

**GENOMIC MUTATIONS IN ORAL POLIOVIRUS VACCINE  
STRAINS: IMPLICATIONS FOR THE ERADICATION OF  
POLIOVIRUS**

**DOBROMIR NIKOLOV PAVLOV**

**GENOMIC MUTATIONS IN ORAL POLIOVIRUS VACCINE  
STRAINS: IMPLICATIONS FOR THE ERADICATION OF  
POLIOVIRUS**

by

**DOBROMIR NIKOLOV PAVLOV**

Submitted in partial fulfilment of the requirements for the degree

**PHILOSOPHIAE DOCTOR**

**PhD (Medical Virology)**

in the Faculty of Health Sciences

Department of Medical Virology

University of Pretoria

Pretoria

South Africa

October 2004

I, the undersigned, declare that the thesis hereby submitted to the University of Pretoria for the degree PhD (Medical Virology) and the work contained therein is my own original work and has not previously, in its entirety or in part, been submitted to any university for a degree.

Signed: \_\_\_\_\_ this \_\_\_\_\_ day of \_\_\_\_\_ 2004

*“In all human affairs... there is a single dominant factor- time. To make sense of the present state of science, we need to know how it got like that: we cannot avoid an historical account... To extrapolate into the future we must look backwards a little into the past.”*

J.M. Ziman

## ACKNOWLEDGEMENTS

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**I would like to sincerely thank:**

**Dr MM Ehlers, Department of Medical Virology, University of Pretoria,** who did not save any energy and time for the successful accomplishment of this project and thesis, for her professional knowledge, advice and assistance

**Prof WOK Grabow, Department of Medical Virology, University of Pretoria,** for his professional assistance, advice and effort to accomplish this project and thesis successfully

**Prof M Kruger, Department of Paediatrics, Kalafong Hospital/University of Pretoria,** for her academic support and assistance, for investigating the clinical status of the immunodeficient children taking part in this project

**Dr L Blignaut, Department of Paediatrics, Kalafong Hospital/University of Pretoria,** for collecting stool specimens from the immunodeficient children and investigating the clinical status of the children taking part in this study

**The colleagues at the Daspoort Sewage Treatment Plant and the East Rand Water Care Company,** for providing the sewage samples used in this project

**The HF Verwoerd Research Trust,** for the Prestigious Award for 2004

**My wife and daughter, Maria and Natalie Pavlovi,** for bringing all the joy, happiness and love in my life

**My parents,** for their loving care and belief in me

**My colleagues and friends,** for their support

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## TABLE OF CONTENTS

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### Acknowledgements

	<b>Page</b>
<b>SUMMARY</b>	1
<b>OPSOMMING</b>	3
<b>LIST OF ABBREVIATIONS</b>	6
<b>LIST OF TABLES</b>	9
<b>LIST OF FIGURES</b>	13
<b>LIST OF PUBLICATIONS AND CONFERENCE CONTRIBUTIONS</b>	18
<b>CHAPTER 1: INTRODUCTION</b>	19
<b>CHAPTER 2: LITERATURE REVIEW</b>	26
2.1 Introduction	26
2.2 History of poliomyelitis	28
2.3 Clinical manifestations of poliovirus infections	29
2.4 Genomic characterisation of poliovirus	31
2.5 Mode of transmission of poliovirus	33
2.6 Survival of poliovirus in nature	34
2.7 Poliovirus vaccines	36
2.7.1 Inactivated poliovirus vaccine	36
2.7.2 Oral poliovirus vaccine	37
2.8 Genetic basis for the attenuation of Sabin vaccine strains of live attenuated poliovirus	38
2.9 Complications resulting from the use of oral poliovirus vaccine	41
2.10 Molecular changes of poliovirus vaccine strains in vaccine recipients	42

2.10.1	Mutations in Sabin poliovirus vaccine strains	43
2.10.2	Recombination in Sabin poliovirus vaccine strains	45
2.11	Vaccine-associated paralytic poliomyelitis and immunodeficiency	46
2.12	Vaccine-derived polioviruses	48
2.12.1	Immunodeficient vaccine-derived polioviruses	48
2.12.2	Circulating vaccine-derived polioviruses	49
2.13	Environmental surveillance of poliovirus circulation	51
2.14	Isolation and identification of polioviruses	52
2.14.1	Recommended cell lines for the isolation of polioviruses	53
2.14.2	Serological diagnosis of poliovirus infection	53
2.14.3	Molecular techniques for the detection of polioviruses	54
2.14.3.1	Reverse transcription multiplex PCR	55
2.14.3.2	Sabin specific RT-triplex PCR	56
2.14.3.3	Restriction fragment length polymorphism	56
2.14.3.4	Nucleotide sequencing of the enteroviral genomes	57
2.14.4	Intratyptic differentiation methods recommended by the WHO	58
2.15	Eradication of poliomyelitis: Progress and Challenges	59
2.16	Summary	62
2.17	References	64
 <b>CHAPTER 3: POLIOVIRUS VACCINE STRAINS IN SEWAGE AND RIVER WATER IN SOUTH AFRICA</b>		 79
3.1	Abstract	79
3.2	Introduction	80
3.3	Materials and methods	83
3.3.1	Virus stock and cell cultures	83
3.3.2	Recovery of viruses from sewage and river water samples	84
3.3.3	Cell culture techniques for assaying plaque forming polioviruses	84
3.3.4	Extraction of the ribonucleic acid from viral isolates	85
3.3.5	Reverse transcription multiplex PCR to distinguish polioviruses from non-polio enteroviruses	86
3.3.6	Sabin specific RT-triplex PCR to distinguish between Sabin PV types 1 to 3	86
3.3.7	Restriction enzymes used in the typing of non-polio enteroviruses	87

3.3.8	Quality control of the amplification method	87
3.4	Results and discussion	87
3.5	Conclusions	91
3.6	References	91
 <b>CHAPTER 4: ISOLATION OF POLIOVIRUS VACCINE STRAINS FROM STOOL SPECIMENS OF IMMUNODEFICIENT CHILDREN IN SOUTH AFRICA</b>		 105
4.1	Abstract	105
4.2	Introduction	106
4.3	Materials and methods	109
4.3.1	Poliovirus stock	109
4.3.2	Sample size	109
4.3.3	Patient specimens	110
4.3.4	Extraction of the ribonucleic acid	110
4.3.5	Reverse transcription polymerase chain reaction	111
4.3.6	Nested polymerase chain reaction	111
4.3.7	Reverse transcription multiplex PCR to distinguish polioviruses from non-polio enteroviruses	112
4.3.8	Restriction enzyme analysis	112
4.3.9	Sabin specific RT-triplex PCR	113
4.3.10	Quality control of the amplification methods	113
4.4	Results and discussion	113
4.5	Conclusions	119
4.6	References	120
 <b>CHAPTER 5: PREVALENCE OF VACCINE-DERIVED POLIOVIRUSES IN SEWAGE AND RIVER WATER IN SOUTH AFRICA</b>		 133
5.1	Abstract	133
5.2	Introduction	134
5.3	Materials and methods	136
5.3.1	Virus stock and cell cultures	136
5.3.2	Isolation of polioviruses from sewage and river water	137
5.3.3	Ribonucleic acid extraction and typing of poliovirus isolates	137



5.3.4	Sabin specific RT-triplex PCR	138
5.3.5	Partial genomic sequencing of the 5' untranslated region of polioviruses	139
5.3.6	Partial genomic sequencing of the VP1 capsid protein of polioviruses	139
5.3.7	Nucleotide sequencing and phylogenetic analysis	140
5.3.8	Nucleotide sequence accession numbers	141
5.4	Results and discussion	141
5.5	Conclusions	145
5.6	References	145
 <b>CHAPTER 6: PREVALENCE OF VACCINE-DERIVED POLIOVIRUSES IN STOOLS OF IMMUNODEFICIENT CHILDREN IN SOUTH AFRICA</b>		 155
6.1	Abstract	155
6.2	Introduction	156
6.3	Materials and methods	159
6.3.1	Poliovirus stock	159
6.3.2	Patient specimens	159
6.3.3	Ribonucleic acid extraction and isolation of polioviruses	160
6.3.4	Typing of the poliovirus isolates	161
6.3.5	Partial genomic sequencing of the 5' untranslated region of polioviruses	161
6.3.6	Partial genomic sequencing of the VP1 capsid protein of polioviruses	162
6.3.7	Nucleotide sequencing and phylogenetic analysis	162
6.3.8	Nucleotide sequence accession numbers	163
6.4	Results and discussion	164
6.5	Conclusions	170
6.6	References	171
 <b>CHAPTER 7: GENERAL DISCUSSION</b>		 183
 <b>APPENDIX I: CULTURE MEDIA, REAGENTS AND MOLECULAR TECHNIQUES</b>		 198

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**SUMMARY**

Large epidemics of poliomyelitis spread across the world in the first half of the 20<sup>th</sup> century. However, polio incidence fell rapidly across the world following the introduction of the oral poliovirus vaccine (OPV). Since the introduction of immunisation with OPV, the vaccine had a remarkable track record of success, because the number of wild-type polio cases decreased from 350 000 to 500 and the number of polio endemic countries declined from 125 to 10. Thus, the global eradication of wild-type poliovirus (PV) seems a realistic goal for the foreseeable future.

Despite its many advantages, one disadvantage of the OPV is the potential risk of revertants of the OPV strains, which may cause neurological complications in vaccine recipients and susceptible contacts. Immunocompetent persons excrete OPV strains for a limited period of time. In contrast, immunodeficient people may become chronically infected and excretion times as long as 10 years have been reported. As a consequence, in the last phase of polio eradication this group of people may serve as potential reservoirs for vaccine-derived polioviruses (VDPVs). Two cases of vaccine-associated paralytic poliomyelitis have been reported in human immunodeficiency virus (HIV)-positive children, although, presently

there is no evidence for prolonged excretion of PV from patients with HIV and acquired immunodeficiency syndrome (AIDS). Highly evolved VDPVs have been isolated from sewage and river water even in the absence of cases of paralytic poliomyelitis.

This study aimed to investigate the prevalence of PVs in sewage and river water as well as in stool specimens of HIV-positive children (including those with an AIDS indicator condition according to the Centers for Disease Control and Prevention classification). Secondly, the study investigated the occurrence of genomic mutations in these OPV isolates.

A total of 49 PV vaccine strains were isolated from the sewage and river water, and 13 PV vaccine strains were detected in the stools of immunodeficient children. Two of the immunodeficient patients (vaccinated 15 months ago) tested positive for Sabin PVs type 1 and 3. Another immunodeficient patient (vaccinated 42 months ago) tested positive for Sabin PV type 1.

The 5'untranslated and the VP1 regions in the genomes of the OPV isolates were partially sequenced. The majority of the OPV strains detected in the sewage and river water displayed >99% VP1 sequence identity to the original PV vaccine strains and were classified as "OPV-like viruses". Two OPV isolates were identified as "suspected" VDPVs, since these isolates showed  $\leq$ 99% VP1 sequence identity to the PV vaccine strains and had probably replicated in one or more people for 12 to 16 months since the administration of the initiating OPV dose. In contrast, three "suspected" immunodeficient VDPVs were identified in the stools of the immunodeficient children. All of the OPV-like and "suspected" VDPV isolates carried genomic mutations, which had been associated with reversion of the attenuated PV phenotypes to increased neurovirulence.

The identification of OPV-like and "suspected" VDPVs in this study emphasised the fundamental importance regarding the control of health risks constituted by OPV vaccination, particularly with regard to immunodeficient individuals such as HIV-positive children, and the possible role of water in the transmission of potentially hazardous VDPVs. These research findings provided valuable data, concerning prolonged excretion of OPV strains by individuals with secondary immunodeficiency and this could have major implications for strategies aimed for the global post-polio eradication era.

**GENOMIESE MUTASIES IN ORALE POLIOVIRUS VAKSIEN  
STAMME: IMPLIKASIES VIR DIE UITWISSING VAN DIE  
POLIOVIRUS**

deur

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**GRAAD:** PhD (Geneeskundige Virologie)

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**OPSOMMING**

Groot epidemies van poliomiëlitis het wêreldwyd gedurende die eerste helfte van die 20<sup>ste</sup> eeu voorgekom. Die voorkoms van polio het skerp afgeneem met die ingebruikneming van die orale poliovaksien (OPV). Sedert die begin van immunisering met OPV, het die vaksien merkwaardige sukses getoon, aangesien die aantal wilde-tipe poliogevallen afgeneem het vanaf 350 000 tot 500 terwyl die aantal lande waar polio endemies voorgekom het, vanaf 125 tot 10 gedaal het. Gevolglik blyk die toekomstige wêreldwye uitwissing van wilde-tipe poliovirus (PV) 'n realistiese doelwit te wees.

Ten spyte van die vele voordele van OPV, blyk die potensiële risiko van terugmutering van die OPV stamme 'n belangrike nadeel te wees, aangesien dit tot neurologiese komplikasies in gevaksinierdes en vatbare kontakte kan lei. Immuunkompetente persone skei OPV stamme vir 'n beperkte tydperk uit. In teenstelling hiermee kan immuunonderdrukte persone kronies geïnfekteer word en gevallen waar OPV vir so lank as 10 jaar uitgeskei is al gerapporteer. As gevolg hiervan kan hierdie groep persone as 'n potensiële reservoir van vaksien-afkomstige poliovirusse (VAPV) optree gedurende die laaste fase van polio-uitwissing. Twee gevallen van vaksien-geassosieerde paralitiese poliomiëlitis is al gerapporteer in menslike immuniteitsgebrek virus (MIV)-positiewe kinders, nogtans is daar

tans geen bewyse van verlengde uitskeiding van PV in pasiënte met MIV en verworwe immuuniteitsgebrek sindroom (VIGS) nie. Hoogs gemuteerde VAPV is al geïsoleer vanuit riool- en rivierwater selfs in die afwesigheid van gevalle van paralitiese poliomiëlitis.

Hierdie studie het gepoog om die voorkoms van poliovirusse in riool- en rivierwater asook in stoelgange van MIV-positiewe kinders (ingesluit die met 'n VIGS indikator kondisie volgens die "Centers for Disease Control and Prevention" klassifikasie) te ondersoek. Tweedens is die voorkoms van genomiese mutasies in hierdie OPV isolate ondersoek.

'n Totaal van 49 PV vaksienstamme is geïsoleer vanuit riool- en rivierwater, en 13 PV vaksienstamme is opgespoor in die stoelgange van immuunonderdrukte kinders. Twee van die immuunonderdrukte pasiënte (15 maande tevore gevaksineer) het positief vir Sabin PV tipe 1 en 3 getoets. 'n Volgende immuunonderdrukte pasiënt (42 maande tevore gevaksineer) het positief vir Sabin PV tipe 1 getoets.

Die nukleotied volgorde van die 5' ongetransleerde en die VP1 gebiede in die genome van die OPV isolate is gedeeltelik bepaal. Die meeste van die OPV stamme wat in die riool- en rivierwater gevind is, het >99% ooreenstemmig getoon tussen die VP1 nukleotied volgordes en die van die oorspronklike PV vaksienstamme en is daarvolgens as "OPV-soortgelyke virusse" geklassifiseer. Twee OPV isolate is geïdentifiseer as "verdagte" VAPV, aangesien hierdie isolate  $\leq 99\%$  ooreenstemmigheid getoon tussen hulle VP1 nukleotied volgordes en die volgordes van die oorspronklike PV vaksienstamme wat aantoon dat hierdie isolate moontlik in een of meer persone vir 12 tot 16 maande vanaf die aanvanklike OPV toediening gerepliseer het. Daarteenoor, is drie "verdagte" immuunonderdrukte VAPV vanuit die stoelgange van immuunonderdrukte kinders geïdentifiseer. Al die OPV-soortgelyke en "verdagte" VAPV isolate het genomiese mutasies bevat wat geassosieer word met terugmutering van die verswakte PV fenotipes wat kan lei tot toenemende neurovirulensie.

Die identifisering van OPV-soortgelyke en "verdagte" VAPV in hierdie studie het die fundamentele belangrikheid van die kontroliering van gesondheidsrisikos wat gepaard gaan met OPV vaksinerings beklemtoon, veral in die geval waar immuunonderdrukte individue soos MIV-positiewe kinders betrokke is, asook die moontlike rol wat water in die oordrag van potensieel gevaarlike VAPV kan speel. Hierdie navorsingsbevindings het waardevolle inligting gelewer, met betrekking tot die verlengde uitskeiding van OPV stamme deur

individue met sekondêre imuunonderdrukking. Dit kan groot implikasies inhou vir strategieë gemik op die wêreldwye post-polio uitwissingsera.

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## LIST OF ABBREVIATIONS

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<b>AFP</b>	Acute flaccid paralysis
<b>AIDS</b>	Acquired immunodeficiency syndrome
<b>AMPS</b>	Ammonium per sulphate
<b>ATCC</b>	American Type Culture Collection
<b>BCG</b>	Bacille Calmette-Guérin vaccine
<b>BGM</b>	Buffalo green monkey kidney
<b>bp</b>	Base pair
<b>C</b>	Concentration
<b>CAV</b>	Coxsackievirus A
<b>CBV</b>	Coxsackievirus B
<b>CDC</b>	Centers for Disease Control and Prevention
<b>cm</b>	Centimetre
<b>CNS</b>	Central nervous system
<b>CO<sub>2</sub></b>	Carbon dioxide
<b>CPE</b>	Cytopathogenic effect
<b>CSF</b>	Cerebrospinal fluid
<b>cVDPV</b>	Circulating vaccine-derived polioviruses
<b>CVID</b>	Common variable immunodeficiency
<b>DNA</b>	Deoxyribonucleic acid
<b>dNTP</b>	DiNucleotide triphosphate
<b>DT</b>	Diphtheria, tetanus vaccine
<b>DTP</b>	Diphtheria, tetanus, pertussis vaccine
<b>ECACC</b>	European Collection of Cell Culture
<b>ECV</b>	Echovirus
<b>EDTA</b>	Ethylenediaminetetraacetate
<b>ELISA</b>	Enzyme linked immunosorbent assay
<b>EMBL</b>	European Bioinformatics Institute
<b>EV</b>	Enteroviruses
<b>FCS</b>	Foetal calf serum
<b>Fig</b>	Figure
<b>g</b>	Gram
<b>g</b>	Gravitational force
<b>h</b>	Hour
<b>HCl</b>	Hydrochloric acid
<b>HEp-2</b>	Human epidermoid carcinoma
<b>HIV</b>	Human immunodeficiency virus
<b>H<sub>2</sub>O</b>	Water

<b>Ile</b>	Isoleucine
<b>ITD</b>	Intratypic differentiation
<b>IPV</b>	Inactivated poliovirus vaccine
<b>iVDPV</b>	Immunodeficient vaccine-derived polioviruses
<b>kb</b>	Kilobytes
<b>KCl</b>	Potassium chloride
<b>L</b>	Litre
<b>L20B</b>	Mouse L cells
<b>M</b>	Molar
<b>mA</b>	Milliampere
<b>MEM</b>	Minimum Essential Medium
<b>µg</b>	Microgram
<b>MgCl<sub>2</sub></b>	Magnesium chloride
<b>MgSO<sub>4</sub></b>	Magnesium sulphate
<b>µl</b>	Microlitre
<b>ml</b>	Millilitre
<b>min</b>	Minute
<b>mm</b>	Millimetre
<b>mM</b>	Millimolar
<b>NaCl</b>	Sodium chloride
<b>ng</b>	Nanogram
<b>NIDs</b>	National immunisation days
<b>NIV</b>	National Institute for Virology
<b>nm</b>	Nanometre
<b>NPEVs</b>	Non-polio enteroviruses
<b>OPV</b>	Oral poliovirus vaccine
<b>ORF</b>	Open reading frame
<b>PBS</b>	Phosphate-buffered saline
<b>PCR</b>	Polymerase chain reaction
<b>PEG</b>	Polyethylene glycol
<b>PEI</b>	Poliomyelitis Eradication Initiative
<b>Pen/strep</b>	Penicillin/streptomycin
<b>PFU</b>	Plaque forming units
<b>Phe</b>	Phenylalanine
<b>PLC/PRF/5</b>	Primary liver carcinoma
<b>pmol</b>	Picomole
<b>ppm</b>	Parts per million
<b>PV</b>	Poliovirus
<b>PVR</b>	Poliovirus receptor
<b>RD</b>	Rhabdomyosarcoma
<b>RE</b>	Restriction enzyme



<b>RFLP</b>	Restriction Fragment Length Polymorphism
<b>RNA</b>	Ribonucleic acid
<b>rpm</b>	Revolutions per minute
<b>RT-PCR</b>	Reverse transcription polymerase chain reaction
<b>s</b>	Second
<b>Ser</b>	Serine
<b>Thr</b>	Threonine
<b>U</b>	Unified atomic mass unit
<b>USA</b>	United States of America
<b>UTR</b>	Untranslated region
<b>UV</b>	Ultraviolet light
<b>V</b>	Volume
<b>Val</b>	Valine
<b>VAPP</b>	Vaccine-associated paralytic poliomyelitis
<b>VDPV</b>	Vaccine-derived poliovirus
<b>VP</b>	Virus protein
<b>V/W</b>	Vaccine/wild
<b>WHO</b>	World Health Organization

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## LIST OF TABLES

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	<b>Page</b>
Table 2.1: Enterovirus and poliovirus specific primers <sup>a</sup> (Egger <i>et al.</i> , 1995)	55
Table 2.2: Sequences of the oligonucleotides used for the detection of Sabin poliovirus types 1, 2 and 3 (Yang <i>et al.</i> , 1991; Yang <i>et al.</i> , 1992)	56
Table 2.3: Restriction enzymes for the genotyping of enteroviruses (Kuan, 1997)	57
Table 2.4: South African immunisation schedule (Department of Health, 1995)	61
Table 3.1: Sewage samples used in the isolation of polioviruses from selected water treatment plants in South Africa	98
Table 3.2: Enterovirus and poliovirus specific primers <sup>a</sup> used in the RT-multiplex PCR (Egger <i>et al.</i> , 1995)	98
Table 3.3: Sabin specific RT-PCR primers used in the detection and differentiation of Sabin PV types 1, 2 and 3 (Yang <i>et al.</i> , 1991; Yang <i>et al.</i> , 1992)	99
Table 3.4: Restriction enzymes (REs) used for the genotyping of enteroviruses (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	99
Table 3.5: Fragments resulting from digestion by <i>Sty</i> I, <i>Bgl</i> I and <i>Xmn</i> I REs of 297 bp amplified EVs (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	99
Table 3.6: Detection of poliovirus vaccine strains in sewage and river water samples in South Africa between 2001 and 2003	100

Table 3.6:	Detection of poliovirus vaccine strains in sewage and river water samples in South Africa between 2001 and 2003 (continued)	101
Table 4.1:	Sample sizes for different levels of confidence and accuracy of estimation for the prevalence of OPV strains in stool specimens of immunodeficient children (Biostatistics Unit, Medical Research Council, South Africa)	126
Table 4.2:	South African childhood immunisation schedule (Department of Health, 1995)	126
Table 4.3:	Primers used in the detection of enteroviruses in stool specimens using RT-PCR and nested PCR methods (Gow <i>et al.</i> , 1991; Kuan, 1997)	126
Table 4.4:	Enterovirus and poliovirus specific primers <sup>a</sup> used in the RT-multiplex PCR (Egger <i>et al.</i> , 1995)	127
Table 4.5:	Restriction enzymes (REs) used for the genotyping of enteroviruses (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	127
Table 4.6:	Fragments resulting from digestion by <i>Sty</i> I, <i>Bgl</i> I and <i>Xmn</i> I REs of 297 bp amplified enteroviruses (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	128
Table 4.7:	Sabin specific RT-PCR primers used in the detection and differentiation of Sabin PV types 1, 2 and 3 (Yang <i>et al.</i> , 1991; Yang <i>et al.</i> , 1992)	128
Table 4.8:	Poliovirus vaccine strains isolated from stool specimens of immunodeficient children from a selected area in South Africa	129

Table 4.9:	Isolation of poliovirus vaccine strains from stool specimens of immunocompetent children (the control group)	130
Table 5.1:	Types of sewage samples used in the isolation of polioviruses from selected water treatment plants in South Africa	150
Table 5.2:	Enterovirus and poliovirus specific primers <sup>a</sup> used in the RT-multiplex PCR (Egger <i>et al.</i> , 1995)	150
Table 5.3:	Sabin specific RT-PCR primers used in the detection and differentiation of Sabin PV types 1, 2 and 3 (Yang <i>et al.</i> , 1991; Yang <i>et al.</i> , 1992)	151
Table 5.4:	Primers used in the RT-PCRs for the amplification of the 5'UTR and VP1 region of the poliovirus genome (Divizia <i>et al.</i> , 1999; Guillot <i>et al.</i> , 2000)	151
Table 5.5:	Extent of nucleotide divergence between the characterised 5'UTR and VP1 regions of polioviruses isolated in this study from their attenuated parental Sabin poliovirus vaccine strains	152
Table 6.1:	Primers used in the detection of enteroviruses in stool specimens using RT-PCR and nested PCR methods (Gow <i>et al.</i> , 1991; Kuan, 1997)	177
Table 6.2:	Restriction enzymes (REs) used for the genotyping of enteroviruses (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	177
Table 6.3:	Fragments resulting from digestion by <i>Sty</i> I, <i>Bgl</i> I and <i>Xmn</i> I REs of 297 bp amplified enteroviruses (Kämmerer <i>et al.</i> , 1994; Kuan, 1997)	177

Table 6.4:	Sabin specific RT-PCR primers used in the detection and differentiation of Sabin PV types 1, 2 and 3 (Yang <i>et al.</i> , 1991; Yang <i>et al.</i> , 1992)	178
Table 6.5:	Primers used in the RT-PCRs for the amplification of the 5'UTR and VP1 region of the poliovirus genome (Divizia <i>et al.</i> , 1999; Guillot <i>et al.</i> , 2000)	178
Table 6.6:	Extent of nucleotide divergence between the characterised 5'UTR and VP1 regions of polioviruses isolated in this study from their attenuated parental Sabin poliovirus vaccine strains	179
Table 6.7:	Poliovirus vaccine strains isolated from stool specimens of immunodeficient children from a selected area in South Africa	180

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## LIST OF FIGURES

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	<b>Page</b>
Figure 2.1: (A) Schematic representation of the icosahedral viral capsid structure of polioviruses. The fivefold (5x) and three fold (3x) axes of symmetry are indicated, as is the position of one of the 60 repeating protomeric units, each comprised of VP1, VP2 and VP3 surface proteins. (B) Line drawing of the VP1 and VP2 proteins in their tertiary configuration (Rotbart, 1997)	31
Figure 2.2: Genomic organisation of poliovirus type 1 (Mahoney). The polyprotein encoded by the single open reading frame is shown as an elongated rectangle, the 5' and 3' untranslated regions are shown as lines and the genome-linked protein (VPg) is indicated by a black arrow. Cleavage sites between individual viral proteins are shown above the genome at appropriate locations; these proteins are described within the rectangle according to the L434 nomenclature (Rueckert and Wimmer, 1984); the capsid proteins 1AB, 1A, 1B, 1C and 1D are commonly referred to as VP0, VP4, VP2, VP3 and VP1, respectively. The proteinases 2A <sup>pro</sup> , 3C <sup>pro</sup> and 3CD <sup>pro</sup> are represented by shaded boxes. The structural protein precursor P1 and the non-structural protein precursors P2 and P3 are indicated above the polyprotein (Muir <i>et al.</i> , 1998)	32

- Figure 2.3: Comparisons of the sequences of polioviruses used to study the basis of attenuation and reversion of the Sabin vaccine strains of poliovirus. (A) Mutations that are involved in deriving the Sabin type 3 vaccine strain from the Leon strain. (B) Attenuating mutations in the Sabin PV type 2. (C) Mutations that are involved in deriving the Sabin PV type 1 strain from the Mahoney strain. The most common base changes and the amino acid differences produced in the encoded protein are shown (Minor, 1999) 40
- Figure 3.1: Plaques formed on HEp-2 cell monolayers by poliovirus isolates 102
- Figure 3.2: Band patterns observed with the RT-multiplex PCR of the Sabin PV types 1 to 3 and the non-polio enteroviruses. Lane 1: 100 bp Marker; Lane 2: CBV1 (297 bp); Lane 3: CBV2 (297 bp); Lane 4: CBV3 (297 bp); Lane 5: CBV4 (297 bp); Lane 6: CBV5 (193 bp and 297 bp); Lane 7: CBV6 (297 bp); Lane 8: CAV9 (297 bp); Lane 9: CAV19 (193 bp and 297 bp); Lane 10: ECV1 (297 bp); Lane 11: Sabin PV type 1 (193 bp, 297 bp, 565 bp and 1 000 bp); Lane 12: Sabin PV type 2 (193 bp, 297 bp, 565 bp and 1 000 bp); Lane 13: Sabin PV type 3 (193 bp and 297bp); Lane 14: 100 bp Marker. In the RT-multiplex PCR, the Sabin PV type 3 strain did not show the Po3-Po4 band (565 bp) 102
- Figure 3.3: Sabin specific RT-triplex PCR of the PV isolates and the positive controls. Lane 1: Marker 100 bp; Lane 2: negative sample; Lane 3: Sabin PV type 1 (positive isolate 97 bp); Lanes 4-8: Negative isolates; Lane 9: Sabin PV type 1 (positive control 97 bp); Lane 10: Sabin PV type 2 (positive control 71 bp); Lane 11: Sabin PV type 3 (positive control 54bp); Lane 12: Marker 100 bp 103

- Figure 3.4: Restriction enzyme digestion of 297 bp products from prototype strains of enteroviruses with three restriction enzymes (*Sty* I, *Bgl* I and *Xmn* I). Lane 1: Marker 100 bp; Lane 2: *Sty* I (226 bp and 71 bp), Lane 3: *Bgl* I (297 bp), Lane 4: *Xmn* I (297bp) - CBV3; Lane 5: PGem Marker; Lane 6: *Sty* I (197 bp and 100 bp), Lane 7: *Bgl* I (297 bp), Lane 8: *Xmn* I (297 bp) - Sabin PV type 2; Lane 9: Marker V; Lane 10: *Sty* I (226 bp and 71 bp), Lane 11: *Bgl* I (217 bp and 80 bp), Lane 12: *Xmn* I (297 bp) - ECV19; Lane 13-18: empty; Lane 19: EV uncut (297 bp); Lane 20: Marker 100 bp 103
- Figure 3.5: Detection of enteroviruses in sewage and river water samples collected from selected areas in South Africa from 2001 to 2003 104
- Figure 4.1: Band patterns observed with the nested PCR in the detection of enteroviruses (EVs) in selected stool samples. Lane 1: Marker 100 bp; Lane 2: EV (297 bp); Lane 3: Negative; Lane 4: EV (297 bp); Lane 5: EV (297 bp); Lane 6: Negative; Lane 7: EV (297 bp); Lanes 8-9: Negative; Lane 10: EV (297 bp); Lane 11: Positive control (297 bp); Lane 12: Marker 100 bp 131
- Figure 4.2: Restriction enzyme digestion of 297 bp products from prototype strains of enteroviruses (EVs) with three restriction enzymes (*Sty* I, *Bgl* I and *Xmn* I). Lane 1: Marker 100 bp; Lane 2: *Sty* I (297 bp), Lane 3: *Bgl* I (297 bp), Lane 4: *Xmn* I (297bp) - PV type 3; Lane 5: *Sty* I (297 bp); Lane 6: *Bgl* I (297 bp), Lane 7: *Xmn* I (297 bp) – PV type 3; Lane 8: PGem marker; Lane 9: *Sty* I (297 bp), Lane 10: *Bgl* I (297 bp), Lane 11: *Xmn* I (297 bp) – PV type 3; Lane 12: *Sty* I (197 bp + 100 bp), Lane 13: *Bgl* I (196 bp + 80 bp + 21 bp), Lane 14: *Xmn* I (297 bp) – CAV17; Lane 15: Marker V; Lane 16: *Sty* I (197 bp + 100 bp), Lane 17: *Bgl* I (297 bp), Lane 18: *Xmn* I (297 bp) – PV type 2; Lane 19: uncut 297 bp product; Lane 20: Marker 100 bp 131



- Figure 4.3: Sabin RT-triplex PCR of the PV isolates and the positive controls. Lane 1: Marker 100 bp; Lane 2: Sabin PV type 2 (positive isolate 71 bp); Lanes 3-4: Negative isolates; Lane 5: PGem Marker; Lane 6: Sabin PV type 1 (97 bp); Lane 7: Sabin PV type 2 (71 bp); Lane 8: Sabin PV type 3 (54bp); Lane 9: Negative control; Lane 10: Marker V; Lane 11: Marker 100 bp 132
- Figure 4.4: Detection of non-polio enteroviruses in stool specimens of immunodeficient children from a selected area in South Africa during a period of one year 132
- Figure 5.1: Unrooted phylogenetic tree re-constructed with the neighbour-joining method from the comparative 5' untranslated region sequence analysis of the sewage isolated oral poliovirus vaccine strains and the poliovirus reference strains. Branch lengths are proportional to the phylogenetic distances, while the vertical branches are non-informative. The scale bar shows 2% nucleotide sequence difference 153
- Figure 5.2: Unrooted phylogenetic tree re-constructed with the neighbour-joining method from the comparative VP1 region sequence analysis of the sewage isolated oral poliovirus vaccine strains and the poliovirus reference strains. Branch lengths are proportional to the phylogenetic distances, while the vertical branches are non-informative. The scale bar shows 10% nucleotide sequence difference 154
- Figure 6.1: Unrooted phylogenetic tree re-constructed with the neighbour-joining method from the comparative 5' untranslated region sequence analysis of the isolated oral poliovirus vaccine strains from immunodeficient children and the poliovirus reference strains. Branch lengths are proportional to the phylogenetic distances, while the vertical branches are non-informative. The scale bar shows 2% nucleotide sequence difference 181

Figure 6.2: Unrooted phylogenetic tree re-constructed with the neighbour-joining method from the comparative VP1 region sequence analysis of the isolated oral poliovirus vaccine strains from immunodeficient children and the poliovirus reference strains. Branch lengths are proportional to the phylogenetic distances, while the vertical branches are non-informative. The scale bar shows 5% nucleotide sequence difference

182

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## LIST OF PUBLICATIONS AND CONFERENCE CONTRIBUTIONS

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### SUBMITTED PUBLICATIONS:

Ehlers, M.M., Grabow, W.O.K., Pavlov, D.N. (2004a) Detection of enteroviruses in untreated and treated drinking water supplies in South Africa. Submitted for publication in *Water Research*.

Pavlov, D.N., Van Zyl, W.B., Grabow, W.O.K., Ehlers, M.M (2004b) Poliovirus vaccine strains in sewage and river water in South Africa. Submitted for publication in *Water Research*.

Pavlov, D.N., Van Zyl, W.B., Kruger, M., Blignaut, L., Grabow, W.O.K., Ehlers, M.M (2004) Isolation of poliovirus vaccine strains from stool specimens of immunodeficient children in South Africa. To be submitted for publication in the *Journal of Clinical Virology*.

Pavlov, D.N., Van Zyl, W.B., Van Heerden, J., Grabow, W.O.K., Ehlers, M.M (2004) Prevalence of vaccine-derived polioviruses in sewage and river water in South Africa. To be submitted for publication in *Water Research*.

Pavlov, D.N., Van Zyl, W.B., Van Heerden, J., Kruger, M., Blignaut, L., Grabow, W.O.K., Ehlers, M.M (2004) Prevalence of vaccine-derived polioviruses in stools of immunodeficient children in South Africa. To be submitted for publication in the *Journal of Clinical Virology*.

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Pavlov, D.N., Grabow, W.O.K., Ehlers, M.M. (2002) Prevalence of enteroviruses in treated and untreated drinking water supplies. Oral presentation on Faculty Day of the Faculty of Health Sciences, University of Pretoria, 21 August 2002.

Ehlers, M.M., Grabow, W.O.K., Pavlov, D.N. (2003) Detection of enteroviruses in raw and treated drinking water supplies in South Africa. Poster presentation (CT-54) at the IWA Health Related Microbiology, 14 -19 September 2003, Cape Town, South Africa.

Pavlov, D.N., Van Zyl, W.B., Grabow, W.O.K., Ehlers, M.M (2003) Poliovirus vaccine strains in sewage and river water in South Africa. Oral (CT-36) and poster presentation (CT-61) at the IWA Health Related Microbiology, 14 - 19 September 2003, Cape Town, South Africa.

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