovirus (PV), the causative agent of poliomyelitis, is a human EV and member of the family of *Picornaviridae*. The PV were first isolated in 1909 by Karl Landsteiner and Erwin Popper (Pallansch and Roos, 2007).

1.4.2 Virology

Human enteroviruses (HEV) are small non-enveloped, positive-strand RNA viruses (Knowles *et al.,* 2012). Enteroviruses are about 27 to 30 nm in diameter with an icosahedral symmetry (Figure 1.8) (Pallansch and Roos, 2007; Knowles *et al.,* 2012). The HEV genome comprises a 5' non-translated region (NTR), a long ORF that encodes a protein of approximately 2100 amino acid residues, a short 3' NTR, and a polyadenylated tail. The post-translational cleavage products include four structural (VP1, VP2, VP3 and VP4) and seven non-structural (2A, 2B, 2C, 3A, 3B, 3C and 3D) viral proteins (Pallansch and Roos, 2007). Among the HEV, PV is one of the most well characterised viruses.

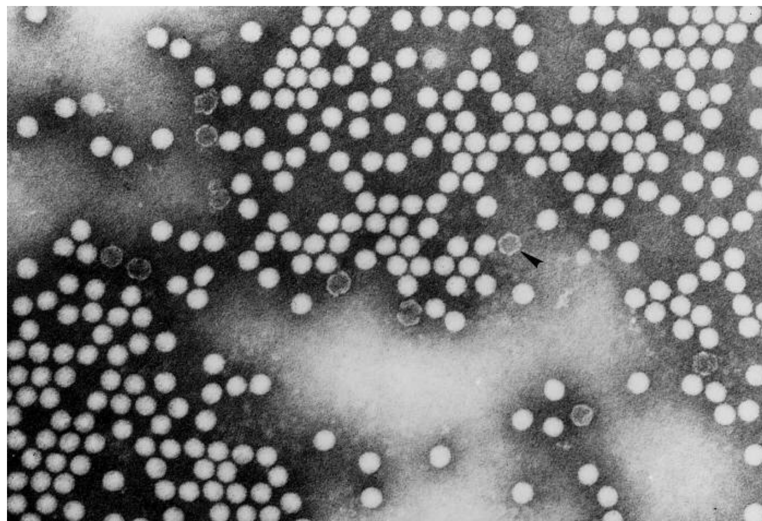

Figure 1.8: Electron micrograph of polioviruses
(http://pathmicro.med.sc.edu/lecture/polio.jpg)

© University of Pretoria

1.4.3 Classification

Enteroviruses (EVs) are classified in the genus *Enterovirus* of the family *Picornaviridae* (Knowles *et al.,* 2012). The other genera in the *Picornaviridae* family are *Cardioviris*, *Aphthovirus*, *Hepatovirus*, *Parechovirus*, *Erbovirus*, *Kobuvirus*, *Teschovirus*, *Sapelovirus*, *Senecavirus*, *Tremovirus* and *Avihepatovirus* (Knowles *et al.,* 2012). Enteroviruses are subgrouped into PVs and non-polioviruses EVs (NPEVs) (Solomon *et al.,* 2010). Poliovirus has three serotypes (PV1-3) and is classified in HEV species C. The NPEVs are divided into HEV-A 22, HEV-B 61, HEV-C 18, HEV-D 4. Other related viruses which may be members of the EVs and have not yet been approved as species by ICTV, include the following, EV-103, EV-108, EV-112 and EV-115, the simian (SV) 6 and SV-47 (Knowles *et al.,* 2012). The EV species can further be classified into serotypes or genotypes by their antigenic properties and phylogenetic sequence similarities (Knowles *et al.,* 2012). Attempts have been made to classify the HEV serotypes by using the partial nucleotide sequences of the HEV genomes, i.e., the 5' NTR (Diedrich *et al.,* 1995; Kopecka *et al.,* 1995; Bailly *et al.,* 1996), the VP4-VP2 junction (Pulli *et al.,* 1995; Huttunen *et al.,* 1996; Shimizu *et al.,* 1999), and VP1 (Brown *et al.,* 1999, Oberste *et al.,* 2000, 2001; Singh *et al.,* 2000). Methods for molecular classification of HEVs should not only identify the serotypes rapidly but also detect antigenic variant strains or new serotypes (Pallansch and Roos, 2007). Comparing the complete nucleotide sequences of the VP1 region a new serotype of HEV was identified and named HEV73 (Oberste *et al.,* 2001).

1.4.4 Pathogenesis and immunity

Enteroviruses first start replication in the gastrointestinal tract and then enter the bloodstream where they can affect other tissues and organs (Pallansch and Roos, 2007). Paralysis caused by EV infection is due to viral damage of the central nervous system (CNS) such as cells of the anterior horn of the spinal cord, with lower motor neuron lesions leading to flaccid paralysis (Pallansch and Roos, 2007). Immunity to EV infection is serotype specific, although reinfection with the same EV serotype may occur. After infection, the gut becomes resistant to reinfection with the same virus due to production in the gut of viral specific neutralising antibody. The humoral immune response plays a dominant role both in response to infection and for prevention of

© University of Pretoria

disease with reinfection (Pallansch and Roos, 2007). However, humoral antibody alone is not sufficient to limit enteroviral replication *in vivo* (Pallansch and Roos, 2007). Secretory IgA immunoglobulin, which appears in mucosal secretions and colostrum approximately two weeks after infection, provides relative protection against infection. Macrophage function plays a critical role in viral clearance, and recent findings in transgenic mice expressing the PV receptor have shown that host interferon response inhibits PV replication in extraneural tissues (Abe *et al.,* 2012). This activity is likely mediated via interaction with the Toll-like receptor (TLR) 3 pathway (Ida-Hosonuma *et al.,* 2005; Richer *et al.,* 2009).

1.4.5 Clinical features

Most EV infections are subclinical, especially in young children, but when they do cause clinically apparent disease, they can cause a wide range of clinical syndromes and can involve many of the body systems (Pallansch and Roos, 2007; Solomon *et al.,* 2010). The incubation period for EV infections is difficult to determine precisely, and may vary according to the clinical syndrome or the specific virus. Brief febrile illnesses mostly occur after an incubation period that averages between three to five days. In comparison, classic cases of paralytic poliomyelitis often exhibit a biphasic pattern with a non-specific febrile illness occurring three to five days after exposure. After nine to 12 days recurrence of fever with CNS manifestations occurs. Non-polio EVs most commonly cause rashes, upper respiratory tract infections (URTIs), aseptic meningitis, acute haemorrhagic conjunctivitis and summer colds. They can also cause neurological disease and are the most common cause of meningitis. In general, coxsackievirus (CV) infections tend to cause more severe complications than echovirus (E) infections resulting in myocarditis, pericarditis, pleurodynia, herpangina, hand-foot-and-mouth disease, and occasionally paralysis (Pallansch and Roos, 2007). As in the case of other EV the PV infection does not always result in clinically apparent disease (Pallansch and Roos, 2007). They usually cause what is known as ''abortive poliomyelitis'' which is a mild febrile illness with or without gastroenteritis and it occurs in about 4% to 8% of infected individuals. Poliovirus infection also causes aseptic meningitis, which is a non-paralytic illness which present with typical clinical features of viral meningitis (Pallansch and Roos, 2007). A small number (1%) of individuals infected with PV will develop acute flaccid paralysis (AFP) (Pallansch and Roos, 2007) due to the death of

© University of Pretoria

muscle-innervating neurons. The muscles become weak and they eventually atrophy as they no longer receive any nerve stimulation (Chumakov *et al.,* 2007). Paralysis of the legs occurs when the lower spinal cord is infected. However, the virus can cause paralysis of the diaphragm and in turn, severe breathing impairment if the infection spreads to the brain stem. Permanent paralysis may occur in approximately 80% of cases, death in about 10% and the remaining 10% may recover completely after infection (Pallansch and Roos, 2007).

1.4.6 Laboratory diagnosis

In most cases of enteroviral infection, virus isolation in cell culture is the method of choice, though not all EV grows in cell cultures, for instance some of the CVs A (CV-A). The samples of choice depends on the site of infection: e.g., for CNS disease, cerebrospinal fluid (CSF), for eye disease, conjunctival swab, for vesicular disease, vescular fluid, and for autopsy materials, brain, intestines, heart, lungs, liver and lymph nodes are collected. Samples collected for PV isolation includes faeces, throat or rectal swabs. Two stool samples are collected 24 h to 48 h apart within four days of onset of paralysis and must reach the laboratory within three days in cooled condition (in ice-packs). The isolated virus can be neutralised by type specific sera which forms the basis for identification (WHO, 2004). Immunological assays are not widely used as the cell culture techniques are so efficient and recommended by WHO for use in routine polio surveillance laboratories throughout the world (WHO, 2004). Other EV infection where there is manifestation of CNS for instance CVs and E usually show the changes typical for that of viral meningitis with lymphocytosis and a high protein level. The wild-type poliovirus (WPV) cases are identified through AFP surveillance and testing of stool specimens for PVs in WHO-accredited laboratories (WHO, 2004). The Global Polio Laboratory Network provides comprehensive genomic sequencing of WPV isolates, which enables tracing of the probable origins of viruses imported into previously polio-free areas (WHO, 2004, 2012).

1.4.7 Management, treatment and prevention

Currently there are no vaccines available for the NPEVs. Prevention includes improved sanitation and general hygiene, in addition to quarantine when epidemics are detected.

© University of Pretoria

In addition to having no vaccines, there are no specific antiviral agents currently available for clinical use (Collett *et al.,* 2008; Oberste *et al.,* 2009). Treatment is symptomatic and focuses on complications associated with infection. Administration of immunoglobulin may be useful in preventing severe disease in immunocompromised individuals or in those with life-threatening disease (Pallansch and Roos, 2007). Currently, there is no treatment for poliomyelitis. In most cases, for those with mild symptoms or aseptic meningitis, supportive care may include medication such as acetaminophen or ibuprofen to control fever or pain. Poliovirus infection can be prevented by immunisation with either one of the approved PV vaccines, i.e., inactivated Salk vaccine - the formalin inactivated polio vaccine (IPV). This vaccine is a trivalent and is administered intramuscularly (Pallansch and Roos, 2007). The IPV does not induce local IgA mediated immunity to PV in the gut. However, IPV has been shown to confer herd immunity against PV (Hird and Grassly, 2012). It reduces pharyngeal and faecal shedding of the virus in vaccinated individuals. In most developed countries that have used IPV a dramatic reduction of circulating PV was recorded (Pallansch and Roos, 2007; Hird and Grassly, 2012). This is the vaccine targeted to replace the live attenuated oral polio vaccine (OPV) (Sabin vaccine) in the proposed polio end-game. Two OPVs are available: the monovalent (mOPV) and the trivalent (tOPV) forms. The mOPV consist of live, attenuated PV strains of either PV1 (mOPV1) or PV3 (mOPV3). The monovalent vaccines were extensively used in the early 1960s. The tOPV consist of live attenuated PVI, PV2 and PV3 strains (Hird and Grassly, 2012). There is also a bivalent OPV (bOPV) that consists of live attenuated PV1 and PV3 strain (Sutter *et al.,* 2010; Hird and Grassly, 2012). The OPV has several advantages over IPV: i) it induces long lasting immunity, similar to that seen after natural infection, ii) it induces secretion of IgA antibodies that is not seen with IPV, thus conferring local immunity against reinfection in the pharynx and gut, and iii) it is inexpensive to use in mass immunisation campaigns especially in developing countries (Hird and Grassly, 2012). The major disadvantage of OPV is that the vaccine strains can reverse to neurovirulence, thus, causing vaccine associated paralytic poliomyelitis (VAPP) (Modlin, 2010).

© University of Pretoria

1.4.8 Epidemiology

Transmission of EVs occurs predominantly via direct or indirect contact with faecally shed virus although transmission from respiratory secretions is known to occur for some serotypes, including CV-A21 and is more likely to occur in crowded living conditions (Pallansch and Roos, 2007). Enteroviruses are distributed worldwide and are influenced by season and climate. Infections occur in summer and early fall in temperate areas, while in tropical and semitropical areas infection occurs throughout the year (CDC, 2006). While EV infections occur in all age groups, infants less than one year of age become infected at rates that exceed those of older children and adults by several-fold. The NPEVs are responsible for 10 to 20 million symptomatic infections per year and are more prevalent among children of lower socioeconomic class, probably because of crowding, poor hygiene, and opportunities for faecal contamination (Pallansch and Roos, 2007). In the USA between 2002 and 2004, E-9 and E-30 were the most commonly reported EV serotypes (CDC, 2006). In contrast, other EV serotypes such as E-1, CV-B6, EV-D68 and EV-B69 were reported to have little epidemic potential (CDC, 2002a). Though this serotype has been detected in other countries and at a high rate (Piralla *et al.,* 2012). The EV-A71 and CV-A16 has been reported to cause major outbreaks of hand, foot, and mouth disease in most parts of the world (Solomon *et al.,* 2010; CDC, 2012).

1.4.8.1 Non-polio enteroviruses

Non-polio enteroviruses remain widespread globally. They cause a wide variety of clinical diseases, especially in children, most of them being mild or asymptomatic, but serious illnesses may occur, such as viral meningitis, paralysis, pericarditis and myocarditis (Laxmivandana *et al.,* 2013). With the eradication of circulating WPVs, other EVs are being more commonly identified as the cause of polio-like illnesses (Kelly *et al.,* 2006; Dhole *et al.,* 2009). As the global eradication of poliomyelitis is in sight, attention towards NPEV infection causing AFP is an equal cause of concern (Laxmivandana *et al.,* 2013). Many NPEV serotypes associated with AFP have been reported (Saeed *et al.,* 2007; Odoom *et al.,* 2012; Laxmivandana *et al.,* 2013). The NPEV isolation rate is reckoned as one of the WHO-recommended indicators that are used to monitor the sensitivity of the PV surveillance programme as NPEVs have a worldwide distribution and are not targeted by any control programme. In India, the

© University of Pretoria

NPEV isolation rate in AFP case is 25% to 35% throughout the year. A few previous study reports have mentioned several cases of paralysis which were attributed to NPEV (Dhole *et al.,* 2009).

1.4.8.2 Polioviruses

Polioviruses, the cause of paralytic poliomyelitis, still causes significant disability in many parts of the world. It is a highly infectious virus that can spread easily from person -to-person (CDC, 2012).

1.4.8.2.1 Global polio eradication initiatives

Since 1988, WPV has been targeted by a global eradication programme based on mass vaccination of the population and a complete virological investigation of all AFP cases (WHO, 2010). Poliomyelitis became very rare at the end of 2012 (CDC, 2012). The PV has been successfully eliminated from many parts of the world by a successful WHO-sponsored Global Poliomyelitis Eradication Initiative (GPEI). The WHO Region of the Americas, the Western Pacific Region, and European Region are certified as polio-free. However, four countries in the European Region (Kazakhstan, Tajikistan, Turkmenistan, and the Russian Federation) experienced WPV outbreaks in 2010 (CDC, 2012). There are, however, three countries, namely Afghanistan, Nigeria and Pakistan, in which the transmission of endemic WPV has never been interrupted (Skern, 2010, CDC, 2011b, WHO, 2012). The WPV has been re-introduced from these countries into countries that have previously been certified as PV-free, and in Africa, 15 previously certified PV-free countries have recorded resurgence in poliomyelitis (Jenkins *et al.,* 2010; Skern, 2010; CDC, 2011b, 2013b). There are recent studies that have reported the outbreak of vaccine-derived PVs (VDPV) in polio-free countries (Rakoto-Andrianarivelo *et al.,* 2005; CDC, 2012) and these VDPVs were due to recombination between PV with circulating HEVs. Recently a recombinant VDPVs in healthy children has been reported in Madagascar (Razafindratsimandresy *et al.,* 2013). Factors which have contributed to the re-emergence of WPV in African countries include armed conflicts, poor infrastructure and widening gaps in immunisation (Skern, 2010).

1.4.8.2.2 Polio eradication efforts in Kenya

The introduction of the OPV into the national immunisation programme and the mass vaccination campaigns of children < 5 years of age resulted in the elimination of the

© University of Pretoria

disease in Kenya. The country was polio-free for 22 years (CDC, 2002b, WHO, 2002b) until its re-introduction in 2006 (CDC, 2009). Poliomyelitis was reported in a three year old girl born in Kenya and who lived in the Dadaab refugee camp in north-eastern Kenya and who had never been to Somalia. Genetic nucleotide sequence analysis indicated that it was a virus of Nigerian origin, imported from Kismayu, Somalia (CDC, 2009). In 2009, there were 19 cases of poliomyelitis confirmed in Turkana, a semi-arid area in Kenya bordering Sudan (CDC, 2009), with the majority of the cases reported being asymptomatic. In 2013, there was emergence of WPV1 in Kenya where 14 cases were reported including in the Dadaab refugee camp (CDC, 2013b) and this strain was of West African origin (CDC, 2013b). There is a large presence of refugees from Somalia in the northern Kenya region and the area is a host to large populations of nomadic pastoralists, crossing the Ethiopian and Sudan borders and therefore is seen to be at risk of importing WPV, which may spread among the young non-immunised generation. As of October 2013 WPV1-associated outbreaks of poliomyelitis also occurred in Ethiopia (six cases), South Sudan (three cases) and Somalia (174 cases) (CDC, 2013b). The WPV can circulate in a well-vaccinated population (Bottinger and Herrstrom, 1992; Swartz and Handsher, 1993-1994; Reichler *et al.,* 1997), with or without clinical cases, and OPV can cause VAPP (Kew *et al.,* 2004; Troy *et al.,* 2011). As the PV eradication campaign reaches its final stages, the emergence of circulating vaccine-derived PVs (cVDPV) is a cause of concern (Modlin, 2010).

1.4.8.2.3 Environmental surveillance of polioviruses

Environmental poliovirus surveillance means monitoring of PV circulation in human populations by examining environmental specimens supposedly contaminated by human faeces (Hovi *et al.,* 2012). The WHO has included environmental surveillance of PV in the new strategic plan of GPEI for years 2010 to 2012 to be increasingly used in PV surveillance, supplementing the AFP surveillance (GPEI, 2010; Hovi *et al.,* 2012). Polioviruses in the environment have been shown to be genetically and epidemiologically related to those circulating in the community (Yoshida *et al.,* 2002; Mueller *et al.,* 2009). The circulation of the PVs in the population can therefore be monitored through the isolation and/or detection of the virus from sewage samples (Hovi *et al.,* 2001, 2005; Horie *et al.,* 2002; Yoshida *et al.,* 2002; Mueller *et al.,* 2009; Tao *et al.,* 2010). Environmental surveillance for PVs has also been applied for the evaluation of the effectiveness of immunisation campaigns and for epidemiological

© University of Pretoria

investigations (Bottinger and Herrstrom, 1992; Tambini, *et al.,* 1993; Yoshida *et al.,* 2002). There are reports indicating that environmental surveillance may be more sensitive in detecting low intensity WPVs in circulation than the surveillance for AFP (Manor *et al.,* 1999; Ranta *et al.,* 2001; Deshpande *et al.,* 2003; El Bassioni *et al.,* 2003; Tao *et al.,* 2010; Hovi *et al.,* 2012). Recently WPV1 was detected in Israel during routine environmental surveillance of PV (Anis *et al.,* 2013). In Kenya EVs were detected in rural and urban rivers and wastewater sources (Kiulia *et al.,* 2010). Therefore, analysis of these water sources for PVs will be a valuable supplementary surveillance system for the presence of circulating WPVs and cVDPVs in high-risk communities in selected areas in Kenya.

1.5 RECOVERY AND DETECTION OF ENTERIC VIRUSES IN WATER

The recovery and detection of viruses in water samples is a multistage process. This includes: i) sample preparation or viral recovery, ii) viral detection, and iii) viral typing or characterisation (Wyn-Jones, 2007; Mattison and Bidawid, 2009).

1.5.1 Recovery of enteric viruses in water

In order to recover viruses in water, the best methods used should be simple, rapid, inexpensive, and consistent (Ikner *et al.,* 2012). The sampling procedures and techniques for the recovery of viruses from water samples are well described (Bosch *et al.,* 2011; Ikner *et al.,* 2012) and include ultracentrifugation, ultrafiltration, adsorption-elution using positively or negatively charged membranes or filters, glass wool or glass powder, flocculation and two-phase separation with polymers (Wyn-Jones, 2007; Mattison and Bidawid, 2009; Bosch *et al.,* 2011). These methods have their own advantages and disadvantages that can affect the efficiency of recovery of viruses (Ikner *et al.,* 2012). The physicochemical quality of water such as pH, conductivity, turbidity, and organic acid should be considered when choosing the method to use for recovery of viruses (Ikner *et al.,* 2012).

1.5.1.1 Ultracentrifugation

Centrifugation with sufficient gravitational forces is capable to pellet and concentrate viruses. Ultracentrifugation may also be used in conjunction with a density gradient to

© University of Pretoria

purify viruses (Ikner *et al.,* 2012). This method has been used for recovery of viruses from water, fresh produce and shellfish (Mattison and Bidawid, 2009). Ultracentrifugation has several advantages over other used concentration methods. The samples can be processed without adjustments to pH and no elution (Prata *et al.,* 2012) or secondary concentration steps is required (Fumian *et al.,* 2010). However, it has several drawbacks. One, the equipment used is highly specialized and expensive and only small volumes of sample can be processed at a time (Fumian *et al.,* 2010; Prata *et al.,* 2012).

1.5.1.2 Entrapment by ultrafiltration

This method recovers viruses from water samples based on their particle size and electrostatic interaction between electronegative viruses (Wyn-Jones and Sellwood, 2001; Wyn-Jones, 2007; Ikner *et al.,* 2012). The main advantages of ultrafiltration is that the sample requires no preconditioning and that a wide range of viruses and bacteriophages are recovered with high efficiency (Wyn-Jones and Sellwood, 2001; Wyn-Jones, 2007; Ikner *et al.,* 2012). This technique is very expensive, since it uses high cost equipment and that turbid waters take a relatively long time to process. The cost of buying the filters is also high and its use in low resource countries is limited (Huang *et al.,* 2000; Ikner *et al.,* 2012).

1.5.1.3 The glass wool adsorption-elution technique

Oiled sodocalcic glass wool is an adsorbent material capable of concentrating viruses from water due to its net positive charge and hydrophobicity. The glass wool adsorption-elution technique has proved to be a cost-effective technique for the recovery of viruses from large volumes of water in the African setting (Taylor *et al.,* 2001; Vivier *et al.,* 2004; Ehlers *et al.,* 2005; van Heerden *et al.,* 2005; van Zyl *et al.,* 2006; Kiulia *et al.,* 2010). With this method viral recovery can be done on laboratories which have minimal facilities like electricity and vacuum pumps without sending or transporting of large volumes of potentially polluted water to a well-equipped or central laboratory for further analysis (Bosch *et al.,* 2011). This method has been used to recover enteric viruses from different water sources, e.g., group A rotavirus (van Zyl *et al.,* 2006; Kiulia *et al.,* 2010), EVs (Ehlers *et al.,* 2005), adenoviruses (van Heerden *et al.,* 2005), NoVs (Kiulia *et al.,* 2010, Mans *et al.,* 2013) and SaVs (Murray *et al.,* 2013). The samples are then eluted using glycine/beef extract buffers. Thereafter a secondary

© University of Pretoria

concentration process is done using polyethylene glycol (PEG)/sodium chloride (NaCl) precipitation. This reduces the volume of the eluate to about 100 mℓ as well as getting rid of the possible inhibitors which may interfere with the downstream application (Ikner *et al.,* 2012).

1.5.2 Detection and molecular characterisation of enteric viruses in water

Currently, a wide range of analytical methods is available for virus detection in environmental water samples (Bosch *et al.,* 2011; Hamza *et al.,* 2011). These include viral isolation, immunological-based assays and molecular-based assays.

1.5.2.1 Viral isolation in cell cultures

Cell culture techniques, the gold standard for isolation and detection of EVs, cannot be applied for the routine detection of potentially infectious viruses in water due to the lack of susceptible cell lines for the isolation of viruses and not all viruses are able to produce a cytopathic effect (CPE). Viruses such as human NoVs have no available cell line for propagation (Formiga-Cruz *et al.,* 2005; Greening, 2006; Green, 2007). This technique is also time consuming, costly and requires training before one embarks on applying it. This method has its advantages as the viruses detected can be identified as infectious because of CPE observed.

1.5.2.2 Electron microscopy and immunological-based assays

Viruses in water are usually in very low titres and the use of EM is limited. Routine conventional viral diagnostic assays such as EIA cannot be applied to the detection of enteric viruses in water as these viruses are usually present in low titres, which are below the detection limits of the assays (Koopmans and Duizer, 2004).

1.5.2.3 Molecular-based assays

Molecular methods, such as PCR and rt RT-PCR, have the highest sensitivity and specificity to investigate virus contamination in water and are most commonly used in environmental virology for both direct detection and typing of the viruses (Fong and Lipp, 2005; Bosch *et al.,* 2011). Despite the high sensitivity of PCR, the main limitation is the lack of correlation between the detected viral genome and viral infectivity, unlike in the cell culture technique**.** The development of these molecular

© University of Pretoria

assays improved the detection of viruses in water (Fong and Lipp, 2005; Hamza *et al.,* 2011). These techniques are now considered to be the gold standard for enteric virus detection in water (Bosch *et al.,* 2008). The development of rt RT-PCR assays, which is now claimed to be the new gold standard for the quantification of enteric viruses (El-Senousy *et al.,* 2007), has further improved the detection of enteric viruses in water samples as the probes used are highly specific. The rt RT-PCR has the added advantage that it is less time consuming, more sensitive and human error is reduced as the post-amplification interpretation of results is automated. In addition contamination is reduced since post-amplification handling of samples is avoided (Manojkumar and Mrudula, 2006). This method also has some limitations: firstly, it is unknown whether or not the viruses are infectious, and. secondly, the reagents and equipment used are very expensive and need well trained personnel to perform the assays.

To maintain accuracy and quality of results when doing detection and characterisation of viruses in water, quality control processes should be applied (Bosch *et al.,* 2011). The quality control and quality assurance measures include the use of both positive and negative controls to validate the process and to know the true positive or negative results, thus, excluding any false results (Bosch *et al.,* 2011). In any process negative controls should be included such as, a negative process control, a negative extraction control, a negative RT-PCR, rt PCR/RT-PCR. Also positive controls which include a positive RT-PCR, rt PCR/RT-PCR control, a positive process control (should have similar features to the target virus), like a sample that has been spiked with the target organism and processed in parallel with the test samples (Bosch *et al.,* 2011). The rt PCR/RT-PCR should also have an internal control that distinguishes a true negative from a false negative result (Rutjes *et al.,* 2005; Bosch *et al.,* 2011).

1.6 MICROBIAL INDICATORS OF FAECAL POLLUTION

The monitoring the microbiological quality of drinking water relies largely on examination of indicator bacteria such as coliforms, *Escherichia coli, Pseudomonas aeruginosa* and enterococci (WHO, 2008). The total coliforms are a group of bacteria that are widespread in nature and are found in the intestinal tracts of warm-blooded animals (Bain *et al.,* 2014; Harwood *et al.,* 2014) but are not specific indicators of faecal pollution (Ashbolt et al., 2001). Thermotolerant or faecal coliforms are more

© University of Pretoria

useful as indicators of faecal pollution (Ashbolt *et al.,* 2001), while the WHO Guidelines for drinking water quality state that as an indicator organism *E. coli* provides conclusive evidence of recent faecal pollution and should not be present in water destined for human consumption (WHO, 2008).

1.7 MOTIVATION FOR THIS INVESTIGATION

Enteric virus diseases cause major health problems in developing countries (Verheyen *et al.,* 2009). In Kenya there are a paucity of data on the occurrence and nature (species and genotypes) of enteric viruses in environmental water sources. Data regarding the presence of enteric viruses in rivers and wastewater will be useful in assessing the potential risk of infection through waterborne transmission. In addition the analysis of water samples is an additional tool to determine the molecular epidemiology of viruses circulating in both symptomatic and asymptomatic individuals in the surrounding community. The introduction of oral RV immunisation has necessitated the continued surveillance of RV types circulating in a given community as circulating RV strains may affect vaccine efficacy. Kenya is planning to introduce the RV vaccine in its EPI programme in mid-2014. As the RV vaccine has already been licensed in Kenya and being used in private sector (Kiulia *et al.,* 2010) there is a need for ongoing surveillance of RVs circulating in urban and rural communities to monitor for the emergence of novel genotypes. Noroviruses are highly infectious, with a low infectious dose (Atmar and Estes, 2006; Teunis *et al.,* 2008) and high attack (>30%) and secondary attack rates (Atmar and Estes, 2006; Atmar, 2010). Worldwide many different types of NoV are circulating in the general population (Atmar, 2010), with individual strains emerging and becoming more prominent (Blanton *et al.,* 2006; Glass *et al.,* 2009; CDC, 2013b). After RVs, NoVs are the second most common cause of gastroenteritis in hospitalised paediatric patients (Matson and Szücs, 2003; Koopmans, 2008; Atmar, 2010; Mans *et al.,* 2010). There are no data regarding the molecular epidemiology of NoVs in Kenya and baseline data is required to establish which genotypes are circulating in the broader Kenyan community. The global eradication of PV cannot be achieved while there is still a risk of importations of WPV from polio-endemic countries into polio-free areas. The risk for importation is great for countries like Kenya which are adjacent to countries such as Somalia and Sudan where WPVs are still circulating (CDC, 2013b). The recent cases of poliomyelitis in Kenya in 2013 (CDC, 2013b), reinforces the need for

© University of Pretoria

environmental surveillance for circulating WPVs in high-risk areas in Kenya. In addition, such surveillance will be useful for monitoring the circulation of VDPVs once vaccination with OPV has been terminated.

1.8 HYPOTHESIS

1.8.1 Null hypothesis (HO)

In Kenya the surface water especially the rivers are not contaminated with clinically relevant enteric viruses and they are not a potential source of waterborne viral diseases.

1.8.2 Alternative hypothesis (HA)

In Kenya the surface water, especially the rivers are contaminated with clinically relevant enteric viruses and are a potential source of waterborne viral diseases.

1.9 AIM OF INVESTIGATION

The aim of this investigation was to determine the nature and prevalence of selected enteric viruses in low and high-risk environmental water samples in Kenya.

Specific objectives

i) To recover and detect, by rt RT-PCR, selected clinically important enteric viruses, namely EVs, NoVs and RVs in environmental water samples in low- and high-risk areas in different geographical regions in Kenya;

ii) To recover and isolate, in cell culture, PVs from environmental water samples from different geographical regions in Kenya;

iii) To characterise the environmental RV, PV and NoV strains genetically, and to compare the data to that of prototype, vaccine and clinical relevant strains from other regions in Africa and the rest of the world.

© University of Pretoria

CHAPTER 2

MATERIALS AND METHODS

2.1 ETHICAL APPROVAL

This study was approved by the Institutional Review Committee (IRC) of the Institute of Primate Research (IPR) (Protocol number: IRC/24/11) and the Student Ethics Committee of the Faculty of Health Sciences, University of Pretoria (Protocol number S172/2011).

2.2 AREA OF STUDY AND SITE SELECTION

The following sites were selected for the collection of water samples:

i) Mboone river in the rural Maua town, Meru County (Figure 2.1A): The town has neither sewerage system nor sewerage treatment plant. This river is highly polluted with sewerage coming from the surrounding overflowing latrines. Although this river was investigated previously for enteric viruses (Kiulia *et al.,* 2010), it was selected for re-investigation to determine whether or not the level of faecal pollution had increased and also to provide molecular epidemiological data on NoVs from rural communities;

ii) Mutoine river in the urban Kibera (Figure 2.1B) which is a densely populated informal housing/slum area of Nairobi. This river passes through the Nairobi dam and joins the Nairobi river downstream. The river is used by many slum dwellers for irrigating their fresh produce;

iii) Nairobi river in urban Nairobi County (Figure 2.1B) and which passes through a second urban slum in Kamukunji which includes the Mathare, Maringo and Eastleigh areas;

iv) Dadaab refugee camp in eastern Kenya (Figure 2.1C). Dadaab is a town in north-eastern Kenya approximately 100 km from the Kenya-Somalia border. Most of the people living in the camp are Somali refugees accounting for about 500 000 people or 97% of the refugees. This refugee camp also caters for refugees from other areas in conflict, namely, Sudan, Uganda and the Democratic Republic of Congo.

© University of Pretoria

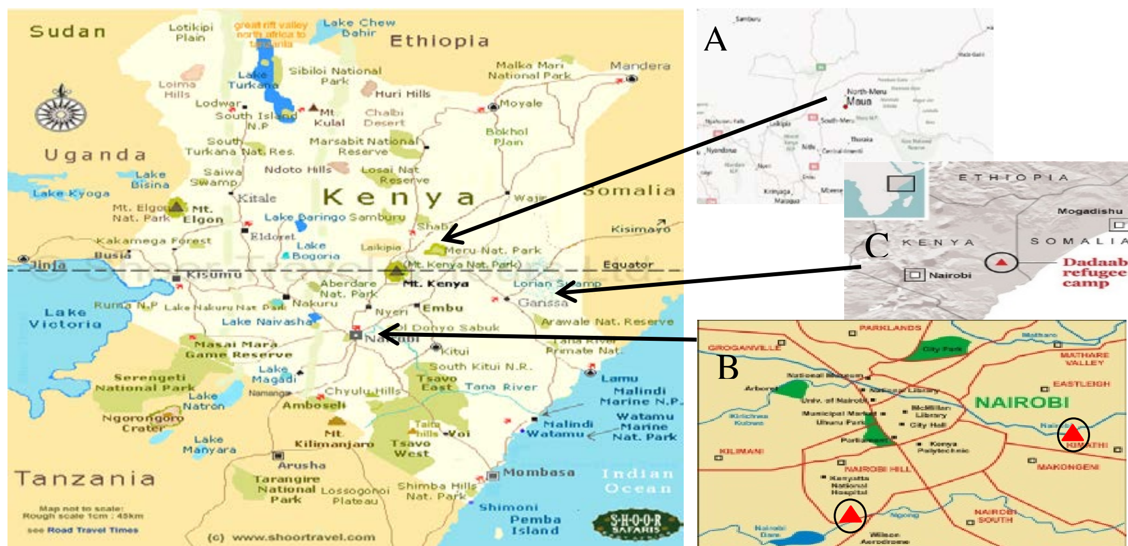

Figure 2.1: Diagrammatic map showing the different geographic regions where water samples were collected: A: Mboone river, Maua, Meru County; B: Mutoine and Nairobi river, Nairobi County; C: Dadaab refugee camp.

2.3 SAMPLE COLLECTION

Water samples (10 litre [ℓ]) were collected in sterile containers from the Nairobi, Mutoine and Mboone rivers. Sampling was done at set intervals on a monthly basis for a full calendar year to cover both the wet and dry seasons. Water samples from household containers (2) and borehole water (2) from the Dadaab refugee camp were collected on two occasions. A separate 500 mℓ water sample was collected in sterile bottles at each site for microbiological indicator analysis. All the samples were transported in cooler bags from the sites to the Enteric Viruses Laboratory, IPR, Nairobi and stored at 4°C until processing.

2.4 SAMPLE PROCESSING AND VIRUS RECOVERY

Viruses were recovered from the river water samples using a glass wool adsorption-elution procedure based on the method of Vilaginès *et al.,* (1993) as described by Wolfaardt *et al.,* (1995) and Vivier *et al.,* (2004), and further modified and optimised by Venter (2004). The glass wool columns, 20 centimetre (cm) in length and with an internal diameter of about 30 millimetre (mm) were packed with 15 grams (g) oiled sodocalcic glass wool (Glass wool Bourre 725 QN, Ouest Isol, Alizay, France). During

© University of Pretoria

packing three 5 g portions of glass wool were compressed into the columns at different angles to each other with a steel sieve grid with pore sizes of about 1 mm^2 inserted between each 5 g portion of compressed glass wool. The packed columns were then treated to positively charge the glass wool by soaking in autoclaved sterile distilled water and then pre-treated with 40 millilitre (mℓ) of 1 molar (M) hydrochloric acid (HCl) (Merck Darmstadt, Germany), 100 mℓ sterile distilled water and then 40 mℓ of 1 M NaOH (Merck) with a final rinse of 100 mℓ sterile distilled water to adjust the pH to 7.0. To recover the viruses, 10 ℓ of water were filtered through the positively charged glass wool columns using negative pressure at a flow rate of 10 ℓ/h. The negatively charged viruses, which adsorbed to the glass wool, were eluted twice with 50 mℓ glycine-beef extract buffer pH 9.5 (GBEB; 0.05 M glycine (Merck); 0.5% beef extract [BBL™ Becton Dickinson and Co., Sparks, MD]). Immediately after elution the pH of the eluate was neutralised to pH 7 using 1 M HCl (Merck). The viruses in 100 mℓ eluate were secondary concentrated using PEG/NaCl precipitation. Briefly, 14 g of PEG 6000 (Merck) and 1.17 g NaCl (Merck) were dissolved in the 100 mℓ eluate and maintained at 4°C overnight. The solution was centrifuged (Beckman GPR, Glenrothes, Scotland) at 6000 x *g* for 30 min at 4°C. The supernatant was discarded and the pellet was resuspended in 10 mℓ phosphate-buffered saline (PBS) pH 7.2 (Sigma-Aldrich Co., St Louis, MO). The recovered virus suspensions were stored at -20°C until further analysis.

2.5 MICROBIAL INDICATOR ORGANISMS

From the 500 mℓ water sample collected 1 mℓ was analysed for indicator organisms within 24 h of collection. *Escherichia coli* and total coliforms were enumerated using the Compact Dry for *E. coli* and coliforms (CD-EC) plate (HyServe GmbH & Co. KG, HechenRainer, Germany). This CD-EC is a ready-to-use test method for the enumeration of *E. coli* and total coliform bacteria in food and water. The plates are pre-sterilised and contained culture medium with a cold water-soluble gelling agent. The CD-EC plate contains the nutrients developed primarily for the rapid growth of coliform, including 5-bromo-6-chloro-3-indoxyl-/3-D-galactopyranoside (Magenta-Gal) for coliform and 5-bromo-4-chloro-3-indoxyl-/3-D-glucuronic acid (X-Gluc) for *E. coli* (Hosokawa and Kodaka, 2010).

© University of Pretoria

One millilitre was inoculated onto the CD-EC plates and the solution was allowed to diffuse by capillary action. After 24 h of incubation at 35°C, *E. coli* and total coliform counts colony forming units (cfu/mℓ) were calculated by counting the total colonies eliciting the obligatory colour production on each medium multiplied by the dilution factor. For the *E. coli* blue and blue purple colonies and for the total coliforms red/pink purple colonies were counted.

2.6 VIRUS ISOLATION

2.6.1 Cell cultures

The cell cultures used in this study were those used routinely at the Department of Medical Virology, University of Pretoria for isolation of selected enteric viruses from water samples. The L20B cell line used, was kindly donated by the WHO Reference Laboratory, National Institute for Communicable Diseases, South Africa, courtesy of Dr. Nicksy Gumede-Moeletsi.

2.6.1.1 Human hepatoma cell line (PLC/PRF/5) (European Collection of Cell Cultures [ECACC 85061113])

The PLC/PRF/5 human liver hepatoma cell line is susceptible to most culturable enteric viruses (Grabow *et al.,* 1999). The PLC/PRF/5 cell line has been used to isolate HAdVs from both environmental and clinical samples (van Heerden *et al.,* 2005).

2.6.1.2 Buffalo green monkey kidney cell line (BGM) (ECACC 90092601)

The BGM cell line is very sensitive to infection by PV and other EVs such as coxsackievirus B virus (CV-B) and is the most common cell line used for the detection of these viruses in water (Dahling, 1991, Balkin and Margolin, 2010).

2.6.1.3 L20B (CDC)

The L20B is a genetically engineered mouse cell line which expresses the human PV receptor and is non-permissive to most other human enteric viruses (Wood and Hull, 1999; WHO, 2004). These cells have shown to be more selective and sensitive for PV isolation than other cell lines used routinely, namely RD or Hep2 cells. The isolation of

© University of Pretoria

PVs from clinical samples is the gold-standard method for virological surveillance in the world-wide initiative to eradicate wild-type PVs.

2.6.1.4 Vero African Green Monkey cell line (ECACC 84113001)

Vero cells are derived from the kidney of an African green monkey, and are one of the more commonly used mammalian continuous cell lines for isolation of viruses (Ammerman *et al.,* 2008).

2.6.2 Cell culture propagation and maintenance

The human hepatoma cell line PLC/PRF/5 and BGM African Green Monkey cell lines were propagated and maintained as described by Taylor *et al.,* (2001), Vivier *et al.,* (2004) and Venter (2004). The L20B cell lines was maintained and propagated using the method described in the WHO polio laboratory manual (WHO, 2004).

2.6.3 Viral isolation and detection

Five milliliters of the recovered virus concentrate was clarified by the addition of 2 mℓ chloroform (Merck) and thereafter treated with 100 μℓ of penicillin/streptomycin/ neomycin (Sigma-Aldrich) and 100 μℓ of nystatin (Sigma-Aldrich) as described by Vivier *et al.,* (2004). Using conventional cell culture techniques, monolayers of the PLC/PRF/5, the L20B and the BGM cell cultures in 25-cm^2 cell culture flasks were inoculated in duplicate with 1 mℓ each of the virus concentrates and incubated at 37ºC as described by Grabow *et al.,* (1999), and Vivier et *al.,* (2004). After a 14-day incubation period at 37ºC, which included a blind-passage at day 7 post-infection, the infected cells were harvested for nucleic acid extraction. The cell cultures were also blind-passaged onto monolayers of the Vero cell line in cell culture tubes with flying coverslips. The infected cell cultures were examined daily for CPE and after seven days incubation, stained with haematoxylin and eosin and examined for virus specific inclusion bodies (Malherbe and Strickland-Cholmley, 1980).

© University of Pretoria

2.7 VIRUS DETECTION

2.7.1 Nucleic acid extraction

Depending on the downstream application and due to the low number of viral particles in water samples genomic viral nucleic acid was extracted using an automated system or a manual extraction method to maximise the rate of detection and characterisation of the selected enteric viruses.

2.7.1.1 Automated nucleic acid extraction

The recovered virus suspensions were clarified by the addition of 200 µℓ of chloroform (Merck) to 1.5 mℓ of the viral suspension followed by rigorous vortexing (Stuart Scientific Autovoltex SA6) for 30 sec. The mixture was then centrifuged at 3000 x *g* (Eppendorf 5402D Microcentrifuge, Hamburg, Germany) for 30 sec. Prior to nucleic acid extraction, 10 µℓ of mengovirus (5 x 10^4 copies/10 µℓ) was added to each sample as a process control to monitor the efficiency of nucleic acid extraction. Genomic viral nucleic acid was extracted directly from the seeded 1 mℓ of recovered virus concentrate using the MagNA Pure LC Total Nucleic Acid Isolation Kit (large volume) (Roche Diagnostics GmbH, Mannheim, Germany) in a MagNA Pure LC Robotic instrument (Roche Diagnostics), following the manufacturer's instructions. The extracted nucleic acid was eluted in 100 µℓ and stored in 10 µℓ aliquots at -70°C until use.

2.7.1.2 Manual nucleic acid extraction

From the 10 mℓ eluate from the viral recovery, 1 mℓ was used for manual nucleic acid extraction using the QIAamp® UltraSens® RNA extraction kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. An extraction negative control (nuclease-free water, Promega Corp, Madison, WI) was included. The extracted nucleic acid was eluted in 100 µℓ and stored in 10 µℓ aliquots at -70°C until use.

2.7.2 Virus amplification

2.7.2.1 Mengoviruses

Mengovirus was detected using a singleplex rt RT-PCR assay based on TaqMan technology and primers and probe described by Pintó *et al.,* (2009). The detection was

© University of Pretoria

performed using the mengo@ceeramTools™ Kit (Ceeram S.A.S, La Chappelle-Sur-Erdre, France) following manufacturer's instructions. The reaction was carried out in a total volume of 20 µℓ in sealed glass capillaries in a Lightcycler® v2.0 Instrument (Roche Diagnostics). The amplification was done on 5 µℓ of RNA extracts and the conditions of the assay were as follows: 45°C for 10 min, 95°C for 10 min and 45 cycles of 95°C for 15 sec, 60°C for 45 sec.

2.7.2.2 Enteroviruses

Enteroviruses were detected using qualitative singleplex rt RT-PCR assays based on TaqMan technology). The primers and probe used targets the EVs 5'-end non-coding region (NCR) and it allows the detection of the different EV species (human EVs A, B, C and D). Reverse transcription was done using 5 µℓ of the purified nucleic acid and amplification was performed using the enterovirus@ceeramTools™ Kit (Ceeram S.A.S) in a Lightcycler® v2.0 Instrument (Roche Diagnostics) following manufacturer's instructions.

2.7.2.3 Rotaviruses

Rotavirus was detected in a qualitative singleplex rt RT-PCR assay based on TaqMan technology and published primers and probe targeting the non-structural protein 3' (NSP3) region of the RV genome (Zeng *et al.,* 2008) using the rotavirus@ceeramTools™ Kit (Ceeram S.A.S) in a Lightcycler® v2.0 Instrument (Roche Diagnostics) following manufacturer's instructions.

2.7.2.4 Noroviruses

Noroviruses (GI and GII) were detected using qualitative singleplex rt RT-PCR assays based on TaqMan technology and published primers and probes, namely, NoV GI (da Silva *et al.,* 2007; Svraka *et al.,* 2007); and NoV GII (Kageyama *et al.,* 2003; Loisy *et al.,* 2005). Reverse transcription for NoV GI and NoV GII was done using 5 µl of the purified nucleic acid template and amplified using the norovirusGI@ceeramTools™ and norovirusGII@ceeramTools™ Kit (Ceeram S.A.S) in a Lightcycler® v2.0 Instrument (Roche Diagnostics) following manufacturer's instructions.

© University of Pretoria

2.7.3 Quality control

Standard precautions were applied in all the manipulations to reduce the possibility of cross-contamination between samples, and between sample and DNA amplicons. Separate laboratories, each equipped with its own apparatus such as, pipettes, using aerosol resistant filter tips and reagents tubes, were used for sample processing and preparation, reagent preparation, reaction preparation and manipulation of amplified fragments. Negative extraction controls i.e molecular grade water, a rt RT-PCR negative and positive control included in the kit was included in each virus detection reaction to monitor for false-positive and false-negative reactions. The mengovirus was used as a process control to validate the extraction procedures. CeeramTools™ kits were used for the detection of, RV, EV, NoV GI and NoV GII using rt RT-PCR. These kits have an internal amplification control to monitor the amplification process and therefore controls for false negatives.

2.8 VIRUS CHARACTERISATION

2.8.1 Rotavirus genotyping

Multiplex nested PCR methods and primers initially developed for the G typing (Gouvea *et al.,* 1990, 1994a; Das *et al.,* 1994; Gault *et al.,* 1999; Iturriza-Gómara *et al.,* 2004) and the P typing (Gentsch *et al.,* 1992; Gouvea *et al.,* 1994b; Mphahlele *et al.,* 1999; Simmonds *et al.,* 2008) of human RVs from clinical samples was applied for the characterisation of the VP7 (G types) and the VP4 (P types) genes of the environmental RV strains. Briefly, the dsRNA was denatured by boiling at 94°C for 5 min followed by chilling on ice. The RNA (8 µℓ) was then reverse transcribed by incubating with 1 µℓ of the forward and reverse primers, 0.8 µℓ 2.5 mM dNTPs, 20 µℓ 5X Avian Myeloblastosis Virus (AMV) buffer and 0.2 µℓ AMV reverse transcriptase (Roche Diagnostics) for 25 to 30 min at 42°C (Little Genius thermocycler (Bioer Technology Co. Ltd, China). The resultant full length 1062 bp (for VP7 gene) and 876 bp (for VP4 gene) cDNA was amplified using primers sBeg9 and End9 (for VP7) and Con2 and Con3 (for VP4) in a 40 µℓ reaction containing, 4 µℓ 2.5 mM dNTPs, 8 µℓ 5X Taq buffer, 2.4 µℓ 25mM $MgCl_2$, 26 µℓ molecular grade water and 0.2 µℓ DNA polymerase (Promega Corp.). A multiplex PCR with genotype specific primers for VP4 (genotype

© University of Pretoria

P), and VP7 (genotype G) was used for the characterisation of the RV. Briefly for VP7, consensus primer 9con 1 as described previously by Das *et al.,* (1994), was used with VP7 genotyping primers (Table 2.1). The Con3 primer was used as the consensus primer for VP4 genotyping with other human and animal primers (1T-1D, 1T-1v, 2T-1, 3T-1, 4T-1, 5T-1, mP11, pGott, pOSU, pUK, pNCDV, pB223) in a 50 µℓ reaction containing, 4 µℓ 2.5 mM dNTPs, 10 µℓ 5X Taq buffer, 3 µℓ 25mM $MgCl_2$, 29.8 µℓ molecular grade water and 0.2 µℓ DNA polymerase (Promega Corp) (Table 2.2). The primers listed in Tables 2.1 and 2.2 are described in the WHO/AFRO laboratory training manual 2010 and the Virology Division, National Institute for Communicable Diseases standard operating procedures for RV characterisation. The PCR was performed at 95°C for 1 min, followed by 30 cycles of 95°C for 1 min, 42°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min, before being stored at 4°C. The PCR products were resolved by electrophoresis on a 2% (m/v) agarose gel (NuSieve® 3:1 Agarose. FMC Bioproducts, Rockland ME), stained with ethidium bromide (Promega Corp.), and visualised under ultraviolet illumination (Gel Doc™.XR+System, Bio-RAD Labortories, Inc, Itercules, CA)

2.8.2 Poliovirus genotyping

Water samples shown to be positive for EV by direct analysis of the water and the L20B CPE-positive cell culture extracts were tested for PV using the rt RT-PCR method currently in routine use in the WHO Global Polio Laboratory Network (GPLN) for the differentiation between vaccine-related PV and WPV isolates (intratypic differentiation [ITD]) (WHO, 2004; Kilpatrick *et al.,* 2009). This was achieved by use of rt RT-PCR CDC analytical kits designed for the identification PV and differentiation between VDPVs and WPVs that included separate reactions with primers that were pan-EV, pan-PV (Kilpatrick *et al.,* 1996), serotype specific (Kilpatrick *et al.,* 1998), and specific for the Sabin type 1, 2, and 3 viruses (Yang *et al.,* 1991). In brief, rt RT-PCR was carried out in a reaction mixture of 25 µℓ containing 2.5 µℓ cDNA, 12.5 µℓ TaqMan Universal PCR Master Mix (Applied Biosystems), a 400 nM concentration of each primer, and 200 nM of each probe. Polymerase chain reaction amplification was performed in an ABI Prism 7500 thermocycler (Applied Biosystems, Foster City, CA) under the following conditions: incubation at 50°C for 2 min, initial denaturation at 95°C for 10 min, and then 45 cycles of amplification with denaturation at 95°C for 15 sec, and

© University of Pretoria

annealing and extension at 56°C for 1 min as described by Kilpatrick *et al.,* (2009). This characterisation was done at the WHO Reference Laboratory, National Institute for Communicable Diseases, Sandringham. This was essential to conform to the South African Department of Health's laboratory containment of PV requirements.

2.8.3 Norovirus genotyping

2.8.3.1 Virus amplification

The NoV strains detected in the water samples were characterised genetically, i.e. genotyped, by DNA sequence and phylogenetic analysis of the 5'-end of the NoV capsid gene (Region C) using a semi-nested RT-PCR. Briefly, amplification of the capsid gene of NoVs was performed as described by Mans *et al.,* (2013). Primers were synthesized by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). The cDNA was synthesized at 42°C for 60 min in a 20 µℓ RT reaction as follows, 10 µℓ of template RNA, 1.5 mM $MgCl_2$, 10 mM concentrations of each dNTPs, 200 U of RevertAid™ Premium Reverse Transcriptase (Thermo Scientific, Waltham, MA), 20 U of RNase inhibitor (Promega Corp.), and 100 pmol/ µℓ concentrations of random hexamer primers. Then, 10 µℓ of the cDNA sample was mixed with 5 µℓ PCR buffer with 1.5 mM $MgCl_2$, 200 µM concentrations of each dNTPs, 0.2 µM concentrations of primers QNIF4 and G1SKR for GI and primers QNIF2 and G2SKR for GII, and 0.25 U/µℓ of AmpliTaq Gold DNA polymerase (Applied Biosystems). After initial incubation at 95°C for 10 min, PCR was performed for 45 cycles (94°C, 30 sec; 50°C, 30 sec; and 72°C, 40 sec), with a final extension step at 72°C for 10 min. At the end of the amplification, 1 µℓ of the first-round PCR product was added to a seminested PCR mix containing the same reagents as the first round of PCR but with the primers G1SKF and G1SKR for GI and the primers G2SKF and G2SKR for GII (Table 2.4). The amplification products were analysed on 1.5% (m/v) agarose gels containing 0.5 ng/mℓ of ethidium bromide (Promega Corp.), using a 100 bp ladder (Thermo Scientific) and visualised under UV illumination (Gel Doc™.XR+System, Bio-RAD).

© University of Pretoria

Table 2.1: Primers used for genotyping of VP7 (G) types of rotaviruses

Primer	**Sequence (5'-3')[a]**	**Nucleotide Position**	**Genotype**
sBeg9	GGCTTTAAAAGAGAGAATTTC	1-21	
9Con1	TAGCTCCTTTTAATGTATGG	37 – 56	
End9	GGTCACATCATACAATTCTAATCTAAG	1062-1036	
dAnEnd9	GGTCACATCAAACAATTCTATTGC	1062-1045	
RVG9	GGTCACATCATACAATTCT	1062-1044	
EndA	ATAGTATAAAATACTTGCCACCA	922-944	
aBT1	CAAGTACTCAAATCAATGATGG	314-335	G1
aCT2	CAATGATATTAACACATTTTCTGTG	411-435	G2
mG3	ACGAACTCAACACGAGAGG	250-269	G3
aDT4	CGTTTCTGGTGAGGAGTTG	480-498	G4
aAT8v	GTCACACCATTTGTAAAYTCAC	178-198	G8
mG9	CTTGATGTGACTAYAAATAC	757-776	G9
mG10	ATGTCAGACTACARATACTGG		
G12b	GGT TAT GTA ATC CGA TGG ACG	504-524	G12
9Con1	TAGCTCCTTTTAATGTATGG	37 – 56	
9T1-1	TCTTGTCAAAGCAAATAATG	176 – 195	G1
9T1-2	GTTAGAAATGATTCTCCACT	262 – 281	G2
9T-3P	GTCCAGTTGCAGTGTTAGC	484 – 503	G3
9T-4	GGGTCGATGGAAAATTCT	423 – 440	G4
aFT5	GACGTAACAACGAGTACATG	779 – 760	G5
aDT6	GATTCTACACAGGAACTAG	499 – 481	G6
MW-8	TCT TCA AAA GTC GTA GTG	670 – 688	G8
aHT8	GTGTCTAATCCGGAACCG	273 – 256	G8
9T-9B	TATAAAGTCCATTGCAC	131 – 147	G9
aET10	GAAGTCGCAACGGCTGAA	714 – 697	G10
aBT11	GCAACTCAGATTGCTGATGAC	336 – 316	G11
sBeg9	GGCTTTAAAAGAGAGAATTTC	1-21	
OR (9Con1)	TAGCTCCTTTTAATGTATGG	37 – 56	

[a]IUPAC codes used to indicate degenerate positions: Y=C/T R=A/G N=A/G/C/T

© University of Pretoria

Table 2.2: Primers used for genotyping of VP4 (P) types of rotaviruses

Primer	Sequence (5'-3')[a]	Nucleotide position	Genotype
Human VP4 typing primers			
Con3	TGGCTTCGCTCATTTATAGACA	11-32	
VP4F	TATGCTCCAGTNAATTGG	132-149	
Con2	ATTTCGGACCATTTATAACC	868-887	
VP4R	ATTGCATTTCTTTCCATAATG	775-795	
1T-1D	TCT ACT GGR TTR ACN TGC	339-356	P[8]
1T-1v	TCT ACT TGG ATA ACG TGC	339-356	P[4]
2T-1	CTATTGTTAGAGGTTAGAGTC	474-494	P[4]
3T-1	TGTTGATTAGTTGGATTCAA	259-278	P[6]
4T-1	TGAGACATGCAATTGGAC	385-402	P[9]
5T-1	ATCATAGTTAGTAGTCGG	575-594	P[10]
mP11	GTAAACATCCAGAATGTG	305-323	P[11]
Con3	TGGCTTCGCTCATTTATAGACA	11-32	
OR (VP4R)	ATTGCATTTCTTTCCATAATG	775-795	
Animal VP4 typing primers			
pNCDV	CGAACGCGGGGGTGGTAGTTG	269-289	P[1]
pUK	GCCAGGTGTCGCATCAGAG	336-354	P[5]
pGott	GCTTCAACGTCCTTTAACATCAG	465-487	P[6]
pOSU	CTTTATCGGTGGAGAATACGTCAC	389-412	P[7]
pB223	GGAACGTATTCTAATCCGGTG	574-594	P[11]
Con2	ATTTCGGACCATTTATAACC	868-887	
OR (VP4R)	ATTGCATTTCTTTCCATAATG	775-795	

[a]IUPAC codes used to indicate degenerate positions: R=A/G N=A/C/G/T

© University of Pretoria

Table 2.3: Real-time RT-PCR primers and probes used for the characterisation of polioviruses.

Virus type	Primer or probe (polarity)[a]	Primer or probe sequence (5'-3')[b]	Nucleotide position[c]
PanEV	PCR-1 (A)	GCGATTGTCACCATWAGCAGYCA	603-581
	PCR-2 (S)	GGCCCCTGAATGCGGCTAATCC	458-480
	pan EV Probe (S)	FAM-CCGACTACTTTGGGWGTCCGTGT-BHQ1	546-568
PanPV	panPV/PCR-1 (A)	AYRTACATIATYTGRTAIAC	2978-2956
	panPV/PCR-2 (S)	CITAITCIMGITTYGAYATG	2876-2895
	panPV probe 21A (A)	FAM-TGRTTNARIGCRTGICCRTTRTTRTT-BHQ1	2957-2935
	seroPV1A (A)	ATCATIYTPTCIARPATYTG	2528-2509
Serotype 1	seroPV 1,2S (S)	TGCGIGAYACIACICAYAT	2459-2477
	seroPV1 probe 16A (A)	FAM-TGICCYAVICCYTGIGMIADYGC-BHQ1	2510-2488
Serotype 2	seroPV2A (A)	AYICCYTCIACIRCICCYTC	2537-2518
	seroPV1,2S (S)	TGCGIGAYACIACICAYAT	2459-2477
	seroPV2 probe5S (S)	FAM-CARGARGCIATGCCICARGGIATNGG-BHQ1	2482-2507
Serotype 3	seroPV3 A (A)	CCCCIAIPTGRTCRTTIKPRTC	3178-3157
	seroPV3S (S)	AAYCCITCIRTITTYTAYAC	3037-3056
	seroPV3 probe11S(S)	FAM-CCRTAYGTNGGITTRGCVAAYGC-BHQ1	3091-3113
Sabin 1	Sab1/PCR-1(A)	CCACTGGCTTCAGTGTTT	2600-2583
	Sab1/PCR-2 (S)	AGGTCAGATGCTTGAAAGC	2505-2523
	Sab1/Probe (A)	CY5-TTGCCGCCCCCACCGTTTCACGGA-BHQ3	2563-2540
Sabin 2	Sab2/PCR-1 (A)	CGGCTTTGTGTCAGGCA	2595-2579
	Sab2/PCR-2 (S)	CCGTTGAAGGGATTACTAAA	2525-2544
	Sab2/Probe (S)	FAM-ATTGGTTCCCCCGACTTCCACCAAT-BHQ1	2550-2572
Sabin 3	Sab3/PCR-1(A)	TTAGTATCAGGTAAGCTATC	2591-2572
	Sab3/PCR-2 (S)	AGGGCGCCCTAACTTT	2537-2552
	Sab3/Probe (S)	ROX-TCACTCCCGAAGCAACAG-BHQ2	2554-2571

[a] Polarity: A, antisense; S, sense.
[b] IUPAC nucleotide codes: V= G/C/A; D= G /A/T; I= inosine; K= G/T; M =A /C;N=A/G/C/T;P,6H,8H-3,4-dihydropyrimido[4,5-c][1,2]oxazin-7-one (3);R, A/G; S, G/C; w, A/T; Y, C/T (Kilpatrick *et al.,* 2009)
[c]Nucleotide positions are numbered according to the consensus numbering system of Toyoda *et al.,* 1984.

2.8.3.2 Cloning and sequencing

The PCR products were cloned using the ClonJET™ PCR cloning kit (Thermo Scientific). Briefly, 5 µℓ of the purified PCR product was used for the ligation reaction according to the manufacturer's instructions. The ligation mixture (4 µℓ) was then transformed in competent *E. cloni*® cells (Lucigen® Corp., Middleleton, WI), where 10-20 µℓ of *E. cloni* competent cells were thawed on ice and added to the ligation mixture and incubated for 30 min on ice. This was followed by heat-shocking of the

© University of Pretoria

mixture at 42°C for 30 min and then cooling the reaction on ice for 2 min. After cooling 200 µℓ of recovery media was added and then the mixture was incubated at 37°C (211D Shaker incubator Labnet International Inc., Woodbridge NJ) for 1 h. One hundred microliters of the transformed cells was then plated on a Luria-Bertani (LB) agar and incubated for 37°C (211D Shaker incubator) for 16 h. Fifteen discrete colonies were randomly selected and colony PCR was performed in a 20 µℓ reaction consisting of 4 µℓ 1X PCR buffer, 1.2 µℓ 1.5 mM $MgCl_2$, 0.4 µℓ 200 µM dNTPs, 0.4 µℓ 0.2 µM pJET1.2 forward and reverse primers and 0.1 GoTaq® Flexi DNA polymerase (Promega Corp.) and nuclease free water (Promega Corp.). The PCR condition were as follows; initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, an extension at 72°C for 40 sec and a final extension at 72°C for 10 min. The amplified product (448 bp) of 10 clones were selected for sequencing using pJet1.2/blunt specific primers (Thermo Scientific). Briefly the PCR products were purified with the DNA Clean and Concentrator kit (Zymo Research, Irvine, CA) and directly sequenced with the ABI PRISM BigDye® Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems) on an ABI 3130 automated analyser (Applied Biosystems). The sequencing reaction (20 µℓ) containing 3 µℓ 5X sequencing buffer, 1 µℓ reaction terminator mix, 1 µℓ of 3.2 pmol of the forward and the reverse primers and 13 µℓ nuclease-free water (Promega Corp). was used. The PCR conditions for sequencing was an initial step at 95°C for 3 min followed by 25 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 10 sec and extension at 60°C for 4 min. This was followed by a precipitation step where the 20 µℓ reaction was mixed with 2 µℓ 125 mM ethylenediaminetetraacetic acid, 2 µℓ 3 M sodium acetate (Merck) (pH 5.2) and 50 µℓ of 100% absolute ethanol vortexed and incubated at room temperature (25°C ±5°C) for 15 min. This was spun for 20 min at 16 000 x *g* (Eppendorf 5402D Microcentrifuge), the supernatant was removed and 100 µℓ of 70% ethanol was added and spun again for 10 min at 16000 x g (Eppendorf 5402D Microcentrifuge). The supernatant was removed and the pellet dried at 95°C in a thermal cycler for about 3 min. It was then dissolved in 10 µℓ of Hi-Di Formamide (Life Technologies Corp, Carlsbad, CA), denatured at 95°C for 2 min and then chilled on ice for 2 min, analysed in the ABI 3130 genetic analyser ((Applied Biosystems) and data collected using the genetic Analyser Data Collection V3.0 software.

© University of Pretoria

Table 2.4: Primers used for genotyping of the noroviruses

Virus	Primer / Probe	Sequence (5'-3')[c]	Nucleotide position	Reference
Norovirus GI	G1SKF	CTGCCCGAATTYGTAAATGA	5342-5361[a]	Kojima *et al.,* 2002
	G1SKR	CCAACCCARCCATTRTACA	5653-5671[a]	Kojima *et al.,* 2002
Or	QNIF4	CGCTGGATGCGNTTCCAT	5291-5308[a]	da Silva *et al.,* 2007
	G1SKR	CCAACCCARCCATTRTACA	5653-5671[a]	Kojima *et al.,* 2002
Norovirus GII	G2SKF	CNTGGGAGGGCGATCGCAA	5046-5064[b]	Kojima *et al.,* 2002
	G2SKR	CCRCCNGCATRHCCRTTRTACAT	5367-5389[b]	Kojima *et al.,* 2002
Or	QNIF2	ATGTTCAGRTGGATGAGRTTCTCWGA	5012-5037[b]	Loisy *et al.,* 2005
	G2SKR	CCRCCNGCATRHCCRTTRTACAT	5367-5389[b]	Kojima *et al.,* 2002

[a]GenBank accession number M87661, [b]GenBank accession number X86557,
[c]IUPAC codes used to indicate degenerate positions. R = A/G, S = G/C, Y = C/T

2.8.3.3 Phylogenetic analysis

Nucleotide sequences for NoVs were edited and analysed using Sequencer™ 4.9 (Gene Codes Corporation, Ann Arbor, MI) and BioEdit Sequence Alignment Editor (V.7.0.9.0) [Hall, 1999]. A Basic Local Alignment Search Tool (BLAST) search (http://blast.ncbi.nlm.nih.gov) was performed to compare the identity of the sequenced clones with the reference strains from the GenBank by pairwise comparison. Where the nucleotide sequence of all 10 clones from a single sample were identical, a representative sequence of each set of clones was used for phylogenetic analyses. The 5'-end of the capsid gene (285 bp for GI and 273 bp for GII) of the NoV strains was aligned with NoV GI and NoV GII reference sequences from GenBank (Table 2.5 and 2.6) using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/). After manual adjustment of the alignment, phylogenetic analysis was performed with MEGA5 using the neighbour-joining methods (Tamura *et al.,* 2011). Genotypes were assigned based on the clustering in the phylogenetic tree (>70% bootstrap support). All the genotypes assigned were confirmed by analysis of the sequences with the NoV Genotyping Tool as described by Kroneman *et al.* (2011) (http://www.rivm.nl/mpf/norovirus/typingtool).

© University of Pretoria

Table 2.5: GenBank accession numbers of the NoV GI isolates used as reference strains for phylogenetic analysis.

Norovirus GI strains			
Genotype	Accession number	Genotype	Accession number
GI.1	EF547392 (Japan)	GI.4	AJ313030 (UK)
	L23828 (Japan)		AB042808 (Japan)
	M87661 (USA)	GI.5	AJ277614 (UK)
	AY502016 (USA)		AF414404 (USA)
	AB447406 (India)		AB039774 (Japan)
	JX416391 (Burkina Faso)	GI.6	AJ277615 (UK)
GI.2	AF435807 (USA)		AY502008 (USA)
	L07418 (UK)		AF093797 (Germany)
GI.3	HE716748 (Vietnam)	GI.7	AJ277609 (UK)
	EF547396 (Japan)		AY675555 (Iraq)
	HQ213844 (South Korea)		AJ844469 (Japan)
	JX416392 (Burkina Faso)	GI.8	AF538679 (USA)
	JN603244 (Sweden)	GI.9	JN871684 (Nigeria)
	AB187514 (Japan)		
	U04469 (USA)		
	GU138161 (Russia)		
	EF630430 (Japan)		
	AF439267 (Germany)		
	AJ277612 (UK)		
	AF414405 (USA)		

© University of Pretoria

Table 2.6: GenBank accession numbers of the NoV GII isolates used as reference strains for phylogenetic analysis.

Norovirus GII strains			
Genotype	Accession number	Genotype	Accession number
GII.1	U07611 (USA)	GII.8	AB039780 (Japan)
	OB2001007		AF195848 (Netherlands)
GII.3	EU187437 (Japan)	GII.9	DQ379715 (Australia)
	AY652979 (USA)		AY038599 (USA)
GII.4	AB445395_2008 (Netherlands)	GII.12	GQ845370 (Australia)
	GQ845367_2010 (Australia)		AJ277618 (UK)
	EF187497_2006a (Australia)		AB032758 (Japan)
	EP200222_Farmington_Hills_2002	GII.14	AY130761(USA)
	AJ004864_US95_96		EF547404 (Japan)
	AY883096_Hunters_2004	GII.16	AY772730 (Germany)
	EF126966_2006b (Netherlands)		AY502010 (USA)
	OB2007254_Sydney_2012	GII.17	AY502009 (USA)
GII.6	AB818398 (Japan)		JQ751044 (China)
	AB039779 (Japan)		DQ438972 (USA)
	AB039777 (Japan)	GII.20	AB542917 (Japan)
	JN183165 (Sweden)		EU373815 (Germany)
	AJ277620 (UK)		

2.8.3.4 Nucleotide sequence accession numbers

The nucleotide sequences determined in this investigation have been deposited in GenBank under the following accession numbers KF793788 to KF793798 for NoV GI and KF808211 to KF808254, KF916584 and KF916585 for NoV GII.

2.8.4 Statistical analysis

Analysis of the viral detection rates in river samples was done using Fisher's exact test using StatView software (version 5.0, SAS Institute Inc, Cary, NC). Differences with p values >0.05 were considered not significant at a 95% confidence interval (Cl).

© University of Pretoria

CHAPTER 3

RESULTS

3.1 WATER SAMPLES

From February 2012 to January 2013, 40 water samples were collected from rural (Maua), urban (Nairobi) and refugee (Dadaab) settings in Kenya. Surface river water samples included 12 from the Mboone river (Maua-rural setting), 12 from the Mutoine river (Kibera, Nairobi-urban setting) and 12 from the Nairobi river (Nairobi-urban setting). Four samples, two from borehole and two from household containers, from the Dadaab refugee camp (refugee setting) were also collected (Table 3.1).

Table 3.1: Summary of the water samples collected during the sampling period February 2012 to January 2013.

Site / Month	Rural setting (Maua)	Urban setting (Nairobi)		Refugee setting Dadaab
	Mboone *(n=12)*	Mutoine *(n=12)*	Nairobi *(n=12)*	Dadaab *(n=4)*
Feb 2012	1	1	1	
Mar 2012	1	1	1	
Apr 2012	1	1	1	2
May 2012	1	1	1	
Jun 2012	1	1	1	
Jul 2012	1	1	1	
Aug 2012	1	1	1	2
Sept 2012	1	1	1	
Oct 2012	1	1	1	
Nov 2012	1	1	1	
Dec 2012	1	1	1	
Jan 2013	1	1	1	
Total	12	12	12	4

© University of Pretoria

3.2 MICROBIOLOGICAL ANALYSIS

Presumptive *E. coli* and total coliforms were detected in all 100% (40/40) the samples (Table 3.2).

Table 3.2: Summary of the presumptive *E. coli* and total coliforms detected during the sampling period February 2012 to January 2013.

Site / Month	**Rural setting (Maua)**		**Urban setting (Nairobi)**				**Refugee setting (Dadaab)**	
	Mboone *(n=12)*		Mutoine *(n=12)*		Nairobi *(n=12)*		Dadaab *(n=4)*	
	cfu[a]/1 mℓ		cfu/1 mℓ		cfu/1 mℓ		cfu/1 mℓ	
	E. coli[b]	Total coliforms	*E. coli*	Total Coliforms	*E. coli*	Total coliforms	*E. coli*	Total coliforms
Feb 2012	24	232	>200	>200	>200	>200		
Mar 2012	130	250	>200	>200	>200	>200		
Apr 2012	160	340	>200	>200	>200	>200		
May 2012	102	200	>200	>200	>200	>200	50	80
Jun 2012	640	672	180	>200	>200	>200		
Jul 2012	320	464	200	>200	>200	>200		
Aug 2012	200	280	160	>200	150	>200	5	20
Sept 2012	136	320	150	>200	>200	>200		
Oct 2012	180	220	180	>200	>200	>200		
Nov 2012	560	800	>200	>200	>200	>200		
Dec 2012	340	760	>200	>200	>200	>200		
Jan 2013	460	980	>200	>200	>200	>200		

[a]cfu = colony forming units

[b]*E. coli = Escherichia coli*

© University of Pretoria

3.3 VIRAL ISOLATION IN CELL CULTURE

Cytopathogenic viruses were detected in 35% (14/40) of the samples (Table 3.3). Viruses were isolated in both BGM and PLC/PRF/5 cells lines in 33% (4/12) of Mutoine river, 17% (2/12) of Mboone river and 33% (4/12) of Nairobi river samples but none in the Dadaab samples (Table 3.3). For the L20B cell line, 25% (3/12) of the Mboone river and 17% (2/12) of the Nairobi river water samples were CPE positive (Table 3.3).

3.4 VIRAL DETECTION

Mengovirus was detected in 100% (40/40) of the samples (Table 3.4 and Table 3.5). Of the 12 samples from Mboone River (Maua- rural setting), NoV GI was detected in 8.3% (1/12) of the samples, NoV GII in 33% (4/12), RV in 83% (10/12) and 50% (6/12) of the samples were positive for EV (Table 3.4 and 3.5). In the 12 samples from Mutoine river (Kibera, Nairobi-urban setting) the following viruses were detected: NoV GI 25% (3/12), NoV GII 100% (12/12), RV 100% (12/12) and EV 83% (10/12) (Table 3.5). Out of the 12 samples collected from the Nairobi river (Nairobi- urban setting), 33% (4/12) were positive for NoV GI, 58% (7/12) for NoV GII, 83% (10/12) for RV and 58% (7/12) for . From the Dadaab (Dadaab-refugee setting) a total of four samples were analysed where NoV GII was detected in 8% (1/4) and RV in 50% (2/4). No NoV GI or EV were detected by direct analysis of the Dadaab samples (Table 3.5). There was a statistically significant difference in the overall prevalence for the viruses detected in the three settings ($p<0.05$).

© University of Pretoria

Table 3.3: Summary of the enterovirus positive results after cell culture amplification.

Site / Month	Rural setting (Maua)			Urban setting (Nairobi)			Urban setting (Nairobi)			Refugee setting (Dadaab)		
	Mboone (*n=12)*			Nairobi (*n=12)*			Mutoine (*n=12)*			Dadaab (*n=4)*		
	Detection			Detection			Detection			Detection		
	BGM	PLC	L20B	BGM	PLC	L20B	BGM	PLC	L20B	BGM	PLC	L20B
Feb 2012	-	-	+	+	+	+	+	+	-			
Mar 2012	-	-	-	-	-	-	-	-	-			
Apr 2012	-	-	+	-	-	+	+	+	-			
May 2012	-	-	-	-	-	-	+	+	-	HH1- HH2-	HH1- HH2-	HH1- HH2-
Jun 2012	-	-	-	+	+	-	-	-	-			
Jul 2012	-	-	-	-	-	-	-	-	-			
Aug 2012	-	-	-	+	+	-	+	+	-	BH1 + BH8 -	BH1 + BH8 -	BH1 - BH8 -
Sept 2012	-	-	-	-	-	-	-	-	-			
Oct 2012	+	+	+	+	+	-	-	-	-			
Nov 2012	+	+	-	-	-	-	-	-	-			
Dec 2012	-	-	-	-	-	-	-	-	-			
Jan 2013	-	-	-	-	-	-	-	-	-			

+ =CPE positive; - =CPE negative; HH1= Household water 1; HH2 =Household water 2; BH1= Borehole water 1; BH8= Borehole water 8

© University of Pretoria

Table 3.4: Summary of results for the enteric viruses detected in water sources from rural, urban and refugee settings from February 2012 to January 2013.

Site / Month	Rural setting (Maua)					Urban setting (Nairobi)					Urban setting (Nairobi)					Refugee setting (Dadaab)				
	Mboone river (*n=12*)					Nairobi river (*n=12*)					Mutoine river (*n=12*)					Dadaab (*n=4*)				
	Mengo-virus	NoV GI	NoV GII	RV	EV	Mengo-virus	NoV GI	NoV GII	RV	EV	Mengo-virus	NoV GI	NoV GII	RV	EV	Mengo-virus	NoV GI	NoV GII	RV	EV
Feb 2012	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+					
Mar 2012	+	-	-	+	-	+	+	+	+	+	+	-	+	+	+					
Apr 2012	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+					
May 2012	+	-	+	+	+	+	-	-	+	-	+	+	+	+	-	+ +	- -	+ -	+ +	- -
Jun 2012	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+					
Jul 2012	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+					
Aug 2012	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+ +	- -	- -	- -	- -
Sept 2012	+	-	-	+	+	+	+	-	+	+	+	-	+	+	-					
Oct 2012	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+					
Nov 2012	+	-	-	+	-	+	-	-	+	-	+	-	+	+	+					
Dec 2012	+	-	-	-	+	+	-	-	-	-	+	+	+	+	+					
Jan 2013	+	-		+	+	+	-	-	-	-	+	-	+	+	+					

NoV GI= norovirus genogroup I; NoV GII= norovirus genogroup II; RV = rotavirus; EV = enterovirus

© University of Pretoria

Table 3.5: Enteric viruses detected in water sources from rural, urban and refugee settings from February 2012 to January 2013.

Site / Virus type	Rural setting (Maua)	Urban setting (Nairobi)		Refugee setting
	Mboone *n=12* (%)	Mutoine *n=12* (%)	Nairobi *n=12* (%)	Dadaab *n=4* (%)
Mengovirus	12 (100%)	12 (100%)	12 (100%)	4 (100%)
NoV GI	1 (8.3%)	3 (25%)	4 (33%)	0
NoV GII	4 (33%)	12 (100%)	7 (58%)	1 (8.3%)
RV	10 (83%)	12 (100%)	10 (83%)	2 (50%)
EV	6 (50%)	10 (83%)	7 (58%)	0

NoV = norovirus; RV = rotavirus; EV = enterovirus; NoV GI = norovirus genotype I; NoV GII = norovirus genotype II

3.5 VIRAL CHARACTERISATION

3.5.1 Rotavirus

Rotavirus strains for both rt RT-PCR positive (n=34) and negative (n=6) water samples were genotyped. The G types were identified in 73% (29/40) of the samples with only G1 and G9 being characterised (Table 3.6). In addition, mixed G types (G1+G9), were detected, i.e. in Nairobi river in July 2012 and August 2012. The G types were non-typeable in 38% (15/40) of the samples. The P types that were identified included P[4], P[6] and P[8] (Table 3.6). Mixed P types were also detected, e.g. in the Mboone river (June 2012 P[4]+P[6]), Mutoine river (February 2012 P[4]+P[8]; May 2012 P[6]+P[8]; October 2012 P[6]+P[8] and January 2013 P[6]+P[8]). In the Nairobi river two to three types were characterised in different months (May 2012 P[4]+P[8]), (June 2012 P[4]+P[6]+P[8]) (July 2012 P[4]+P[6]), (September 2012 P[6]+P[8]) and October 2012 P[6]+P[8] (Table 3.6).

© University of Pretoria

Table 3.6: Summary of rotavirus G and P genotypes detected in rural, urban and refugee settings.

Site / Months	Rural Setting	Urban Setting		Refugee setting
	Mboone (n=12)	Mutoine (n=12)	Nairobi (n=12)	Dadaab (n=4)
Feb 2012	-	G?P[4]+P[8]	G?P[8]	
Mar 2012	-	G9P[8]	-	
Apr 2012	-	G1P[8]	-	
May 2012	G?P[8]	G1P[6]+P[8]	G1P[4]+P[8]	G?P[4]
Jun 2012	G?P[8]	G1P[4]	G?P[4]+P[6]+P[8]	
Jul 2012	G1P[?]	G?P[8]	G1+G9P[4]+P[6]	
Aug 2012	-	G?P[4]	G1+G9P[8]	-
Sept 2012	G?P[4]+P[6]	G?P[4]	G?P[6]+P[8]	
Oct 2012	G9P[6]	G1P[6]+P[8]	G1P[6]+P[8]	
Nov 2012	G1P[8]	G?P[6]	G1P[8]	
Dec 2012	G1P[8]	-	G?P[4]	
Jan 2013	-	G?P[6]+P[8]	G?P[4]	

3.5.2 Poliovirus

The results of the analysis for PV on the EV-positive samples are summarised in Table 3.7. No PVs were identified in any of the sample analysed using the WHO recommended techniques for clinical specimens.

3.5.3 Norovirus

3.5.3.1 Nucleotide sequence analysis of region C of the NoV capsid gene

Strains from the 24 samples positive for NoV GII were selected for amplification and sequencing and of these, 87.5% (21/24) were sequenced successfully (Table 3.8). Strains from four samples that were NoV GI positive were selected for amplification

© University of Pretoria

Table 3.7: Summary of poliovirus typing results.

Site / Virus type	Rural setting	Urban setting		Refugee setting
	Mboone *(n=12)*	Mutoine *(n=12)*	Nairobi *(n=12)*	Dadaab *(n=4)*
PanEV	-	-	-	-
PanPV	-	-	-	-
Serotype 1	-	-	-	-
Serotype 2	-	-	-	-
Serotype 3	-	-	-	-
Sabin 1	-	-	-	-
Sabin 2	-	-	-	-
Sabin 3	-	-	-	-
rt RT-PCR[c] ITD[b]	NEV[a]	NEV	NEV	NEV

[a] NEV – non-enteroviruses; [b]ITD - intratypic differentiation;
[c]rt RT-PCR- real time, reverse transcriptase-polymerase chain reaction

and sequencing and all strains were successfully sequenced (Table 3.8). A neighbor-joining tree constructed from an alignment of the 285-base nucleotide sequence for NoV GI and NoV GII and NoV reference strains (Figures 3.1 to 3.6) of the capsid gene revealed multiple genotypes. Three different GI genotypes were identified, namely, GI.1, GI.3 and GI.9. The predominant GI genotype being GI.3 and detected in the Nairobi river during June 2012 and August 2012 (Table 3.8). The GI.1 was detected in Mutoine river (Kibera) during the month of May 2012 and GI.9 was detected in Nairobi river in June 2012. The NoV GII strains clustered into five genotypes, namely, GII.4, GII.6, GII.12, GII.16 and GII.17 (Figures 3.2 to 3.7). Norovirus GII.17 was the most predominant genotype detected in the surface water sources. In Mboone river (Maua) it was detected in May 2012 to July 2012 while in the Mutoine river (Kibera) it was detected in February 2012, March 2012, August 2012 and October 2012 to January 2013 (Table 3.8). In the Nairobi river NoV GII.17 strains was detected in February 2012 to April 2012, June 2012, July 2012 and October 2012 (Table 3.8). Only NoV GII.6 was detected in the Dadaab household water in May 2012 (Table 3.8).

© University of Pretoria

Table 3.8: Summary of the norovirus genotypes detected in water samples.

Site / Month	Rural Setting	Urban setting		Refugee setting
	Mboone	Mutoine	Nairobi	Dadaab
Month	NoV GI+GII	NoV GI+GII	NoV GI+GII	NoV GI+GII
Feb 2012	-	GII.17	GII.17	
Mar 2012	-	GII.4; GII.6; GII.16; GII.17	GII.17	
Apr 2012	-	GII.6	GII.17	
May 2012	GII.16; GII.17	GI.1	-	GII.6
Jun 2012	GII.17	GII.6	GI.3; GI.9; GI1.17	
Jul 2012	GII.17	GII.6	GII.17	
Aug 2012	-	GII.12; GII.17	GI.3; GII.4	-
Sept2012	-	-	-	
Oct 2012	-	GII.17	GII.17	
Nov 2012	-	GII.6;GII.17	-	
Dec 2012	-	GII.6;GII.17	-	
Jan 2013	-	GII.17	-	

Norovirus GII.4 strains were detected in both urban and rural settings. In the Mboone river it was detected in June 2012, in the Mutoine river in March 2012 and in Nairobi river in February 2012 and August 2012 (Table 3.8). Norovirus GII.6 was detected in urban and refugee settings where it was detected in the Mutoine river in March 2012-July 2012, November 2012 to December 2012 and in Dabaab household water in May 2012 (Table 3.8). Genotype GII.16 was detected in both Mboone (Maua) in May 2012 and the Mutoine (Kibera) river in March 2012. The GII.12 strain was only detected in the Mutoine river in August 2012 (Table 3.8).

© University of Pretoria

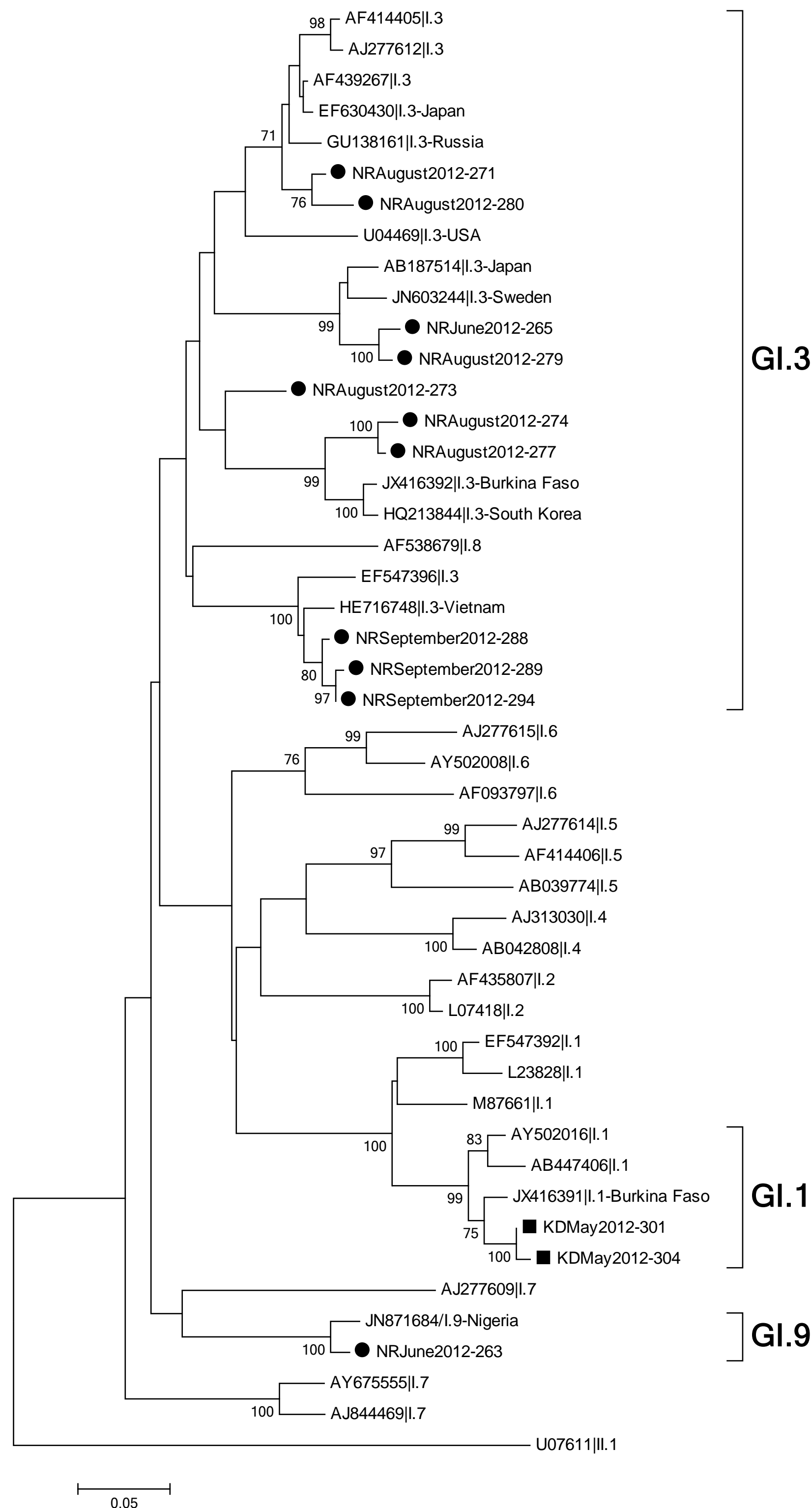

Figure 3.1**:** Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoVs GI strains detected in Kenyan river water samples (● Nairobi river ■ Mutoine river). The sampling sites NR (Nairobi river), KD (Mutoine river). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.05) represent the number of substitution per site.

© University of Pretoria

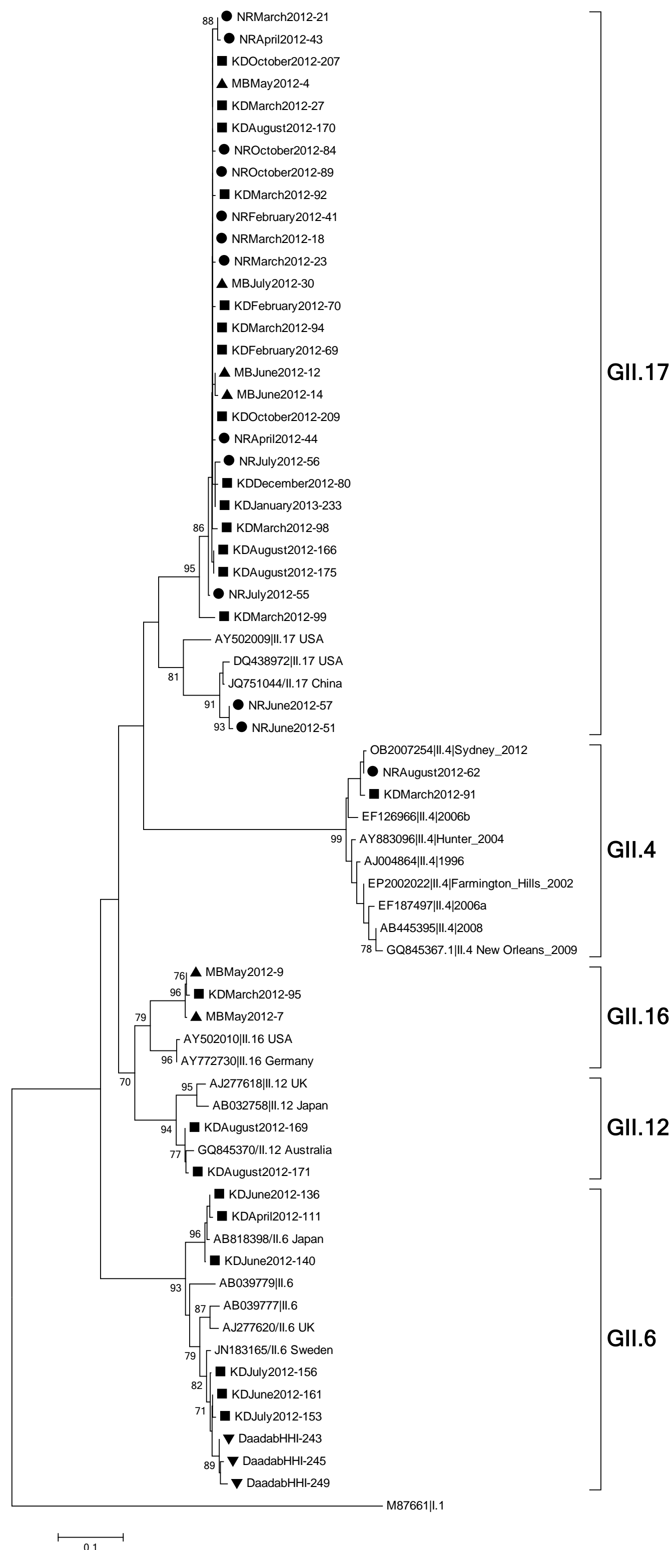

Figure 3.2: Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII strains detected in Kenyan river water samples (●Nairobi river ■ Mutoine river ▲Mboone river ▼Dadaab Household water). The sampling sites NR (Nairobi river), KD (Mutoine river), MB (Mboone river) and HHI (Daadab Household water one). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.1) represents the number of substitution per site.

© University of Pretoria

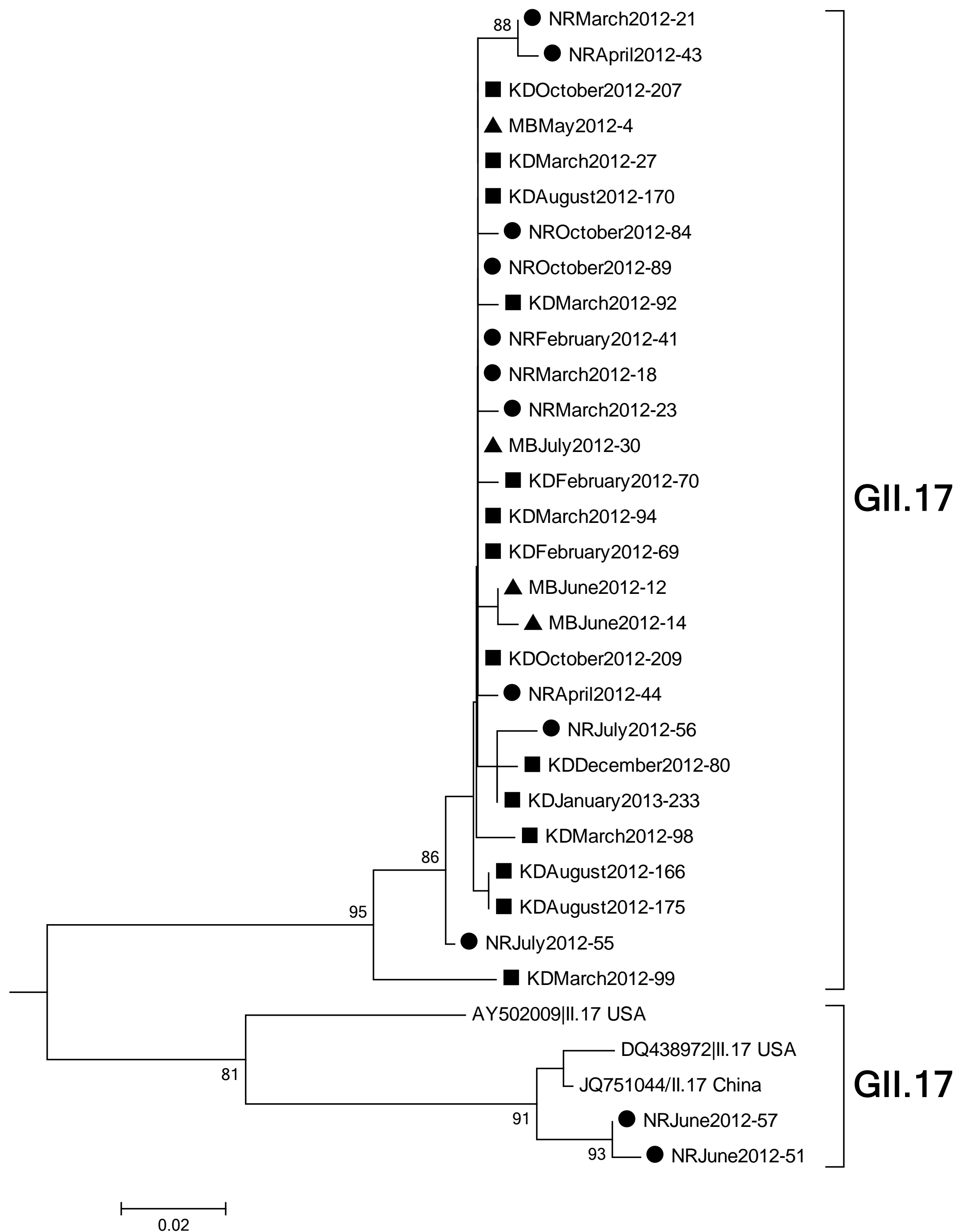

Figure 3.3: Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.17 strains detected in Kenyan river water samples (●Nairobi river ■ Mutoine river ▲Mboone river). The sampling sites NR (Nairobi river), KD (Mutoine river) and MB (Mboone river). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.02) represent the number of substitutions per site.

© University of Pretoria

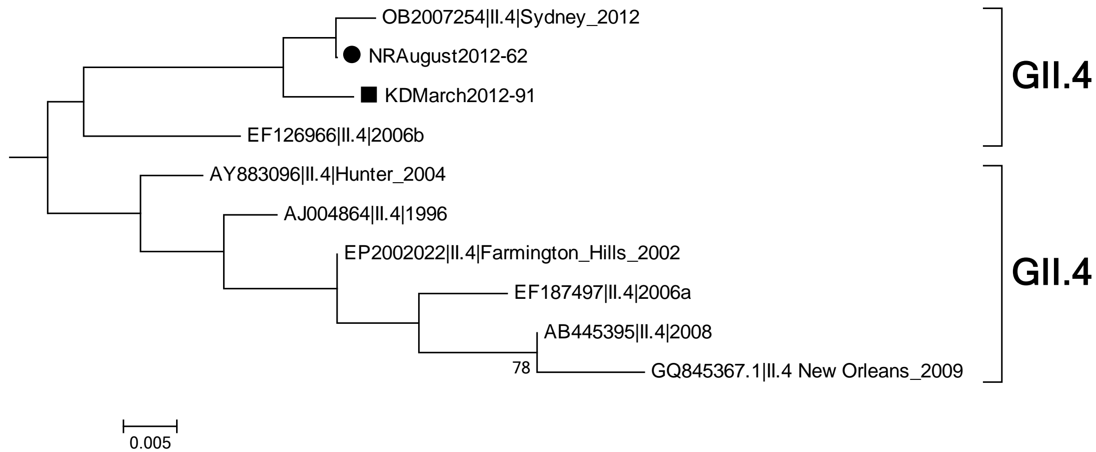

Figure 3.4: Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.4 strains detected in Kenyan river water samples (● Nairobi river ■ Mutoine river). The sampling sites KD (Mutoine river) and NR (Nairobi river). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.005) represents the number of substitutions per site.

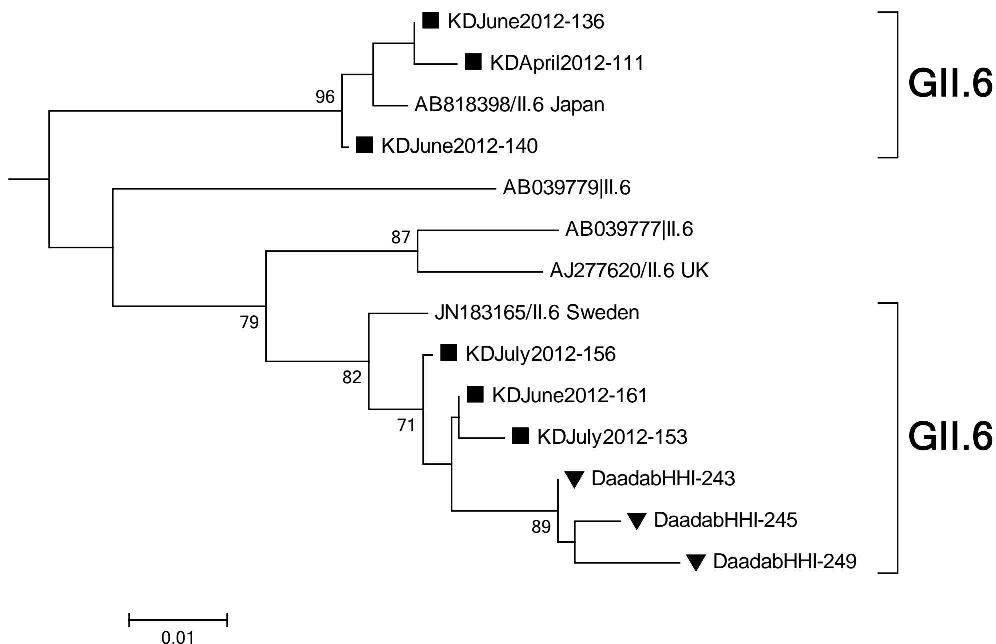

Figure 3.5: Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.6 strains detected in Kenyan river water samples (■ Mutoine river and ▼Dadaab Household water one). The sampling sites KD (Mutoine river) and HHI (Daadab Household water one). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.01) represents the number of substitutions per site.

© University of Pretoria

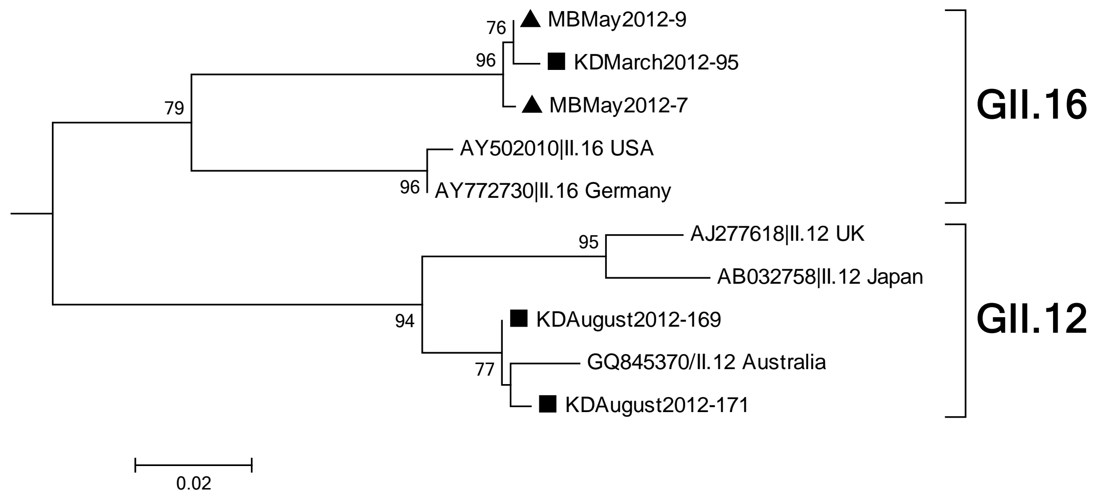

Figure 3.6: Unrooted phylogenetic tree based on partial nucleotide sequences (320 bp) of the capsid gene of NoV GII.12 and GII.16 strains detected in Kenyan river water samples (▲Mboone river ■ Mutoine river). The sampling sites MB (Mboone river) and KD (Mutoine river). Collection month/year and clone number for different genotypes are indicated. Numbers at each branch indicate bootstrap values for the clusters supported by that branch. Bootstrap values of >70 are shown. The scale (0.02) represents the number of substitutions per site.

© University of Pretoria

CHAPTER 4

DISCUSSION AND CONCLUSIONS

Waterborne illness associated with viruses are more common than those caused by bacteria making it important to estimate the prevalence and diversity of enteric viruses in environmental water sources in order to assess the public health risks associated with exposure to these pathogens (Espinosa *et al.,* 2008). Enteric viruses are transmitted by the faecal-oral route and their spread has in part been attributed to the consumption of contaminated food and drinking water (La Rosa *et al.,* 2012). Waterborne infections have been shown to be an important cause of gastroenteritis outbreaks throughout the world (Lund and O'Brien, 2011). However, current water quality monitoring is based on the levels of faecal bacteria indicators rather than viruses resulting in a paucity of data regarding waterborne viruses. This makes it hard to develop and implement plans to prevent viral transmission through contact with environmental water (Lee and Kim, 2002). In Kenya, despite the potential public health risk posed by enteric viruses, data regarding the occurrence of these pathogens in environmental water sources is limited, and in addition, data on molecular epidemiology of NoVs is lacking. Therefore, the aim of this investigation was to determine the prevalence and molecular epidemiology of selected enteric viruses namely, EVs, NoVs and RVs in Kenyan water sources. The surface water sampling sites for the Mutoine, Nairobi and Mboone rivers were located close to highly populated areas. The sample collection site for the Mboone river was located close to Maua town. This town does not have a sewage plant and most of the households have pit latrines. It is thus highly probable that enteric virus contamination of the river by run-off water or overflowing latrines may increase during the rainy season. The Mutoine river passes through the Kibera informal settlement, one of the largest informal settlements in the south and east of sub-Saharan Africa and it is polluted by household waste and raw sewage from residential estates. The development of an informal housing settlement has impacted negatively on the water quality of the stream and has led to the loss of biodiversity, especially in the Nairobi dam, which was earlier a good water source for irrigation, recreation and domestic use.

© University of Pretoria

Enteric virus detection in water sources is a multistep process involving sample preparation or viral recovery, viral detection and viral characterisation (Wyn-Jones, 2007; Mattison and Bidawid, 2009). In this study the glass wool adsorption- elution method (Lambertini *et al.,* 2008), which is considered to be the most cost effective to use in developing countries, was applied for the recovery of viruses. For the detection of viruses a combination of cell cultures and molecular-based methods was applied. Commercial rt RT-PCR kits were used to detect viruses directly in the recovered virus concentrate as the kits have an internal positive control to monitor for any inhibitors that might be present in polluted water. Mengovirus, which was used as a process control to monitor the efficiency of the nucleic acid extraction procedure, was detected in all (100%) of the samples indicating that the processing and nucleic acid extractions procedures were adequate and valid. The isolation of viruses in cell culture was used to establish if there were any infectious viruses present and also to monitor specifically for for PVs. The detected viruses were characterised to identify the circulating strains since this gives an indication on the strains circulating in the surrounding communities close to the water source therefore providing new data on the molecular epidemiology of the selected enteric viruses. The analysis of bacterial indicators provides general information on microbial water quality at each source. In this study *E coli* and total coliforms were detected in 100% (40/40) of the samples at levels >200 cfu/mℓ, thereby exceeding the WHO recommended guidelines for drinking water (<1 most probable number (MPN)/100 mℓ) (Table 3.2). This suggests that there is gross faecal contamination in these water sources, including the household water.

Globally, group A RVs remain the major cause of morbidity and mortality and are associated with an estimated 453 000 deaths (range 420 000 to 494 000) annually in infants and children younger than five years of age (Tate *et al.,* 2012). Rotavirus has been detected in surface water sources in many countries namely, South Africa (van Zyl *et al*., 2006; Sibanda and Okoh, 2013), Egypt and Spain (Villena *et al*., 2003), Brazil (Miagostovich *et al.,* 2008), the Netherlands (Rutjes *et al.,* 2009) and Venezuela (Rodríguez-Díaz *et al.,* 2009). Recently RV was detected in flood water in Thailand (Ngaosuwankul *et al.,* 2013). In this investigation RVs were detected in 85% (34/40) of the samples analysed. It was the most predominant virus being detected in 83% (10/12) of the Mboone river samples, 100% (12/12) of the Mutoine river samples, 83% (10/12) of the Nairobi river samples and 50% (2/4) of the household water from the Dadaab

© University of Pretoria

refugee camp (Table 3.5). The prevalence of RVs in this investigation is similar to an earlier study in 2007-2008 where RVs were detected in 100% of Mutoine water samples (Kiulia *et al.,* 2010). In the Mboone river the current study reported a higher prevalence of 100% compared to 25% prevalence detected in the earlier study (Kiulia *et al.,* 2010). This study corresponds to other studies conducted in surface water e.g., a study in China where 100% (40/40) of river water samples were RV positive (He *et al.,* 2008), but differs from a study in SA where RVwas detected in surface water at a lower rate of 14% (Chigor and Okoh, 2012) while in Benin no RV was detected in surface water (Verheyen *et al.,* 2009). In this study RV was detected in 50% (1/2) of household water samples in the Dadaab refugee site and these results differs to a study in Korea where no RV was detected in untreated groundwater (Jung *et al.,* 2011). The RV strains detected in this study were further characterised and 75% were successfully typed (Table 3.6). This typing rate is comparable to earlier studies in Kenya and SA where 96% (Kiulia *et al.,* 2010) and 95% (van Zyl *et al*. 2006) of RVs were successfully typed, respectively. The G types identified in this study were G1 and G9, either individually or as mixed types (Table 3.6). These strains were also reported in a South African study where G1, G2, G8, and G9 were identified in river water samples (van Zyl *et al*. 2006). Of note is that no G2, G3, G4 and G8 were detected in this study, as these strains have been circulating in the Kenyan population for over 10 years (Kiulia *et al.,* 2006, 2008, 2009; Mwenda *et al.,* 2010; Nokes *et al.,* 2010). The G9 RV strain identified in this study has been the predominant RV strain circulating in the eastern region of Kenya for the last 10 years (Kiulia *et al.,* 2006, 2014). Of interest is that this strain is not included in either of the current licensed oral RV vaccines, i.e. Rotarix (G1P[8]) and RotaTeq (G1, G2, G3, G4, and P[8]) (Ruiz-Palacios *et al.,* 2006; Vesikari *et al.,* 2006). The following P types were identified, P[4], P[6] and P[8], with mixed P types P[4]+P[6], P[4]+P[8], P[6]+P[8] and P[4]+P[6]+P[8] present (Table 3.6). The rate of mixed RV types in this study is comparable to that observed in an earlier study in the environmental samples where the same mixed P types were detected (Kiulia *et al.,* 2010), and in a study in Venezuela where mixed P[4]+P[8] types were detected in sewage-polluted water (Rodríguez-Díaz *et al.,* 2009).

Noroviruses have been identified as aetiological agents of acute gastroenteritis in all age groups worldwide (Glass *et al.,* 2009; Vega *et al.,* 2014). Noroviruses are highly infectious and transmitted primarily via the faecal-oral route. Worldwide, water and

© University of Pretoria

foodborne outbreaks of acute gastroenteritis associated with NoVs have been frequently reported (Riera-Montes *et al.,* 2011; Bitler *et al.,* 2013; Hoa Tran *et al.,* 2013; Stals *et al.,* 2013; Larsson *et al.,* 2014). In this study NoVs were identified in 80% (32/40) of the samples analysed (Table 3.5). These high rates of NoV detection correspond with a study in SA where NoV was detected in 66% of surface water samples (Mans *et al.,* 2013). Norovirus GII was detected at all the sampling sites with prevalence rates of 33% in the Mboone river, 100% in the Mutoine river, 58% in the Nairobi river and 8.3% in the Dadaab household water (Table 3.5). The NoV GI was detected in 8.3% of the Mboone, 25% of the Mutoine and 33% of the Nairobi river samples but it was not detected in the Dadaab borehole and household water. Comparing the results from this study to an earlier study (Kiulia *et al.,* 2010), the rate of both NoV GI and NoV GII was similar in the Mboone river (NoV GI 8.3% vs 8.3%; NoV GII 33% vs 25%). In the Mutoine river the rate of NoV GI detection in the earlier studydiffered from the current study with NoV GI being detected in 25% of the samples while in the earlier study it was detected in 90% of the samples analysed (Table 3.5, Kiulia *et al.,* 2010). The rate of NoV detection (8.3%) in the household (Dadaab) in this study corresponds with a study in South Korea where a detection rate of 18% was reported in borehole water (Jung *et al.,* 2011). While NoV GI predominated (71%) in the Korean study (Jung *et al.,* 2011) this study showed that NoV GII was the predominant strain identified in 8.3% while no NoV GI was detected in neither household or borehole water (Table 3.5).

Based on nucleotide sequence analysis of the capsid region (320 bp) the following NoV GI strains were detected, NoV GI.1, GI.3 and GI.9 (Figure 3.1). Two NoV GI.1 strains (KDMarch2012-301 and KDMarch2012-304) detected in this study were closely related to Burkina Faso NoV GI.1 strain (JX416391) showing high nucleotide sequence identity (97%). Phylogenetic analysis of the GI.3 strains showed that it grouped into four clusters (Figure 3.1), and were closely related to strains from Russia (GU138161), Japan (AB187514), Sweden (JN603244), Burkina Faso (JX416392), South Korea (HQ213844) and Vietnam (HE716748) with nucleotide sequence identities ranging from 94% to 97%. The GI.9 strain detected was related to a Nigerian (JN871684) strain showing a 98% nucleotide sequence identity. Phylogenetic analysis showed that GII.17 was the predominant strain detected in this investigation and strains grouped into two clusters (Figure 3.3) with most of the strains detected from different sampling sites resorting in one cluster and the other cluster comprising strains from the Nairobi river

© University of Pretoria

and were closely related to strains from the USA (DQ438972) and China (JQ751044), respectively. The GII.17 strain has also recently been identified in clinical specimens collected during 1999 to 2000 from immunocompromised children at a care center in Kenya (Mans *et al.,* 2014). This could suggest this strain has been in circulation for a considerable period of time. The detection of GII.17 in this study is of clinical relevance as it has been implicated in nosocomial NoV infection (Sukhrie *et al.,* 2011) and chronic NoV infection in a kidney transplant patient (Schorn *et al.,* 2010). The genotype has also been widely reported in children with gastroenteritis in Central and South America (Gomes *et al.,* 2008; Bucardo *et al.,* 2009; Ferreira *et al.,* 2012), Korea (Park *et al.,* 2011) and Thailand (Kittigul *et al.,* 2010). The other NoV GII strains detected were GII.4, GII.6, GII.7, GII.12 and GII.16 (Table 3.8), which are similar to types detected in a study conducted in HIV seropositive children in Kenya (Mans *et al.,* 2014). The GII.4 strains in this study (NRAugust2012-62 and KDMarch2012-91) were closely related to the Sydney variant (OB2007254_Sydney_2012) (Figure 3.4), while the GII.4 strain identified in the clinical study was closely related to the non-epidemic GII.4 Kaiso 2003 variant (Mans *et al.,* 2014). Of note is that GII.4 detected in the Nairobi river was closely related to the Sydney_2012 variant, which is currently the most predominant strain identified in most NoV-associated gastroenteritis outbreaks worldwide (CDC, 2013a; Kim *et al.,* 2013; Maritschnik *et al.,* 2013, Silva *et al.,* 2013), highlighting the significance of the presence of these strains in Kenyan surface waters used for domestic purposes. The GII.6 strains were the second most predominant strains and were closely related to the Swedish (JN183165) and Japanese (AB818398) strains. The GII.6 strain was also identified in the clinical study in Kenya as reported by Mans *et al.,* (2014), indicating the presence of clinically relevant strains in the water sources. After NoV GII.4, NoV GII.6 has been showed in many studies to be the second most dominant strain in clinical studies in a number of countries namely, SA, Brazil, Japan, and Finland (Phan *et al.,* 2005; Ferreira *et al.,* 2010; Mans *et al.,* 2010; Puustinen *et al.,* 2011; Chan-It *et al.,* 2012). This shows the need to monitor this strain closely in both environmental and clinical specimens. The NoV GII.12 strains were detected in the Mutoine river in August 2012 (KDAugust2012-169/171) and were related to an Australian (GQ845370) strain with a nucleotide sequence identity of 99% which was identified in Australia during 2008. The GII.16 was also identified in the Mboone (MBMay2012-7/9) and the Mutoine rivers (KDMarch2012-95) and were closely related to strains from Germany (AY772730) and USA (AY502010). This

© University of Pretoria

study correlates with other studies conducted in Africa where NoVs GI.1, GI.3 and NoVs GII.4, GII.12, GII.17 have been identified in South African water sources (Mans *et al.,* 2013). Using BLAST analysis and the NoV typing tool, one of the clones (NRJuly2012-46) was assigned to be a GII.7 and it was closely related to strains from Japan (AB258331) and UK (AJ277608). This sequence was not used for phylogenetic analysis and the nucleotide sequence was not submitted to GenBank due to various mutations identified, therefore this sample need further analysis to confirm the nucleotide sequence. Overall, the results in this study suggest that the rivers are contaminated with clinically relevant NoV strains namely, NoVs GII.4, GII.6 and GII.12.

In assessing the prevalence and isolation of EV in cell culture it was noted the detection of viruses by cell culture methods was more sensitive than the molecular-based methods with the advantage that infectious viruses were detected. Enterovirus strains from samples where CPE was noted on L20B, BGM and PLC cell cultures were further characterised for PV using the method used in the WHO reference laboratories to characterise clinical samples but no PV was identified. In this study EV was detected by rt RT-PCR directly in 58% (23/40) of the samples and it was identified in the three rivers (Mboone, Mutoine and Nairobi rivers) accounting for 50% (6/12), 83% (10/12) and 58% (7/12) of EV-positive water samples, respectively, but not in the borehole or household water from Dadaab (Table 3.5). There were no PVs identified by direct analysis of the water samples using the rt RT-PCR analytical kits designed for identification of PV and differentiation between VDPVs and WPVs. Due to the low titers of viruses in water samples nucleic acid was re-extracted again from all the samples using a more sensitive QIAamp UltraSens RNA kit to determine if it would be possible to detect any PV from the EV positive samples. This also did not yield any positive result for EVs and neither VDPVs nor WPVs were detected (Table 3.7).

This study outlines several interesting aspects regarding the virological quality of environmental water in Kenya. First, is the high prevalence of viral pollution in the Mutoine river in the Nairobi urban setting, i.e., 100% for NoV GII, 100% for RV, 83% for EV and 25% of NoV GI (Table. 3.5). The results differ from other epidemiological studies of enteric viruses in a middle income country like Brazil where a study on enteric viruses in stream waters reported that RVs were detected in 44.2% and NoV in

© University of Pretoria

5.8% of the samples analysed (Miagostovich *et al.,* 2008). These studies indicate that regardless of the status of a country, either middle income or developing, continuous epidemiological survey of enteric viruses in environmental water samples will be an important indicator of the level of contamination and also providing information on viruses circulating in the community. Surface waters may be directly or indirectly contaminated with human pathogenic viruses by untreated sewage or run-off of animal manure. The high level of viral pollution demonstrated in the Mutoine river provides evidence that these viruses circulate at high frequencies among the population of Kibera, while also reflecting the effects of increased population densities and anthropogenic activities on fresh water resources. The diversity of enteric viruses detected in the surface waters from different geographical areas was variable and appeared to be dependent on factors such as population density and drinking water and sanitation facilities. In Kenya, the majority of the population lacks access to treated drinking water and rely on surface water for their domestic needs. Thus, considering the levels of pollution present in the Mboone, Mutoine and the Nairobi rivers, the levels of enteric virus present could possibly pose a public health risk if the water was used for domestic or recreation purposes.

In conclusion, this is the first comprehensive report on the molecular epidemiology of NoVs in Kenyan water sources. Furthermore, it is the first study to assess environmental water sources in Kenya for the presence of PV. The study also contributes additional important information on the circulating RV strains in the Kenyan population before the introduction of the RV vaccine into the EPI schedule. The results of this study therefore provide valuable information on selected enteric viruses in Kenyan water sources and surrounding communities. From these data it is evident that further nationwide studies are necessary to fully establish the prevalence and distribution of enteric viruses in the Kenyan water sources and surrounding communities

© University of Pretoria

CHAPTER 5

REFERENCES

Abe Y, Fujii K, Nagata N, Takeuchi O, Akira S, Oshiumi H, Matsumoto M, Seya T, Koike S. The toll-like receptor 3-mediated antiviral response is important for protection against poliovirus infection in poliovirus receptor transgenic mice. Journal of Virology 2012;86:185-194.

Aboudy Y, Shif I, Silberstein I, Gotlieb-Stematsky T. Efficiency of isolation of human rotavirus in primary African green monkey kidney cells. Journal of Virological Methods 1989;25:251-257.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acid Research 1997;25:3389-3402.

Ammerman NC, Beier-Sexton M, Azad AF. Growth and maintenance of Vero cell lines. Current Protocols in Microbiology 2008; Appendix 4E. doi:10.1002/9780471729259.mca04es11.

Anderson EJ, Weber SG. Rotavirus infection in adults. The Lancet Infectious Diseases 2004;4:91-99.

Anis E, Kopel E, Singer SR, Kaliner E, Moerman L, Moran-Gilad J, Sofer D, Manor Y, Shulman LM, Mendelson E, Gdalevich M, Lev B, Gamzu R, Grotto I. Insidious reintroduction of wild poliovirus into Israel, 2013. Euro Surveillance 2013;9;18(38).

Armah GE, Mingle JA, Dodoo AK, Anyanful A, Antwi R, Commey J, Nkrumah FK. Seasonality of rotavirus infection in Ghana. Annals of Tropical Paediatrics 1994;14:223-230.

© University of Pretoria

Arnold M, Patton JT, McDonald SM. Culturing, storage, and quantification of rotaviruses. Current Protocol on Microbiology 2009;Chapter 15:Unit 15C.3.

Aryal KK, Joshi HD, Dhimal M, Singh SP, Dhakal P, Dhimal B, Bhusal CL. Environmental burden of diarrhoeal diseases due to unsafe water supply and poor sanitation coverage in Nepal. Journal of Nepal Health Research Council 2012;21:125-129.

Ashbolt NJ. Microbial contamination of drinking water and disease outcomes in developing regions. Toxicology 2004;198:229-238.

Ashbolt NJ, Grabow WOK, Snozzi M. Indicators of microbial water quality. In: Fewtrell L, Bartram J, editors. Water Quality: Guidelines, Standards and Health London: IWA Publishing; 2001. p. 287-315.

Atmar RL. Noroviruses: State of the art. Food and Environmental Virology 2010;2:117-126.

Atmar RL, Estes MK. Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. Clinical Microbiology Review 2001;14:15-37.

Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. Gastroenterology Clinics of North America 2006;35:275-290.

Atmar, RL, Estes, MK. Human caliciviruses. In Richman DD, Whitely RJ, Hayden FG, editors. Clinical Virology. 3rd ed. Washington, DC: ASM Press.; 2009. p. 1109-1126.

Attoui H, Mertens PPC, Becnel J, Belaganahalli S, Bergoin M, Brussaard CP, Chappell JD, Ciarlet M, del Vas M, Dermody TS, Dormitzer PR, Duncan R, Fang Q, Graham R, Guglielmi KM, Harding RM, Hillman B, Makkay A, Marzachi C, Matthijnssens J, Milne RG, Mohd Jaafar F, Mori H, Noordeloos AA, Omura T, Patton JT, Rao S, Maan M, Stoltz D, Suzuki N, Upadhyaya NM, Wei C, Zhou H. Reoviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus Taxonomy: Classification and

© University of Pretoria

nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2012. p. 541-637.

Bagci S, Eis-Hübinger AM, Yassin AF, Simon A, Bartmann P, Franz AR, Mueller A. Clinical characteristics of viral intestinal infection in preterm and term neonates. European Journal of Clinical Microbiology and Infectious Diseases 2010;29:1079-1084.

Baggi F, Peduzzi R. Genotyping of rotaviruses in environmental water and stool samples in southern Switzerland by nucleotide sequence analysis of 189 base pairs at the 5' end of the VP7 gene. Journal of Clinical Microbiology 2000;38:3681-3685.

Bailly JL, Borman AM, Peigue-Lafeuille H, Kean KM. Natural isolates of echovirus type 25 with extensive variations in IRES sequences and different translational efficiencies. Virology 1996;215:83–96.

Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. PLoS Medicine 2014;11(5):e1001644.

Balkin HB, Margolin AB. Detection of poliovirus by ICC/qPCR in concentrated water samples has greater sensitivity and is less costly using BGM cells in suspension as compared to monolayers. Virology Journal 2010;7:282.

Bartolini L, Mardari R, Toldo I, Calderone M, Battistella PA, Laverda AM, Sartori S. Norovirus gastroenteritis and seizures: an atypical case with neuroradiological abnormalities. Neuropediatrics 2011;42:167-169.

Benabbes L, Ollivier J, Schaeffer J, Parnaudeau S, Rhaissi H, Nourlil J, Le Guyader FS. Norovirus and other human enteric viruses in Moroccan shellfish. Food and Environmental Virology 2013;5:35-40.

Bern C, Glass RI. Impact of diarrheal diseases worldwide. In: Kapikian AZ, editor. Viral Infections of the Gastrointestinal Tract. New York: Marcel Dekker Inc.; 1994. p. 1-26.

© University of Pretoria

Bernstein DI. Rotavirus overview. Pediatric Infectious Disease Journal 2009;28 [Suppl 3]:S50-S53.

Birch CJ, Rodger SM, Marshall JA, Gust ID. Replication of human rotavirus in cell culture. Journal of Medical Virology 1983;11:241–250.

Bishop RF, Davidson GP, Holmes IH, Ruch BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet 1973;1:181-183.

Bitler EJ, Matthews JE, Dickey BW, Eisenberg JN, Leon JS. Norovirus outbreaks: a systematic review of commonly implicated transmission routes and vehicles. Epidemiology and Infection 2013;141:1563-1571.

Blanton LH, Adam SM, Beard RS, Wei G, Bulens SN, Widdowson M, Glass RI, Monroe SS. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United State, 2000-2004. Journal of Infectious Diseases 2006;193:413-421.

Bok K, Green KY. Norovirus gastroenteritis in immunocompromised patients. New England Journal of Medicine 2012;367:2126-2132

Boone SA, Gerba C. Significance of fomites in the spread of respiratory and enteric viral disease. Applied and Environmental Microbiology 2007;73:1687-1696.

Bosch A, Guix S, Sano D, Pintó RM. New tools for the study and direct surveillance of viral pathogens in water. Current Opinion in Biotechnology 2008;19:295-301.

Bosch A, Sánchez G, Abbaszadegan M, Carducci A, Guix S, Le Guyader FS, Netshikweta R, Pintó RM, van der Poel WHM, Rutjes S, Sano D, Taylor MB, van Zyl WB, Rodríguez-Lázaro D, Kovač K, Sellwood J. Analytical methods for virus detection in water and food. Food Analytical Methods 2011;4:4-12.

© University of Pretoria

Bottinger M, Herrstrom E. Isolation of polioviruses from sewage and their characteristics. Experience over two decades in Sweden. Scandinavian Journal of Infectious Diseases 1992;24:151-155.

Brown BA, Oberste MS, Alexander JP Jr, Kennett ML, Pallansch MA. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. Journal of Virology 1999;73:9969-9975.

Bruijning-Verhagen P, Quach C, Bonten M. Nosocomial rotavirus infections: a meta-analysis. Paediatrics 2012;129:1011-1019.

Bucardo F, Kindberg E, Paniagua M, Grahn A, Larson G, Vildevall M, Svensson L. Genetic susceptibility to symptomatic norovirus infection in Nicaragua. Journal of Medical Virology 2009;81:728-735.

Butot S, Le Guyader FS, Krol J, Putallaz T, Amoroso R, Sánchez G. Evaluation of various real-time RT-PCR assays for the detection and quantitation of human norovirus. Journal of Virological Methods 2010;167:90-94.

Cáceres VM, Kim DK, Bresee JS, Horan J, Noel JS, Ando T, Steed CJ, Weems JJ, Monroe SS, Gibson JJ. A viral gastroenteritis outbreak associated with person-to-person spread among hospital staff. Infection Control Hospital Epidemiology 1998;19:162-167.

Cardemil CV, Cortese MM, Medina-Marino A, Jasuja S, Desai R, Leung J, Rodriguez-Hart C, Villarruel G, Howland J, Quaye O, Tam KI, Bowen MD, Parashar UD, Gerber SI; Rotavirus Investigation Team. Two rotavirus outbreaks caused by genotype G2P[4] at large retirement communities: cohort studies. Annals of Internal Medicine 2012;157:621-631.

Carling PC, Bruno-Murtha LA, Griffiths JK. Cruise ship environmental hygiene and the risk of norovirus infection outbreaks: an objective assessment of 56 vessels over 3 years. Clinical Infectious Diseases 2009;49:1312-1317.

© University of Pretoria

Caro V, Guillot S, Delpegroux F, Crainic R. Molecular strategy for 'serotyping' of human enteroviruses. Journal of General Virology 2002;82:79-91.

Carter MJ. Enterically infecting viruses: Pathogenicity, transmission and significance for food and waterborne infection. Journal of Applied Microbiology 2005;98:1354-1380.

Centers for Disease Control and Prevention. Enterovirus surveillance-United States, 2000-2001. Morbidity and Mortality Weekly Report 2002a;51:1047-1049.

Centers for Disease Control and Prevention. Progress towards global poliomyelitis eradication, 2001. Morbidity and Mortality Weekly Report 2002b;51:253-256

Centers for Disease Control and Prevention. Enterovirus surveillance-United States, 2002-2004. Morbidity and Mortality Weekly Report 2006;55:153-156.

Centers for Disease Control and Prevention. Wild poliovirus type 1 and type 3 importations-15 countries, Africa, 2008-2009. Morbidity Mortality Weekly Report 2009;58:357-362.

Centers for Disease Control and Prevention. Outbreaks following wild poliovirus importations - Europe, Africa, and Asia, January 2009 - September 2010. Morbidity and Mortality Weekly Report 2010;59:1393-1399.

Centers for Disease Control and Prevention. Updated norovirus outbreak management and disease prevention guidelines. Morbidity and Mortality Weekly Report 2011a;60:1-15.

Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission-worldwide, January 2010-March 2011. Morbidity and Mortality Weekly Report 2011b;60:582-586

© University of Pretoria

Centers for Disease Control and Prevention. Progress toward poliomyelitis eradication--Nigeria, January 2011-September 2012. Morbidity and Mortality Weekly Report 2012;61:899–904.

Centers for Disease Control and Prevention. Emergence of new norovirus strain GII.4 Sydney--United States, 2012. Morbidity Mortality Weekly Report 2013a;62:55

Centers for Disease Control and Prevention. Assessing the risks for poliovirus outbreaks in polio-free countries--Africa, 2012-2013. Morbidity Mortality Weekly Report 2013b;62:768-772.

Chang KO, George DW. Interferons and ribavirin effectively inhibit Norwalk virus replication in replicon-bearing cells. Journal of Virology 2007;81:12111-12118.

Chan-It W, Thongprachum A, Khamrin P, Kobayashi M, Okitsu S, Mizuguchi M, Ushijima H. Emergence of a new norovirus GII.6 variant in Japan, 2008–2009. Journal of Medical Virology 2012;84:1089-1096.

Chen SY, Tsai CN, Lai MW, Chen CY, Lin KL, Lin TY, Chiu CH. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. Clinical Infectious Diseases 2009;48:849-855

Chen SY, Chiu CH. Worldwide molecular epidemiology of norovirus infection. Paediatrics and International Child Health 2012;32:128-131

Chigor VN, Okoh AI. Quantitative RT-PCR detection of hepatitis A virus, rotaviruses and enteroviruses in the Buffalo River and source water dams in the Eastern Cape Province of South Africa. International Journal of Environment Research and Public Health 2012;9:4017-4032.

Choi UY, Lee SY, Ma SH, Jang YT, Kim JY, Kim HM, Kim JH, Kim DS, Kim YS, Kang JH. Epidemiological changes in rotavirus gastroenteritis in children under 5 years of age after the introduction of rotavirus vaccines in Korea. European Journal of Pediatrics 2013;172:947-952.

© University of Pretoria

Chumakov K, Ehrenfeld E, Wimmer E, Agol VI. Vaccination against polio should not be stopped. Nature Reviews in Microbiology 2007;5:952-958

Clarke, IN, Estes MK, Green KY, Hansman GS, Knowles NJ, Koopmans MK, Matson DO, Meyers G, Neill JD, Radford A, Smith AW, Studdert MJ, Thiel HJ, Vinje J. Caliciviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus Taxonomy: Classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2012. p. 977-986.

Collett MS, Neyts J, Modlin JF. A case for developing antiviral drugs against polio. Antiviral Research. 2008;79:179-187.

Cubitt WD, Holzel H. Hospital acquired rotavirus infection in adults. Who is at risk? Journal of Hospital Infection 1980;1:327-331

Cunliffe NA, Dove W, Bunn JE, Ben Ramadam M, Nyangao JW, Riveron RL, Cuevas LE, Hart CA. Expanding Global distribution of Rotavirus serotype G9: Detection in Libya, Kenya, and Cuba. Emerging Infectious Diseases 2001;5:890-892

da Silva AK, Le Saux JC, Parnaudeau S, Pommepuy M, Elimelech M, Le Guyader FS. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviours of genogroups I and II. Applied and Environmental Microbiology 2007;73:7891-7897.

Dahling, D. Detection and enumeration of enteric viruses in cell culture. Critical Reviews in Environmental Contamination 1991;21:237-263.

Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI. Characterization of rotavirus strains from newborns in New Delhi, India. Journal of Clinical Microbiology 1994;32:1820-1822.

Dennehy PH. Transmission of rotavirus and other enteric pathogens in the home. Pediatric Infectious Disease Journal 2000;19 [Suppl]:S103-S105.

© University of Pretoria

Dennehy PH, Hartin M, Nelson SM, Reising SF. Evaluation of the ImmunoCardSTAT! rotavirus assay for detection of group A rotavirus in fecal specimens. Journal of Clinical Microbiology 1999;37:1977-1979.

Desai R, Oliveira LH, Parashar UD, Lopman B, Tate JE, Patel MM. Reduction in morbidity and mortality from childhood diarrhoeal disease after species A rotavirus vaccine introduction in Latin America - a review. Memorias Instituto Oswaldo Cruz 2011;106:907-911.

Desai R, Hembree CD, Handel A, Matthews JE, Dickey BW, McDonald S, Hall AJ, Parashar UD, Leon JS, Lopman B. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clinical Infectious Diseases 2012;55:189-193.

Deshpande JM, Shetty SJ, Siddiqui ZA. Environmental surveillance system to track wild poliovirus transmission, Applied and Environmental Microbiology 2003;69:2919-2927.

Desselberger U, Gray J. Viral gastroenteritis: causes, pathophysiology, immunology, treatment and epidemiology. In: Desselberger U, Gray J, editors. Viral Gastroenteritis. Amsterdam: Elsevier Science B.V.; 2003; p. 1-8.

Dhole TN, Ayyagari A, Chowdhary R, Shakya AK, Shrivastav N, Datta T, Prakash V. Non-polio enteroviruses in acute flaccid paralysis children of India: vital assessment before polio eradication. Journal of Paediatric and Child Health 2009;45:409-413.

Diedrich S, Driesel G, Schreier E. Sequence comparison of echovirus type 30 isolates to other enteroviruses in the 5’ noncoding region. Journal of Medical Virology 1995;46:148-152.

Diggle L. Rotavirus diarrhoea and future prospects for prevention. British Journal of Nursing 2007;16: 970–974.

© University of Pretoria

Dudareva-Vizule S, Koch J, An der Heiden M, Oberle D, Keller-Stanislawski B, Wichmann O. Impact of rotavirus vaccination in regions with low and moderate vaccine uptake in Germany. Human Vaccines and Immunotherepeutics 2012;8:1407-1415.

Ehlers MM, Grabow WOK, Pavlov DN. Detection of enteroviruses in untreated water and treated drinking water supplies in South Africa. Water Research 2005;39:2253-2258.

El Bassioni L, Barakat I, Nasr E, de Gourville EM, Hovi T, Blomqvist S, Burns C, Stenvik M, Gary H, Kew OM, Pallasch MA, Wahdan MH. Prolonged detection of indigenous wild poliovirus in sewage from communities in Egypt. American Journal of Epidemiology 2003;158:807–815.

El-Senousy WM, Guix S, Abid I, Pintó RM, Bosch A. Removal of astrovirus from water and sewage treatment plants, evaluated by a competitive reverse transcription-PCR. Applied and Environmental Microbiology 2007;73:164-167.

Espinosa AC, Mazari-Hiriart M, Espinosa R, Maruri-Avidal L, Méndez E, Arias CF. Infectivity and genome persistence of rotavirus and astrovirus in groundwater and surface water. Water Research 2008;42:2618-2628.

Estes M, Kapikian A. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. Fields Virology. 5th ed., Vol. 2. Philadelphia: Wolters Kluwer/Lippincott, Williams and Wilkins; 2007. p. 1917–1974.

Ferreira MS, Victoria M, Carvalho-Costa FA, Vieira CB, Xavier MP, Fioretti JM, Andrade J, Volotão EM, Rocha M, Leite JP, Miagostovich MP. Surveillance of norovirus infections in the state of Rio De Janeiro, Brazil 2005-2008. Journal of Medical Virology 2010;82:1442-1448.

Ferreira MS, Xavier Mda P, Tinga AC, Rose TL, Fumian TM, Fialho AM, de Assis RM, Carvalho Costa FA, de Oliveira SA, Leite JP, Miagostovich MP. Assessment of gastroenteric viruses frequency in a children's day care center in Rio De Janeiro, Brazil: a fifteen year study (1994-2008). Plos One 2012;7(3):e33754.

© University of Pretoria

Flewett TH. Diagnosis of enteritis virus. Proceedings of the Royal Society for Medicine 1976;69:693-696.

Florescu DF, Hill LA, McCartan MA, Grant W. Two cases of Norwalk virus enteritis following small bowel transplantation treated with oral human serum immunoglobulin. Pediatric Transplantation 2008;12:372-375.

Fong TT, Lipp EK. Enteric viruses of humans and animals in aquatic environments: Health risks, detection, and potential water quality assessment tools. Microbiology and Molecular Biology Reviews 2005;69:357-371

Formiga-Cruz M, Hundesa A, Clemente-Casares P, Albiñana-Gimenez N, Allard A, Girones R. Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage. Journal of Virological Methods 2005;125:111-118.

Friesema IH, Vennema H, Heijne JC, de Jager CM, Teunis PF, van der Linde R, Duizer E, van Duynhoven YT. Differences in clinical presentation between norovirus genotypes in nursing homes. Journal of Clinical Virology 2009;46:341-344.

Fumian TM, Leite JP, Castello AA, Gaggero A, Caillou MS, Miagostovich MP Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. Journal of Virological Methods 2010;170:42-46.

Gatheru Z, Kobayashi N, Adachi N, Chiba S, Muli J, Ogaja P, Nyangao J, Kiplagat E, Tukei PM. Characterization of human rotavirus strains causing gastroenteritis in Kenya. Epidemiology and Infection 1993;110:419-423.

Gault E, Chikhi-Brachet R, Delon S, Schnepf N, Albiges L, Grimprel E, Girardet JP, Begue P, Garbarg-Chenon A. Distribution of human rotavirus G types circulating in Paris, France, during the 1997-1998 epidemic: high prevalence of type G4. Journal Clinical Microbiology 1999;37:2373-2375.

© University of Pretoria

Gentsch JR, Glass RI, Woods PA, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK. Identification of group A rotavirus gene 4 types by polymerase chain reaction. Journal Clinical Microbiology 1992;30:1365-1373.

Gerba CP. Virus occurrence and survival in environmental waters. In: Bosch A, editor. Human Viruses in Water. Amsterdam: Elsevier B.V.; 2007. p. 91-108.

Gibson KE, Opryszko MC, Schissler JT, Guo Y, Schwab KJ. Evaluation of human enteric viruses in surface water and drinking water resources in southern Ghana. American Journal of Tropical Medicine and Hygiene 2011;84:20-29.

Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. New England Journal of Medicine 2009;361:1776-1785.

Global Polio Eradication Initiative. Polio eradication and endgame strategic plan (2010-2012). Geneva, Switzerland: World Health Organization, Global Polio Eradication Initiative;2010.(http://www.polioeradication.org/content/publications/GPEI.StrategicPlan.2010-2012.ENG.May.2010.pdf). Accessed 22 October 2013.

Gomes KA, Stupka JA, Diana A, Parra GI. Molecular characterization of calicivirus strains detected in outbreaks of gastroenteritis occurring in Argentina during 2005 and 2006. Revista Argentina de Microbiologia 2008;40:222-228.

Götz H, de Jong B, Lindbäck J, Parment PA, Hedlund KO, Torvén M, Ekdahl K. Epidemiological investigation of a food-borne gastroenteritis outbreak caused by Norwalk-like virus in 30 day-care centres. Scandinavian Journal of Infectious Diseases 2002;34:115-121.

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. Journal Clinical Microbiology 1990;28:276-282.

Gouvea V, Santos N, Timenetsky Mdo C. Identification of bovine and porcine rotavirus G types by PCR. Journal Clinical Microbiology 1994a;32:1338-1340.

© University of Pretoria

Gouvea V, Santos N, Timenetsky Mdo C. VP4 typing of bovine and porcine group A rotaviruses by PCR. Journal Clinical Microbiology 1994b;32:1333-1337.

Grabow WOK. Overview of health-related water virology. In: Bosch A, editor. Human Viruses in Water. Amsterdam: Elsevier B.V.; 2007. p.1-25.

Grabow WOK, Botma KL, de Villiers JC, Clay CG, Erasmus B. Assessment of cell culture and polymerase chain reaction procedures for the detection of polioviruses in wastewater. Bulletin of the World Health Organization 1999;77:973-980.

Gratacap-Cavallier B, Genoulaz O, Brengel-Pesce K, Soule H, Innocenti-Francillard P, Bost M, Gofti L, Zmirou, D, Seigneurin JM. Detection of human and animal rotavirus sequences in drinking water. Applied and Environmental Microbiology 2000;66:2690-2692.

Green K. Caliciviridae: The Noroviruses. In: Knipe DM, Howley, PM, Griffin, DE, Martin MA, Lamb RA, Roizman B, Straus SE, editors. Fields Virology. Philadelphia PA: Lippincott Williams & Wilkins; 2007. p. 967-979.

Greening GE. Human and animal viruses in food (including taxonomy of enteric viruses). In: Goyal SM, editor. Viruses in Food. New York: Springer Science and Business Media; 2006. p. 5-42

Gunn L, Feeney SA, Cashman O, Collins PJ, Coyle PV, O'Shea H. Molecular characterization of group A rotavirus found in elderly patients in Ireland; predominance of G1P[8], continued presence of G9P[8], and emergence of G2P[4]. Journal of Medical Virology 2012;84:2008-2017.

Hall TA. Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acid Symposium Series 1999;41:95-98.

Hamza IA, Jurzik L, Überla K, Wilhelm M. Methods to detect infectious human enteric viruses in environmental water samples International Journal of Hygiene and Environment Health 2011;214:424-36.

© University of Pretoria

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiology Reviews 2014;38:1-40.

Harris, J.P., Edmunds, W.J., Pebody, R., Brown, D.W., & Lopman, B.A. Deaths from norovirus among the elderly, England and Wales. Emerging Infectious Diseases 2008;10:1546-1552.

Harris JP, Lopman BA, O'Brien SJ. Infection control measures for norovirus: a systematic review of outbreaks in semi-enclosed settings. Journal of Hospital Infection. 2010;74:1-9.

Hassine-Zaafrane M, Sdiri-Loulizi K, Kaplon J, Salem IB, Pothier P, Aouni M, Ambert-Balay K. Prevalence and genetic diversity of norovirus infection in Tunisian children (2007-2010). Journal of Medical Virology 2013;85:1100-1110.

He XQ, Cheng L, Li W, Xie XM, Ma M, Wang ZJ. Detection and distribution of rotavirus in municipal sewage treatment plants (STPs) and surface water in Beijing. Journal of Environmental Science and Health 2008;43:424-429.

Hemming M, Räsänen S, Huhti L, Paloniemi M, Salminen M, Vesikari T. Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of RotaTeq vaccine into the National Immunization Programme in Finland. European Journal of Pediatrics 2013;172:739-746.

Hird TR, Grassly NC. Systematic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge. PLoS Pathogens 2012;8:e1002599.

Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. Journal of Clinical Virology 2013;56:185-193.

© University of Pretoria

Hollinger B, Emerson SU. Hepatitis A virus. In: Knipe, D.M., Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, Straus SE, editors. Fields Virology Philadelphia PA: Lippincott Williams & Wilkins; 2007. p. 911-947.

Holzel H, Cubitt DW, McSwiggan DA, Sanderson PJ, Church J. An outbreak of rotavirus infection among adults in a cardiology ward. Journal of Infection 1980;2:33-37.

Horie H, Yoshida H, Matsuura K, Miyazawa M, Ota Y, Nakayama T, Doi Y, Hashizume S. Neurovirulence of type 1 polioviruses isolated from sewage in Japan. Applied and Environmental Microbiology 2002;68:138-142.

Hoshino Y, Kapikian AZ. Rotavirus serotypes: classification and importance in epidemiology, immunity, and vaccine development. Journal of Health, Population, and Nutrition 2000;18:5-14.

Hosokawa S, Kodaka H. Efficacy of Compact Dry EC for coliform detection in seafood. International Journal of Food Microbiology 2010;27:80-85.

Hot D, Legeay O, Jacgues J, Gantzer C, Caudrelier Y, Guyard K, Lange M, Andréoletti L. Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Research 2003;37:4703-4710

Hovi T, Stenvik M, Partanen H, Kangas A. Poliovirus surveillance by examining sewage specimens. Quantitative recovery of virus after introduction into sewerage at remote upstream location. Epidemiology and Infection 2001;127:101-106.

Hovi T, Blomqvist S, Nasr E, Burns CC, Sarjakoski T, Ahmed N, Savolainen C, Roivainen M, Stenvik M, Laine P, Barakat I, Wahdan MH, Kamel FA, Asghar H, Pallansch MA, Kew OM, Gary HE Jr, deGourville EM, Bassioni LE.. Environmental surveillance of wild poliovirus circulation in Egypt-Balancing between detection sensitivity and workload. Journal of Virological Methods 2005;126:127-134.

© University of Pretoria

Hovi T, Shulman LM, van der Avoort H, Deshpande J, Roivainen M, DE Gourville EM. Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiology and Infection 2012;140:1-13.

Huang PW, Laborde D, Land VR, Matson DO, Smith AW, Jiang X. Concentration and detection of caliciviruses in water samples by reverse transcription-PCR. Applied and Environmental Microbiology 2000;66:4383-4388.

Huang J, Xu X, Weng Q, Hong H, Guo Z, He S, Niu J. Serial foodborne norovirus outbreaks associated with multiple genotypes. PLoS One 2013;8(5):e63327.

Huttunen P, Santii J, Pulli T, Hyypia T. The major echovirus group is genetically coherent and related to coxsackie B viruses. Journal of General Virology 1996;77:715-725.

Huynen P, Mauroy A, Martin C, Savadogo LG, Boreux R, Thiry E, Melin P, De Mol P. Molecular epidemiology of norovirus infections in symptomatic and asymptomatic children from Bobo Dioulasso, Burkina Faso. Journal of Clinical Virology 2013;58:515-521.

Ikner LA, Gerba CP, Bright KR. Concentration and recovery of viruses from water: a comprehensive review. Food and Environmental Virology 2012;4:41-67.

Ida-Hosonuma M, Iwasaki T, Yoshikawa T, Nagata N, Sato Y, Sata T, Yoneyama M, Fujita T, Taya C, Yonekawa H, Koike S. The alpha/beta interferon response controls tissue tropism and pathogenicity of poliovirus. Journal of Virology 2005;79:4460-4469.

Iturriza-Gómara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. Journal of Clinical Virology 2004;31:259-265.

© University of Pretoria

Jenkins HE, Aylward RB, Gasasira A, Donnelly CA, Mwanza M, Corander J, Garnier S, Chauvin C, Abanida E, Pare MA, Adu F, Baba M, Grassly NC. Implications of a circulating vaccine-derived poliovirus in Nigeria. New England Journal of Medicine 2010;362:2360-2369.

Jiang B, Gentsch JR, Glass RI. The role of serum antibodies in the protection against rotavirus disease: an overview. Clinical Infectious Diseases 2002;34:1351-1361.

Jung JH, Yoo CH, Koo ES, Kim HM, Na Y, Jheong WH, Jeong YS. Occurrence of norovirus and other enteric viruses in untreated groundwaters of Korea. Journal of Water Health 2011;9:544-55.

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. Journal of Clinical Microbiology 2003;41:1548-1557.

Kahler AM, Cromeans TL, Roberts JM, Hill VR. Source water quality effects on monochloramine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. Water Research 2011;45:1745-1751

Katayama H, Haramoto E, Oguma K, Yamashita H, Tajima A, Nakajima H, Ohgaki S. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in waste water collected from six plants in Japan. Water Research 2008; 42:1441-1448.

Kelly H, Brussen KA, Lawrence A, Elliot E, Pearn J, Thorley B. Polioviruses and other enteroviruses isolated from faecal samples of patients with acute flaccid paralysis in Australia, 1996-2004. Journal of Paediatric Child Health 2006;42:370-376.

Kew OM, Wright PF, Agol VI, Delpeyroux F, Shimizu H, Nathanson N, Pallansch MA. Circulating vaccine-derived polioviruses: current state of knowledge. Bulletin of the World Health Organization 2004;82:16-23

© University of Pretoria

Khan RR, Lawson AD, Minnich LL, Martin K, Nasir A, Emmett MK, Welch CA, Udall JN Jr. Gastrointestinal norovirus infection associated with exacerbation of inflammatory bowel disease. Journal of Pediatric Gastroenterology and Nutrition 2009;48:328-333.

Kilpatrick DR, Nottay B, Yang CF, Yang SJ, Mulders MN, Holloway BP, Pallansch MA, Kew OM. Group-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residue at positions of codon degeneracy. Journal of Clinical Microbiology 1996;34:2990-2996.

Kilpatrick DR, Nottay B, Yang CF, Yang SJ, Da Silva E, Peñaranda S, Pallansch M, Kew O. Serotype-specific identification of polioviruses by PCR using primers at positions of codon degeneracy. Journal of Clinical Microbiology 1998;36:352-357.

Kilpatrick DR, Yang CF, Ching K, Vincent A, Iber J, Campagnoli R, Mandelbaum M, De L, Yang SJ, Nix A, Kew OM. Rapid group-, serotype-, and vaccine strain-specific identification of poliovirus isolates by real-time reverse transcription-PCR using degenerate primers and probes containing deoxyinosine residues. Journal of Clinical Microbiology 2009;47:1939-1941

Kim HS, Hyun J, Kim HS, Kim JS, Song W, Lee KM. Emergence of GII.4 Sydney norovirus in South Korea during the winter of 2012-2013. Journal of Microbiology and Biotechnology 2013;23:1641-1643.

Kittigul L, Ekchaloemkiet S, Utrarachkij F, Siripanichgon K, Sujirarat D, Pungchitton S, Boonthum A. An efficient virus concentration method and RT-nested PCR for detection of rotaviruses in environmental water samples. Journal of Virological Methods 2005;124:117-122.

Kittigul L, Pombubpa K, Taweekate Y, Diraphat P, Sujirarat D, Khamrin P, Ushijima H. Norovirus GII-4 2006b variant circulating in patients with acute gastroenteritis in Thailand during a 2006-2007 study. Journal of Medical Virology 2010;82:854-860.

© University of Pretoria

Kiulia NM, Peenze I, Dewar J, Nyachieo A, Galo M, Omolo E, Steele AD, Mwenda JM. Molecular characterization of the rotavirus strains prevalent in Maua, Meru North district, Kenya. East African Medical Journal 2006;83:360-365.

Kiulia NM, Mwenda JM, Nyachieo A, Taylor MB. Astrovirus infection in young Kenyan children with diarrhoea. Journal of Tropical Pediatrics 2007;53:206-209.

Kiulia NM, Kamenwa R, Irimu G, Nyangao JWO, Gatheru Z, Nyachieo A, Steele AD, Mwenda JM. The epidemiology of human rotavirus diarrhoea in Kenya. A review. Journal of Tropical Pediatrics 2008;54:401-405.

Kiulia NM, Nyaundi JK, Peenze I, Nyachieo A, Musoke RN, Steele AD. Mwenda JM. Rotavirus infections among HIV-infected children in Nairobi, Kenya. Journal of Tropical Pediatrics 2009;55:318-323.

Kiulia NM, Netshikweta R, Page NA, van Zyl WB, Kiraithe MM, Nyachieo A, Mwenda JM, Taylor MB. The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special reference to rotaviruses. Journal of Applied Microbiology 2010;109:818-828.

Kiulia NM, Nyaga MM, Seheri ML, Wolfaardt M, van Zyl WB, Esona MD, Irimu G, Inoti M, Gatinu BW, Njenga PK, Taylor MB, Nyachieo A. Rotavirus G and P types circulating in the eastern region of Kenya: predominance of G9 and emergence of G12 genotypes. Pediatrics Infectious Disease Journal 2014;33 [Suppl 1]:S85-88.

Knowles NJ, Hovi T, Hyypia T, Kind AMQ, Lindberg AM, Pallansch MA, Palmenberg AC, Simmonds P, Skern T, Stanway G, Yamashita T, Zell R. Picornaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus Taxonomy: Classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2012. p. 855-880.

Kojima, S., Kageyama, T., Fukushi, S., Hoshino, F.B., Shinohara, M., Uchida, K., et al. (2002). Genogroup-specific PCR primers for detection of Norwalk-like viruses. Journal of Virological Methods 2002;100:107-114.

© University of Pretoria

Komoto S, Maeno Y, Tomita M, Matsuoka T, Ohfu M, Yodoshi T, Akeda H, Taniguchi K. Whole genomic analysis of a porcine-like human G5P[6] rotavirus strain isolated from a child with diarrhoea and encephalopathy in Japan. Journal of General Virology 2013;94:1568-1575.

Koopmans M. Progress in understanding norovirus epidemiology. Current Opinion in Infectious Diseases 2008;21:544-552.

Koopmans M, Duizer E. Foodborne viruses: an emerging problem. International Journal of Food Microbiology 2004;90:23-41.

Kopecka H, Brown B, Pallansch M. Genotypic variation in coxsackie B5 isolates from three different outbreaks in the United States. Virus Research 1995;38:125–136.

Koroglu M, Yakupogullari Y, Otlu B, Ozturk S, Ozden M, Ozer A, Sener K, Durmaz R. A waterborne outbreak of epidemic diarrhoea due to group A rotavirus in Malatya, Turkey. New Microbiologica 2011;34:17-24.

Kroneman A, Vennema H, Deforche K, v d Avoort H, Peñaranda S, Oberste MS, Vinjé J, Koopmans M. An automated genotyping tool for enteroviruses and noroviruses. Journal of Clinical Virology 2011;51:121-125.

Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M. Proposal for a unified norovirus nomenclature and genotyping. Archives of Virology 2013:158:2059-2068.

Lambertini E, Spencer SK, Bertz PD, Loge FJ, Kieke BA, Borchardt MA. Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. Applied and Environmental Microbiology 2008;74:2990-2996.

La Rosa G, Fratini M, della Libera S, Iaconelli M, Muscillo M. Emerging and potentially emerging viruses in water environments. Annali dell'Istituto Superiore Sanita 2012;48:397-406.

© University of Pretoria

Larsson C, Andersson Y, Allestam G, Lindqvist A, Nenonen N, Bergstedt O. Epidemiology and estimated costs of a large waterborne outbreak of norovirus infection in Sweden. Epidemiology and Infection 2014;142:592-600.

Laxmivandana R, Yergolkar P, Gopalkrishna V, Chitambar SD. Characterization of the non-polio enterovirus infections associated with acute flaccid paralysis in South-Western India. PLoS One 2013;8:e61650.

Lee SH, Kim SJ. Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. Water Research 2002;36:248-256.

Le Guyader FS1, Neill FH, Dubois E, Bon F, Loisy F, Kohli E, Pommepuy M, Atmar RL. A semiquantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak. International Journal of Food Microbiology 2003;87:107-112.

Le Guyader FS, Mittelholzer C, Haugarreau L, Hedlund KO, Alsterlund R, Pommepuy M, Svensson L. Detection of noroviruses in raspberries associated with a gastroenteritis outbreak. International Journal of Food Microbiology 2004;97:179-186.

Le Guyader FS, Le Saux JC, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, Giraudon H, Delmas G, Pommepuy M, Pothier P, Atmar RL. Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. Journal of Clinical Microbiology. 2008;46:4011-4017.

Le Guyader FS, Parnaudeau S, Schaeffer J, Bosch A, Loisy F, Pommepuy M, Atmar RL. Detection and quantification of noroviruses in shellfish. Applied and Environmental Microbiology 2009;75:618-624.

Lewis D, Ando T, Humphrey CD, Monroe SS, Glass RI. Use of solid-phase immune electron microscopy for classification of Norwalk-like viruses into six antigenic groups from 10 outbreaks of gastroenteritis in the United States. Journal of Clinical Microbiology 1995;33:501-504.

© University of Pretoria

Loisy F, Atmar RL, Guillon P, Le Cann P, Pommepuy M, Le Guyader FS. Real-time RT-PCR for norovirus screening of shellfish. Journal of Virological Methods 2005; 123:1-7.

Lopman B, Armstrong B, Atchison C, Gray JJ. Host, weather and virological factors drive norovirus epidemiology: time-series analysis of laboratory surveillance data in England and Wales. PLoS One 2009;4:e6671.

Lou F, Neetoo H, Chen H, Li J. Inactivation of a human norovirus surrogate by high-pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. Applied and Environmental Microbiology 2011;77:1862-1871.

Lund BM, O'Brien SJ. The occurrence and prevention of foodborne disease in vulnerable people. Foodborne Pathogen and Disease 2011;8:961-973.

Makino Y, Matsumoto I, Chiba Y, Mohammed OA, Ogaja PO, Kibue AM, Muli JM, Nakitare GW. Virological survey of children in Nyeri and Mombasa. Monthly survey of rotavirus in faeces. East African Medical Journal 1983; 60:536-541.

Malherbe H, Strickland-Cholmley M. Viral Cytopathology. Boca Raton: CRC Press, Inc., 1980.

Manojkumar R, Mrudula V. Applications of real-time reverse transcription polymerase chain reaction in clinical virology laboratories for the diagnosis of human diseases. American Journal of Infectious Diseases 2006;2:204-209

Manor Y, Handsher R, Halmut T. Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority. Journal of Clinical Microbiology 1999;37:1670–1675.

Mans J, de Villiers JC, du Plessis N, Avenant T, Taylor MB. Emerging norovirus GII.4 2008 variant detected in hospitalised paediatric patients in South Africa. Journal of Clinical Virology 2010;49:258-264.

© University of Pretoria

Mans J, Netshikweta R, Magwalivha M, van Zyl WB, Taylor MB. Diverse norovirus genotypes identified in sewage-polluted river water in South Africa. Epidemiology and Infection 2013;141:303-313.

Mans J, Murray TY, Kiulia NM, Mwenda JM, Musoke RN, Taylor MB. Human caliciviruses detected in HIV-seropositive children in Kenya Journal of Medical Virology 2014;86:75-81

Maritschnik S, Kanitz EE, Simons E, Höhne M, Neumann H, Allerberger F, Schmid D, Lederer I. A food handler-associated, foodborne norovirus GII.4 Sydney 2012-Outbreak following a wedding dinner, Austria, October 2012. Food Environmental Virology 2013;5:220-225.

Marshall JA, Bruggink LD. Laboratory diagnosis of norovirus. Clinical Laboratory 2006;52: 571–81.

Martella V, Campolo M, Lorusso E, Cavicchio P, Camero M, Bellacicco AL. Norovirus in captive lion cub (*Panthera leo*). Emerging Infectious Diseases 2007;13:1071-1073.

Martella V, Lorusso E, Decaro N, Elia G, Radogna A, D'Abramo M. Detection and molecular characterization of a canine norovirus. Emerging Infectious Diseases 2008; 14:1306-1308.

Mathijs E, Stals A, Baert L, Botteldoorn N, Denayer S, Mauroy A, Scipioni A, Daube G, Dierick K, Herman L, Van Coillie E, Uyttendaele M, Thiry E. A review of known and hypothetical transmission routes for noroviruses. Food and Environmental Virology 2012;4:131-152.

Matson DO, Szücs G. Calicivirus infections in children. Current Opinion in Infectious Diseases 2003;16:241-246.

© University of Pretoria

Matthews JE, Dickey BW, Miller RD, Felzer JR, Dawson BP, Lee AS, Rocks JJ, Kiel J, Montes JS, Moe CL, Eisenberg JN, Leon JS. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. Epidemiology and Infection 2012;140:1161-1172.

Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Archives of Virology 2011;156:1397-1413.

Mattison K, Bidawid S. Analytical methods for food and environmental viruses. Food and Environmental Virology 2009;1:107-122.

Mena KD. Waterborne viruses: Assessing the risks. In: Bosch A, editor. Human Viruses in Water. Amsterdam: Elsevier B.V.; 2007. p. 163-175.

Miagostovich MP, Ferreira FFM, Guimarães FR, Fumian TM, Diniz-Mendes L, Luz SLB, Silva LA, Leite JPG. Molecular detection and characterization of gastroenteritis viruses occurring naturally in the stream waters of Manaus, Central Amazônia, Brazil. Applied and Environmental Microbiology 2008;74:375–382.

Miles SL, Gerba CP, Pepper IL, Reynolds KA. Point-of-use drinking water devices for assessing microbial contamination in finished water and distribution systems. Environmental Science and Technology 2009;43:1425-1429.

Miura T, Parnaudeau S, Grodzki M, Okabe S, Atmar RL, Le Guyader FS. Environmental detection of genogroup I, II, and IV noroviruses by using a generic real-time reverse transcription-PCR assay. Applied and Environmental Microbiology. 2013;79:6585-6592.

Modlin JF. The bumpy road to polio eradication. New England Journal of Medicine 2010;362:2346-2349.

© University of Pretoria

Mphahlele MJ, Peenze I, Steele AD. Rotavirus strains bearing the VP4P[14] genotype recovered from South African children with diarrhoea. Archives of Virology 1999;144:1027-1034.

Msimang VM, Page N, Groome MJ, Moyes J, Cortese MM, Seheri M, Kahn K, Chagan M, Madhi SA, Cohen C. Impact of rotavirus vaccine on childhood diarrheal hospitalization after introduction into the South African public immunization program. Pediatric Infectious Disease Journal 2013;32:1359-1364.

Mueller JE, Bessaud M, Huang QS, Martinez LC, Barril PA, Morel V, Balanant J, Bocacao J, Hewitt J, Gessner BD, Delpeyroux F, Nates SV. Environmental poliovirus surveillance during oral poliovirus vaccine and inactivated poliovirus vaccine use in Córdoba province, Argentina. Applied and Environmental Microbiology 2009;75:1395-1401.

Munos MK, Walker CL, Black RE. The effect of oral rehydration solution and recommended home fluids on diarrhoea mortality. International Journal of Epidemiology 2010;39 [Suppl 1]:i75-87.

Murray TY, Mans J, Taylor MB. First detection of human sapoviruses in river water in South Africa. Water Science and Technology 2013;67:2776-2783.

Mutanda LN, Kinoti SN, Gemert W, Lichenga EO. Age distribution and seasonal pattern of rotavirus infection in children in Kenya. Journal of Diarrhoeal Disease Research 1984;2:147-150.

Mutanda LN, Patel A, Masudi AM, Maina G. Aetiology of diarrhoea in pre-term neonate at Kenyatta National Hospital nursery, Nairobi, Kenya. East African Medical Journal 1990;67:223-230.

Mwenda JM, Peenze I, Omollo E, Galo M, Steele AD. Human group C rotaviruses identified in Kenya. East African Medical Journal. 2003;80:73-76.

© University of Pretoria

Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, Kisakye A, Mpabalwani EM, Pazvakavambwa I, Armah GE, Seheri LM, Kiulia NM, Page N, Widdowson MA, Steele AD. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. Journal of Infectious Diseases 2010;202 [Suppl]:S5-S11.

Nakagomi T, Doan YH, Dove W, Ngwira B, Iturriza-Gómara M, Nakagomi O, Cunliffe NA. G8 rotaviruses with conserved genotype constellations detected in Malawi over 10 years (1997-2007) display frequent gene reassortment among strains co-circulating in humans. Journal of General Virology 2013;94:1273-295.

Nakata S, Gatheru Z, Ukae S, Adachi N, Kobayashi N, Honma S, Muli J, Ogaja P, Nyangao J, Kiplagat E, Tukei PM, Chiba S. Epidemiological study of the G-serotype distribution of group A in Kenya from 1991 to 1994. Journal of Medical Virology 1999; 58:296-303.

Ndze VN, Achidi EA, Papp H, Kovács E, Farkas S, Adiogo D, Kisfali P, Ngeng MB, Abena MT, Martella V, Esona MD, Bányai K. Shared G12 VP7 gene among human and bovine rotaviruses detected in Cameroonian villages. Acta Microbiologica et Immunologica Hungarica 2013;60:21-28.

Nenonen NP, Hannoun C, Larsson CU, Bergström T. Marked genomic diversity of norovirus genogroup I strains in a waterborne outbreak. Applied and Environmental Microbiology 2012;78:1846-1852.

Ngaosuwankul N, Thippornchai N, Yamashita A, Vargas RE, Tunyong W, Mahakunkijchareon Y, Ikuta K, Singhasivanon P, Okabayashi T, Leaungwutiwong P. Detection and characterization of enteric viruses in flood water from the 2011 thai flood. Japanese Journal of Infectious Disease 2013;66:398-403.

Nicolay N, McDermott R, Kelly M, Gorby M, Prendergast T, Tuite G, Coughlan S, McKeown P, Sayers G. Potential role of asymptomatic kitchen food handlers during a food-borne outbreak of norovirus infection, Dublin, Ireland, March 2009. European Surveillance 2011;16.pii: 19931.

© University of Pretoria

Nokes DJ, Peenze I, Netshifhefhe L, Abwao J, De Beer MC, Seheri M, Williams TN, Page N, Steele D. Rotavirus genetic diversity, disease association, and temporal change in hospitalized rural Kenyan children. Journal of Infectious Diseases 2010;202 [Suppl]:S180-S186.

Oberste MS, Maher K, Flemister MR, Marchetti G, Kilpatrick DR, Pallansch MA. Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. Journal of Clinical Microbiology 2000;38:1170–1174.

Oberste MS, Schnurr D, Maher K, al-Busaidy S, Pallansch MA. Molecular identification of new picornaviruses and characterization of a proposed enterovirus 73 serotype. Journal of General Virology 2001;82:409–416.

Oberste MS, Moore D, Anderson B, Pallansch MA, Pevear DC, Collett MS. In vitro antiviral activity of V-073 against polioviruses. Antimicrobial Agents and Chemotherapy. 2009;53:4501-4503.

Odoom JK, Obodai E, Barnor JS, Ashun M, Arthur-Quarm J, Osei-Kwasi M. Human enteroviruses isolated during acute flaccid paralysis surveillance in Ghana: implications for the post eradication era. Pan African Medical Journal 2012;12:74-80.

Offit PA. Host factors associated with protection against rotavirus disease: the skies are clearing. Journal of Infectious Diseases 1996;174 [Suppl 1]:S59-S64.

Okabayashi T, Yokota S, Ohkoshi Y, Ohuchi H, Yoshida Y, Kikuchi M, Yano K, Fujii N. Occurrence of norovirus infections unrelated to norovirus outbreaks in an asymptomatic food handler population. Journal of Clinical Microbiology 2008;46:1985-1988

O'Ryan M, Prado V, Pickering LK. A millennium update on pediatric diarrheal illness in the developing world. Seminars in Pediatrics Infectious Diseases 2005;16:125-136.

Pallansch M, Roos R. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe, D.M., Howley PM, Griffin DE, Martin MA, Lamb RA,

© University of Pretoria

Roizman B, Straus SE, editors. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 839-894.

Pammi M, Haque KN. Oral immunoglobulin for the prevention of rotavirus infection in low birth weight infants. Cochrane Database Systematic Reviews 2011;11:CD003740.
Parashar UD, Glass RI. Viral causes of gastroenteritis. In: Desselberger U, Gray J, editors. Viral Gastroenteritis. Amsterdam:Elsevier Science B.V.; 2003. p.9-21.

Parashar UD, Holman RC, Clarke MJ, Bresee JS, Glass RI. Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. Journal of Infectious Diseases 1998;177:13-17.

Parashar UD, Quiroz ES, Mounts AW, Monroe SS, Fankhauser RL, Ando T, Noel JS, Bulens SN, Beard SR, Li JF, Bresee JS, Glass RI. "Norwalk-like viruses". Public health consequences and outbreak management. Morbidity and Mortality Weekly Report 2001;50(RR-9):1-17.

Parashar UD1, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. Global mortality associated with rotavirus disease among children in 2004. Journal of Infectious Diseases 2009;200 [Suppl 1]:S9-S15.

Park SH, Kim EJ, Oh SA, Kim CK, Choi SS, Cho SJ, Han KY, Lee JI, Kim MY, Jung HS, Chun DS, Kim HS. Viral agents associated with acute gastroenteritis in Seoul, Korea. Clinical Laboratory 2011;57:59-65.

Patel MM, Hall AJ, Vinjé J, Parashar UD. Noroviruses: a comprehensive review. Journal of Clinical Virology 2009;44:1–8.

Pazzaglia G, Bourgeois AL, Araby I, Mikhail I, Podgore JK, Mourad A, Riad S, Gaffar T, Ramadan AM. Campylobacter-associated diarrhoea in Egyptian infants: epidemiology and clinical manifestations of disease and high frequency of concomitant infections. Journal of Diarrhoeal Diseases Research 1993;11:6-13.

© University of Pretoria

Phan TG, Nguyen TA, Kuroiwa T, Kaneshi K, Ueda Y, Nakaya S, Nishimura S, Nishimura T, Yamamoto A, Okitsu S, Ushijima H. Viral diarrhea in Japanese children: results from a one-year epidemiologic study. Clinical Laboratory 2005;51:183–191.

Pintó RM, Saiz JC. Enteric hepatitis viruses. In: Bosch A, editor. Human Viruses in Water. Amsterdam: Elsevier B.V.; 2007. p. 39-67.

Pintó R.M., Costafreda I., Bosch A. Risk assessment in shellfish-borne outbreaks of hepatitis A. Applied and Environmental Microbiology 2009;75:7350-7355.

Piralla A, Lilleri D, Sarasini A, Marchi A, Zecca M, Stronati M, Baldanti F, Gerna G. Human rhinovirus and human respiratory enterovirus (EV68 and EV104) infections in hospitalized patients in Italy, 2008-2009. Diagnostic Microbiology and Infectious Disease 2012;73:162-167.

Prata C, Ribeiro A, Cunha Â, Gomes NC, Almeida A. Ultracentrifugation as a direct method to concentrate viruses in environmental waters: virus-like particle enumeration as a new approach to determine the efficiency of recovery. Journal of Environmental Monitoring 2012;14:64-70.

Pulli T, Koskimies P, Hyypia T. Molecular comparison of coxsackie A virus serotypes. Virology 1995;211:30-38.

Pusch D, Oh DY, Wolf S, Dumke R, Schröter-Bobsin U, Höhne M, Röske I, Schreier E. Detection of enteric viruses and bacterial indicators in German environmental waters. Archives of Virology 2005;150:929-947.

Puustinen L, Blazevic V, Salminen M, Hämäläinen M, Räsänen S, Vesikari T. Noroviruses as a major cause of acute gastroenteritis in children in Finland, 2009–2010. Scandinavia Journal of Infectious Diseases 2011;43:804–808.

Rakoto-Andrianarivelo M, Rousset D, Razafindratsimandresy R, Chevaliez S, Guillot S, Balanant J, Delpeyroux F. High frequency of human enterovirus species C circulation in Madagascar. Journal of Clinical Microbiology 2005;43:242–249.

© University of Pretoria

Ranta J, Hovi T, Arjas E, Poliovirus surveillance by examining sewage water specimens: studies on detection probability using simulation models. Risk Analysis 2001;21:1087-1096.

Razafindratsimandresy R, Joffret ML, Rabemanantsoa S, Andriamamonjy S, Heraud JM, Delpeyroux F. Reemergence of recombinant vaccine-derived polioviruses in healthy children, Madagascar. Emerging Infectious Disease 2013;19:1008-1010.

Reichler MR, Abbas A, Kharabsheh S, Mahafzah A, Alexander JP, Rhodes P, Faouri S, Ptoum H, Bloch S, Abdel-Magid M, Mulders M, Aslanian R, Hull HF, Pallansch M, Patriarca PA. Outbreak of paralytic poliomyelitis in a highly immunized population in Jordan. Journal of Infectious Diseases 1997;175[Suppl. 1]:S62-S70.

Richer MJ, Lavallée DJ, Shanina I, Horwitz MS. Toll-like receptor 3 signaling on macrophages is required for survival following coxsackievirus B4 infection. PLoS One 2009;4(1):e4127.

Riera-Montes M, Brus Sjölander K, Allestam G, Hallin E, Hedlund KO, Löfdahl M. Waterborne norovirus outbreak in a municipal drinking-water supply in Sweden. Epidemiology and Infection 2011;139:1928-1935.

Rodríguez-Díaz J, Querales L, Caraballo L, Vizzi E, Liprandi F, Takiff H, Betancourt WQ. Detection and characterization of waterborne gastroenteritis viruses in urban sewage and sewage-polluted river waters in Caracas, Venezuela. Applied Environmental and Microbiology 2009;75:387-394.

Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, López P, Macías-Parra M, Ortega-Barría E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavía-Ruz N, Salmerón J, Rüttimann R, Tinoco JC, Rubio P, Nuñez E, Guerrero ML, Yarzábal JP, Damaso S, Tornieporth N, Sáez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M; Human Rotavirus Vaccine Study

© University of Pretoria

Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. New England Journal of Medicine 2006;354:11-22.

Rutjes SA, Italiaander R, van den Berg HH, Lodder WJ, de Roda Husman AM. Isolation and detection of enterovirus RNA from large-volume water samples by using the NucliSens miniMAG system and real-time nucleic acid sequence-based amplification. Applied and Environmental Microbiology. 2005;71:3734-3740.

Rutjes SA, Lodder WJ, van Leeuwen AD, de Roda Husman AM. Detection of infectious rotavirus in naturally contaminated source waters for drinking water production. Journal of Applied Microbiology 2009;107:97-105.

Saeed M, Zaidi SZ, Naeem A, Masroor M, Sharif S, Shaukat S, Angez M, Khan A. Epidemiology and clinical findings associated with enteroviral acute flaccid paralysis in Pakistan. BMC Infectious Diseases 2007;7:6.

Sakai Y, Nakata S, Honma S, Tatsumi M, Numata-Kinoshita K, Chiba S. Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. Pediatric Infectious Disease Journal 2001;20:849–853.

Sakamaki N, Ohiro Y, Ito M, Makinodan M, Ohta T, Suzuki W, Takayasu S, Tsuge H. Bioluminescent enzyme immunoassay for the detection of norovirus capsid antigen Clinical and Vaccine Immunology 2012;19:1949-1954.

Samadi AR, Islam R, Huq MI: Replacement of intravenous therapy by oral rehydration solution in a large treatment centre for diarrhoea with dehydration. Bulletin of the World Health Organization 1998;76:319-324.

Schorn R, Höhne M, Meerbach A, Bossart W, Wüthrich RP, Schreier E, Müller NJ, Fehr T. Chronic norovirus infection after kidney transplantation: molecular evidence for immune-driven viral evolution. Clinical Infectious Diseases 2010;51:307-314.

© University of Pretoria

Scipioni A, Mauroy A, Vinjé J, Thiry E. Animal noroviruses. Veterinary Journal 2008;178:32-45.

Shen Z, Wang G, Zhang W, Qian F, Li Y, Zhang M, Gu S, Wang M, Lin F, Hu Y, Yuan Z, Zhang J. Rotavirus infection and its genetic characterization in non-hospitalized adults with acute gastroenteritis in Shanghai, China. Archives of Virology 2013; 158:1671-1677.

Sherchan JB, Ohara H, Sherpa K, Sakurada S, Gurung B, Tandukar S, Pradhan L, Burlakoti T, Pokharel BM, Sherchand JB. Rotavirus nosocomial infection in children under 5 years of age: A preliminary study in Nepal. Journal of Nepal Paediatric Society 2010;31:30-34.

Shimizu H, Utama A, Yoshii K, Yoshida H, Yoneyama T, Sinniah M, Enterovirus 71 from fatal and nonfatal cases of hand, foot and mouth disease epidemics in Malaysia, Japan and Taiwan in 1997-1998. Japan Journal of Infectious Diseases. 1999;52:12-15.

Sibanda,T, Okoh AI. Real-time PCR quantitative assessment of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River located in the Eastern Cape Province, South Africa. Water SA 2013;39:295-304.

Siebenga JJ, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. Journal of Infectious Diseases 2009;200:802-812.

Silva LD, Rodrigues EL, Lucena MS, Lima IC, Oliveira DD, Soares LS, Mascarenhas JD, Linhares AD, Gabbay YB. Detection of the pandemic norovirus variant GII.4 Sydney 2012 in Rio Branco, state of Acre, northern Brazil. Memorias do Instituto Oswaldo Cruz 2013;108:1068-1070.

© University of Pretoria

Simmonds MK, Armah G, Asmah R, Banerjee I, Damanka S, Esona M, Gentsch JR, Gray JJ, Kirkwood C, Page N, Iturriza-Gómara M. New oligonucleotide primers for P typing of rotavirus strains: Strategies for typing previously untypeable strains. Journal of Clinical Virology 2008;42:368-373.

Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerging Infectious Diseases 2013;19:1260-1267.

Singh S, Chow VTK, Chan KP, Ling AE, Poh CLRT-PCR. nucleotide, amino acid and phylogenetic analyses of enterovirus type 71 strains from Asia. Journal of Virological Methods 2000;88:193-204.

Skern T. 100 years poliovirus: from discovery to eradication. A meeting report. Archives of Virology 2010;155:1371-1381.

Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. The Lancet Infectious Diseases 2010;10:778-790.

Stals A, Uyttendaele M, Baert L, Van Coillie E. Norovirus transfer between foods and food contact materials. Journal of Food Protection. 2013;76:1202-1209.

Steele AD, Ivanoff B. Rotavirus strains circulating in Africa during 1996-1999: emergence of G9 strains and P[6] strains. Vaccine. 2003;21:361-367.

Steele M, Odumeru J. Irrigation water as source of foodborne pathogens on fruit and vegetables Journal of Food Protection 2004;67:2839-2849.

Sukhrie FH, Beersma MF, Wong A, van der Veer B, Vennema H, Bogerman J, Koopmans M. Using molecular epidemiology to trace transmission of nosocomial norovirus infection. Journal of Clinical Microbiology 2011;49: 602-606.

© University of Pretoria

Sutter RW, John TJ, Jain H, Agarkhedkar S, Ramanan PV, Verma H, Deshpande J, Singh AP, Sreevatsava M, Malankar P, Burton A, Chatterjee A, Jafari H, Aylward RB. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. Lancet 2010;376:1682-1688.

Svraka S, Duizer E, Vennema H, de Bruin E, van der Veer B, Dorresteijn B, Koopmans M. Etiological role of viruses in outbreaks of acute gastroenteritis in the Netherlands from 1994 through 2005. Journal of Clinical Microbiology 2007;45:1389-1394.

Swartz TA, Handsher R. Israel in the elimination phase of poliomyelitis--achievements and remaining problems. Public Health Review 1993-1994;21:99-106.

Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, Gray JJ, Letley LH, Rait G, Tompkins DS, O'Brien SJ; IID2 Study Executive Committee. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 2012;61:69-77

Tambini G, Andrus JK, Marques E, Boshell J, Pallansch M , de Quadros CA, Kew OM. Direct detection of wild poliovirus circulation by stool survey of healthy children and analysis of community wastewater. Journal of Infectious Diseases 1993;168:1510-1514.

Tamura K, Peterson D, Peterson N, Stecher G, Ne, M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 2011;28:2731-1739.

Tao Z, Wang H, Xu A, Zhang Y, Song L, Zhu S, Li Y, Yan D, Liu G, Yoshida H, Liu Y, Feng L, Chosa T, Xu W. Isolation of a recombinant type 3/type 2 poliovirus with a chimeric capsid VP1 from sewage in Shandong, China. Virus Research 2010;150:56-60.

Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. The Lancet Infectious Diseases 2012;12:136-141.

© University of Pretoria

Tate JE, Haynes A, Payne DC, Cortese MM, Lopman BA, Patel MM, Parashar UD. Trends in national rotavirus activity before and after introduction of rotavirus vaccine into the national immunization program in the United States, 2000 to 2012. Pediatric Infectious Disease Journal. 2013;32:741-744.

Taylor MB, Cox N, Vrey MA, Grabow WOK. The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. Water Research 2001; 35:2653-2660.

Tazi-Lakhsassi L1, Garbarg-Chenon A, Nicolas JC, Soubhi H, Benbachir M, el Mdaghri N, Tazi M, Bricout F, Huraux JM. Epidemiological and clinical study and electrophoretyping survey of rotavirus acute diarrhoea in a children's infectious disease unit in Casablanca, Morocco. Annales de I'Institut Pasteur Virology. 1988;139:205-215.

Teunis PFM, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. Norwalk virus: How infectious it is. Journal of Medical Virology 2008;80:1468-1476.

Toyoda H, Kohara M, Kataoka Y, Suganuma T, Omata T, Imura N, Nomoto A. Complete nucleotide sequences of all three poliovirus serotype genomes. Implication for genetic relationship, gene function and antigenic determinants. Journal of Molecular Biology. 1984;174:561-585.

Trainor E, Lopman B, Iturriza-Gomara M, Dove W, Ngwira B, Nakagomi O, Nakagomi T, Parashar U, Cunliffe N. Detection and molecular characterisation of noroviruses in hospitalised children in Malawi, 1997-2007. Journal of Medical Virology 2013;85:1299-1306.

Trivedi TK, Desalvo T, Lee L, Palumbo A, Moll M, Curns A, Hall AJ, Patel M, Parashar UD, Lopman BA. Hospitalizations and mortality associated with norovirus outbreaks in nursing homes, 2009-2010. Journal of the American Medical Association 2012;18:1-8.

© University of Pretoria

Troy SB, Ferreyra-Reyes L, Huang C, Mahmud N, Lee YJ, Canizales-Quintero S, Flaster H, Báez-Saldaña R, García-García L, Maldonado Y. Use of a novel real-time PCR assay to detect oral polio vaccine shedding and reversion in stool and sewage samples after a Mexican national immunization day. Journal of Clinical Microbiology 2011;49:1777-1783.

Turcios-Ruiz RM, Axelrod P, St John K, Bullitt E, Donahue J, Robinson N, Friss HE. Outbreak of necrotizing enterocolitis caused by norovirus in a neonatal intensive care unit. Journal of Pediatrics. 2008;153:339-344.

Urasawa T, Urasawa S, Chiba Y, Taniguchi K, Kobayashi N, Mutanda LN, Tukei PM Antigenic characterisation of rotaviruses isolated in Kenya from 1982 to 1983 Journal of Clinical Microbiology 1987;25:1891-1896.

Van Heerden J, Ehlers MM, Heim A, Grabow WOK. Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. Journal of Applied Microbiology 2005;99:234-242.

Van Zyl WB, Page NA, Grabow WOK, Steele AD, Taylor MB. Molecular epidemiology of group A rotaviruses in water sources and selected raw vegetables in southern Africa. Applied and Environmental Microbiology 2006;72:4554-4560.

Vantakaris AC, Papapetropoulou M. Detection of enteroviruses and adenoviruses in coastal waters of SW Greece by nested polymerase chain reaction. Water Research 1998;30:2365-2372.

Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinjé J. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009-2013. Journal of Clinical Microbiology 2014;52:147-155.

Venter JME. The incidence of hepatitis A virus in selected water sources and associated risk of infection in South Africa. [MSc dissertation]. Pretoria: University of Pretoria; 2004.

© University of Pretoria

Verheyen J, Timmen-Wego M, Laudien R, Boussaad I, Sen S, Koc A, Uesbeck A, Mazou F, Pfister H. Detection of adenoviruses and rotaviruses in drinking water sources used in rural areas of Benin, West Africa. Applied and Environmental Microbiology 2009;75:2798-2801.

Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB, Shinefield HR, Christie CD, Ylitalo S, Itzler RF, Coia ML, Onorato MT, Adeyi BA, Marshall GS, Gothefors L, Campens D, Karvonen A, Watt JP, O'Brien KL, DiNubile MJ, Clark HF, Boslego JW, Offit PA, Heaton PM; Rotavirus Efficacy and Safety Trial (REST) Study Team. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. New England Journal of Medicine 2006;354:23-33.

Vesikari T, Uhari M, Renko M, Hemming M, Salminen M, Torcel-Pagnon L, Bricout H, Simondon F. Impact and effectiveness of RotaTeq® vaccine based on 3 years of surveillance following introduction of a rotavirus immunization program in Finland. Pediatrics Infectious Diseases Journal. 2013;32:1365-1373.

Vilaginès Ph, Sarrette B, Husson G, Vilaginès R. Glass wool for virus concentration at ambient pH level. Water Science and Technology 1993;27:299-306.

Villena C, El-Senousy WM, Abad FX, Pintó RM, Bosch A. Group A rotavirus in sewage samples from Barcelona and Cairo: emergence of unusual genotypes. Applied and Environmental Microbiology 2003;69:3919-3923.

Vivancos R, Keenan A, Sopwith W, Smith K, Quigley C, Mutton K, Dardamissis E, Nichols G, Harris J, Gallimore C, Verhoef L, Syed Q, Reid J. Norovirus outbreak in a cruise ship sailing around the British Isles: investigation and multi-agency management of an international outbreak. Journal of Infection 2010;60:478-485.

Vivier JC, Ehlers MM, Grabow. Detection of enteroviruses in treated drinking water. Water Research 2004;38:2699-2705.

© University of Pretoria

Ward RL, Knowlton DR, Pierce MJ. Efficiency of human rotavirus propagation in cell culture. Journal of Clinical Microbiology 1984;19:748-753.

Wikswo ME, Hall AJ. Outbreaks of acute gastroenteritis transmitted by person-to-person contact--United States, 2009-2010. Morbidity and Mortality Weekly Report 2012;61:1-12.

Wikswo ME, Cortes J, Hall AJ, Vaughan G, Howard C, Gregoricus N, Cramer EH. Disease transmission and passenger behaviors during a high morbidity norovirus outbreak on a cruise ship, January 2009. Clinical Infectious Diseases 2011;52:1116-1122.

Wolfaardt M, Moe CL, Grabow WOK. Detection of small rounded structured viruses in clinical and environmental samples by polymerase chain reaction. Water Science and Technology 1995;31:375-382.

Wood DJ, Hull B. L20B cells simplify culture of polioviruses from clinical samples. Journal of Medical Virology 1999;58:188-192.

World Health Organization. Generic protocols for (i) hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and (ii) a community-based survey on utilization of health care services for gastroenteritis in children. Field test version. WHO/V&B/02.15, Geneva: World Health Organization; November 2002a. http://whqlibdoc.who.int/hq/2002/WHO_V&B_02.15.pdf: accessed date 2013-10-11

World Health Organization. Progress towards global poliomyelitis eradication 2001. Weekly Epidemiological Records 2002b;77:98-107

World Health Organization. 4th Edition, Polio laboratory manual. WHO/IVB/04.10, Geneva: November 2004 http://whqlibdoc.who.int/hq/2008/who_ivb_08.17_eng.pdf: accessed date 2013-10-11

World Health Organization 2008. Guidelines for Drinking-Water Quality. Geneva, Switzerland: World Health Organization, 1-668.

© University of Pretoria

World Health Organization. Manual of rotavirus detection and characterisation. WHO/IVB/08.17, Geneva: World Health Organization; October 2009a. http://whqlibdoc.who.int/hq/2008/who_ivb_08.17_eng.pdf: accessed date 2013-10-11

World Health Organization. Rotavirus vaccines: an update. Weekly Epidemiological Records. 2009b;84:533-540.

World Health Organization. The polio eradication and endgame strategic plan 2013–2018. Geneva, Switzerland: World Health Organization; 2010. Available at http://www.polioeradication.org/resourcelibrary/strategyandwork.aspx access date 2011-11-29.

World Health Organization. Progress towards poliomyelitis eradication, Nigeria, January 2011– September 2012. Weekly Epidemiological Records 2012;87:439-446.

Wyn-Jones AP. The detection of waterborne viruses. In: Bosch A, editor. Human Viruses in Water. Amsterdam: Elsevier B.V.; 2007. p.177-203.

Wyn-Jones AP, Sellwood J. A review: enteric viruses in the aquatic environment. Journal of Applied Microbiology 2001;91:945-962

Yang C, De L, Holloway B, Pallansch M, Kew O. Detection and identification of vaccine-related polioviruses by the polymerase chain reaction. Virus Research 1991;20:159-179.

Yap J, Qadir A, Liu I, Loh J, Tan BH, Lee VJ. Outbreak of acute norovirus gastroenteritis in a military facility in Singapore: a public health perspective. Singapore Medical Journal 2012;53:249-254.

Yee EL, Palacio H, Atmar RL, Shah U, Kilborn C, Faul M, Gavagan TE, Feigin RD, Versalovic J, Neill FH, Panlilio AL, Miller M, Spahr J, Glass RI. Widespread outbreak of norovirus gastroenteritis among evacuees of Hurricane Katrina residing in a large "megashelter" in Houston, Texas: lessons learned for prevention. Clinical Infectious Diseases 2007;44:1032-1039.

© University of Pretoria

Yoshida H, Horie H, Matsuura K, Kitamura T, Hashizume S, Miyamura T. Prevalence of vaccine-derived polioviruses in the environment. Journal of General Virology 2002;83:1107-1111.

Yu JH, Kim NY, Lee EJ, Jeon IS. Norovirus infections in asymptomatic food handlers in elementary schools without norovirus outbreaks in some regions of Incheon, Korea. Journal of Korean Medical Science 2011;26:734-739

Zakikhany K, Allen DJ, Brown D, Iturriza-Gómara M. Molecular evolution of GII-4 norovirus strains. PLoS One 2012;7(7):e41625.

Zeng S-Q, Halkosalo A, Salminen M, Szakal ED, Puustinin L, Vesikari T. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. Journal of Virological Methods 2008;153:238-240.

Zheng DP, Ando T, Fankauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. Virology 2006;346:312-323.

© University of Pretoria

APPENDIX A

© University of Pretoria

A.1: **Kiulia NM,** Mans J, Page NA, Gumede-Moeletsi N, Mwenda JM, Taylor MB. Enteric viruses in selected surface waters in urban and rural Kenya [Presentation]. 19th International Scientific Conference on "Basic and Clinical Research for Improved Health hosted by the Institute of Primate Research and Kenya Agricultural Research Institute-Trypanosomiasis Research Centre 11-13 September 2013 Southern Sun, Mayfair, Nairobi, Kenya

Background: Human enteric viruses, associated with various clinical syndromes are shed in high concentrations in faecal matter of infected patients. Viruses are discharged into sewage which may contaminate surface water sources for drinking water, recreational activities and irrigation.

Objectives: To investigate the prevalence and predominant genotypes of selected enteric viruses, namely rotaviruses (RVs), noroviruses (NoVs) and enteroviruses (EVs) in surface water from high risk water catchment areas in urban and rural Kenya.

Methods and Results: River (10 L) and wastewater samples (2 L) were collected in urban (Nairobi river, Kibera stream) and rural (Mboone river) areas. Enteric viruses were recovered from the water samples by glass-wool adsorption elution and/or PEG/NaCl precipitation. Nucleic acid was extracted using the automated MagNA Pure platform and screened using commercial singleplex real-time RT-PCR assays. Detected virus strains were characterised by type-specific polymerase chain reactions or nucleotide sequence analysis. Among the 36 water samples collected, at least one virus was detected in each of the samples except for the samples collected from Mboone river in August 2012 with RV being the most commonly detected in 85% (34/40), and NoV genogroup II in 60% (24/40), NoV genogroup I in 20% (8/40) and EVs in 58% (23/40) of the samples. No polioviruses were identified in all the EVs positive samples. Rotavirus G9 and P[8] were the most predominant type detected. Norovirus GII.4, GII.7 and GII.17 - like strains were detected in both rural and urban rivers.

Conclusions: This study reports for the first time the NoV strains circulating in environmental waters in Kenya thus, indicating the importance of this pathogen in Kenya. Continued surveillance of environmental samples in Kenya is therefore crucial to supplement the ongoing clinical surveillance of these viruses.

© University of Pretoria

APPENDIX B

© University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

8/12/2011

Number	:	**S172/2011**
Title	:	Assessment of selected environmental water samples in Kenya for the presence of clinically important enteric viruses
Investigator	:	N M Kiulia, Department of Medical Virology, University of Pretoria (SUPERVISOR: Prof M B Taylor)
Sponsor	:	**MERCK Project – Enteric Virus Research Group**
Study Degree:		**MSc. Medical Virology**

This Student Protocol was reviewed by the Faculty of Health Sciences, Student Research Ethics Committee, University of Pretoria on 8/12/2011 and found to be acceptable. The approval is valid for a period of 3 years.

Prof M J Bester	BSc (Chemistry and Biochemistry); BSc (Hons)(Biochemistry); MSc (Biochemistry); PhD (Medical Biochemistry)
Prof R Delport	(female)BA et Scien, B Curationis (Hons) (Intensive care Nursing), M Sc (Physiology), PhD (Medicine), M Ed Computer Assisted Education
Prof J A Ker	MBChB; MMed(Int); MD – Vice-Dean (ex officio)
Dr NK Likibi	MBB HM – (Representing Gauteng Department of Health) MPH
Dr MP Mathebula	Deputy CEO: Steve Biko Academic Hospital
Prof A Nienaber	(Female) BA (Hons) (Wits); LLB (Pretoria); LLM (Pretoria); LLD (Pretoria); PhD; Diploma in Datametrics (UNISA)
Prof L M Ntlhe	MBChB(Natal); FCS(SA)
Mrs M C Nzeku	(Female) BSc(NUL); MSc Biochem(UCL,UK)
Snr Sr J. Phatoli	(Female) BCur (Et.Al); BTech Oncology
Dr R Reynders	MBChB (Pret), FCPaed (CMSA) MRCPCH (Lon) Cert Med. Onc (CMSA)
Dr T Rossouw	(Female) MBChB.(cum laude); M.Phil (Applied Ethics) (cum laude), MPH (Biostatistics and Epidemiology (cum laude), D.Phil
Mr Y Sikweyiya	MPH (Umea University Umea, Sweden); Master Level Fellowship (Research Ethics) (Pretoria and UKZN); Post Grad. Diploma in Health Promotion (Unitra); BSc in Health Promotion (Unitra)
Dr L Schoeman	(Female) BPharm (NWU); BAHons (Psychology)(UP); PhD (UKZN); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) - MBChB; MMed (Int); MPharMed.
Prof T J P Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE
Prof C W van Staden	**Chairperson** - MBChB; MMed (Psych); MD; FCPsych; FTCL; UPLM; Dept of Psychiatry

Student Ethics Sub-Committee

Prof R S K Apatu	MBChB (Legon,UG); PhD (Cantab); PGDip International Research Ethics (UCT)
Mrs N Briers	(female) BSc (Stell); BSc Hons (Pretoria); MSc (Pretoria); DHETP (Pretoria)
Prof M M Ehlers	(female) BSc (Agric) Microbiology (Pret); BSc (Agric) Hons Microbiology (Pret); MSc (Agric) Microbiology (Pret); PhD Microbiology (Pret); Post Doctoral Fellow (Pret)
Dr R Leech	(female) B.Art et Scien; BA Cur; BA (Hons); M (ECI); PhD Nursing Science
Dr S A S Olorunju	BSc (Hons). Stats (Ahmadu Bello University –Nigeria); MSc (Applied Statistics (UKC United Kingdom); PhD (Ahmadu Bello University – Nigeria)
Dr L Schoeman	CHAIRPERSON: (female) BPharm (North West); BAHons (Psychology)(Pretoria); PhD (KwaZulu-Natal); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) MBChB; M.Med (Int); MPhar.Med
Prof L Sykes	(female) BSc, BDS, MDent (Pros)

....

DR L SCHOEMAN; BPharm, BA Hons (Psy), PhD;
Dip. International Research Ethics
CHAIRPERSON of the Faculty of Health Sciences
Student Research Ethics Committee, University of Pretoria

....

DR R SOMMERS; MBChB; M.Med (Int); MPhar.Med.
VICE-CHAIR of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

© University of Pretoria