# 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

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**PhD (Medical Virology)**

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Department of Medical Virology

University of Pretoria

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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 entirely 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

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## SUMMARY

Large epidemics of poliomyelitis spread across the world in the first half of the 20$^{th}$ 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 ≤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

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## OPSOMMING

Groot epidemies van poliomiëlitis het wêreldwyd gedurende die eerste helfte van die 20ste 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 poliogevalle 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 gevaksineerdes 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 gevalle waar OPV vir so lank as 10 jaar uitgeskei is 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 gevalle 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 immuniteitsgebrek 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 imuunonderdrukte kinders. Twee van die imuunonderdrukte pasïente (15 maande tevore gevaksineer) het positief vir Sabin PV tipe 1 en 3 getoets. 'n Volgende imuunonderdrukte pasïent (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 ≤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 “vedagte” imuunonderdrukte VAPV vanuit die stoelgange van imuunonderdrukte 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 kontrolering van gesondheidsrisikos wat gepaard gaan met OPV vaksinering beklemtoon, veral in die geval waar imuunonderdrukte 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

| | |
|---|---|
| **AFP** | Acute flaccid paralysis |
| **AIDS** | Acquired immunodeficiency syndrome |
| **AMPS** | Ammonium per sulphate |
| **ATCC** | American Type Culture Collection |
| **BCG** | Bacille Calmette-Guérin vaccine |
| **BGM** | Buffalo green monkey kidney |
| **bp** | Base pair |
| **C** | Concentration |
| **CAV** | Coxsackievirus A |
| **CBV** | Coxsackievirus B |
| **CDC** | Centers for Disease Control and Prevention |
| **cm** | Centimetre |
| **CNS** | Central nervous system |
| **$CO_2$** | Carbon dioxide |
| **CPE** | Cytopathogenic effect |
| **CSF** | Cerebrospinal fluid |
| **cVDPV** | Circulating vaccine-derived polioviruses |
| **CVID** | Common variable immunodeficiency |
| **DNA** | Deoxyribonucleic acid |
| **dNTP** | DiNucleotide triphosphate |
| **DT** | Diphtheria, tetanus vaccine |
| **DTP** | Diphtheria, tetanus, pertussis vaccine |
| **ECACC** | European Collection of Cell Culture |
| **ECV** | Echovirus |
| **EDTA** | Ethylenediaminetetraacetate |
| **ELISA** | Enzyme linked immunosorbent assay |
| **EMBL** | European Bioinformatics Institute |
| **EV** | Enteroviruses |
| **FCS** | Foetal calf serum |
| **Fig** | Figure |
| **g** | Gram |
| ***g*** | Gravitational force |
| **h** | Hour |
| **HCl** | Hydrochloric acid |
| **HEp-2** | Human epidermoid carcinoma |
| **HIV** | Human immunodeficiency virus |
| **$H_2O$** | Water |

| | |
|---|---|
| **Ile** | Isoleucine |
| **ITD** | Intratypic differentiation |
| **IPV** | Inactivated poliovirus vaccine |
| **iVDPV** | Immunodeficient vaccine-derived polioviruses |
| **kb** | Kilobytes |
| **KCl** | Potassium chloride |
| **L** | Litre |
| **L20B** | Mouse L cells |
| **M** | Molar |
| **mA** | Milliampere |
| **MEM** | Minimum Essential Medium |
| **µg** | Microgram |
| **$MgCl_2$** | Magnesium chloride |
| **$MgSO_4$** | Magnesium sulphate |
| **µl** | Microlitre |
| **ml** | Millilitre |
| **min** | Minute |
| **mm** | Millimetre |
| **mM** | Millimolar |
| **NaCl** | Sodium chloride |
| **ng** | Nanogram |
| **NIDs** | National immunisation days |
| **NIV** | National Institute for Virology |
| **nm** | Nanometre |
| **NPEVs** | Non-polio enteroviruses |
| **OPV** | Oral poliovirus vaccine |
| **ORF** | Open reading frame |
| **PBS** | Phosphate-buffered saline |
| **PCR** | Polymerase chain reaction |
| **PEG** | Polyethylene glycol |
| **PEI** | Poliomyelitis Eradication Initiative |
| **Pen/strep** | Penicillin/streptomycin |
| **PFU** | Plaque forming units |
| **Phe** | Phenylalanine |
| **PLC/PRF/5** | Primary liver carcinoma |
| **pmol** | Picomole |
| **ppm** | Parts per million |
| **PV** | Poliovirus |
| **PVR** | Poliovirus receptor |
| **RD** | Rhabdomyosarcoma |
| **RE** | Restriction enzyme |

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

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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*.

**CONFERENCE CONTRIBUTIONS:**

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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