



**MOLECULAR  
CHARACTERISATION OF WILD  
AND SABIN-LIKE POLIOVIRUSES  
CIRCULATING IN AFRICA AFTER  
2000**

By

Heronyma Nelisiwe “Nicksy” Gumede-Moeletsi

A Thesis Submitted To The Faculty Of Health Sciences

At University Of Pretoria, Pretoria,

In Fulfilment Of The Requirements For The

Degree of Doctor of Philosophy

Supervisor

Prof M Venter

2012



## DECLARATION

I declare that this thesis is my own, unaided work. It is submitted for the degree of Doctor of Philosophy at the University of Pretoria, Pretoria. It has not been submitted before for any degree or examination at this or any other university.

.....

Heronyma N Gumede-Moeletsi

On this..... day of..... 2012



---

## **To my family**

My husband Joe and my sons Lesego and Thato with sincere thanks  
for their love, encouragement and understanding  
during the writing of this thesis

“Nothing is too high for a man to reach, but he must climb with care and confidence”.

Hans Christian Andersen

---

## PUBLICATIONS

**Nicksy Gumede**, Vongani Muthambi and Barry D Schoub. Type 3 Immunodeficiency-associated vaccine-derived polioviruses (iVDPV) in chronically infected agammaglobulinemic infant, South Africa, 2011. (Submitted: MS#EID-12-0037).

**Nicksy Gumede**, Marietjie Venter, Jagadish Deshpande, Cara Burns, Mark Pallansch, Riziki Yogolelo, Jean Jacques Muyembe-Tamfum and Barry D Schoub. Identification and molecular characterisation of an Indian genotype of poliovirus type 1 isolated during outbreaks of poliomyelitis in the DRC from 2006 - 2008. (in preparation).

**Nicksy Gumede**, Olivia Skweit, Jagadish Deshpande, Cara Burns, Mark Pallansch, Riziki Yogolelo, Jean Jacques Muyembe-Tamfum, Esther de Gourville, Adrian Puren, Barry Schoub and Marietjie Venter. Vaccine Derived Polioviruses (VDPV) threatening the eradication of polio in the Democratic Republic of Congo (DRC). (in preparation).

**Nicksy Gumede**, Cara Burns, Mark Pallansch, Berhane Beyene, Barry Schoub and Marietjie Venter. Identification of type 2 and type 3 circulating vaccine derived polioviruses isolated during an outbreak of Poliomyelitis in Ethiopia in 2008-2010. (in preparation).

Rakoto-Andrianarivelo M, **Gumede N**, Jegouic S, Balanant J, Andriamamonjy SN, Rabemanantsoa S, Birmingham M, Randriamanalina B, Nkolomoni L, Venter M, Schoub BD, Delpeyroux F, Reynes JM. Re-emergence of Recombinant Vaccine-Derived Poliovirus Outbreak in Madagascar. *Journal of Infectious Diseases* 2008:197 (15 May).

---

Gouandjika-Vasilache I, Burns CC, **Gumede N**, Guillot S, Ménard D, Dosseh A, Akoua-Koffi C, Pallansch MA, Kew OM, Delpeyroux F. Molecular epidemiology of wild poliovirus type 1 circulating in West and Central Africa from 1997-1999, using genotyping with a restriction fragment length polymorphism assay. Archives of virology 2008; 153(3):409-16. 2006.

Adu F, Iber J, Bukbuk D, **Gumede N**, Yang SJ, Jorba J, Campagnoli R, Sule WF, Yang CF, Burns C, Pallansch M, Harry T, Kew O. Isolation of recombinant type 2 vaccine-derived poliovirus cVDPV from a Nigerian child. Virus Research, 2007; 127(1): 17 - 25.

NP Sithebe, **HN Gumede-Moeletsi**, NM Bida, MJ Mphahlele, N Ngcobo, NE Khomo and BD Schoub. Laboratory Containment of Polioviruses in South Africa. The Southern African Journal of Epidemiology and Infection 2006;21(1):3-4

B. D. Schoub, **H. N. Gumede**, G. Besselaar, N. K. Blackburn, E. M. Webb, O. Tomori, M. Otten. (CDC 2001) Progress towards polio eradication: An African Perspective. Dev. Biol. 105: 9-19.

---

## PRESENTATIONS

### (Oral)

- HN Gumede and M Rakoto-andrianarivelo. "Outbreak in Ethiopia, Somalia, Mozambique and detection in Chad, Niger and Nigeria". Informal consultation on Global polio network, 20-24 September 2011, Geneva, Switzerland.
- HN Gumede. "Evaluation of FTA card for referral specimen for Mycoplasma testing or ITD and sequencing: Experience in South Africa". Informal consultation on Global polio network, 20-24 September 2010, Geneva, Switzerland.
- HN Gumede. "Evaluation of real-time PCR tests in South Africa". 13th Meeting of laboratory Directors, 12-14 July 2010, Harare, Zimbabwe. HN Gumede. "Detection of VDPVs in other countries in the African Region". Informal consultation on Global polio laboratory network, 23-25 June 2009, Geneva, Switzerland.
- HN Gumede. "Molecular studies of genetic variability of Sabin viruses in Africa". Vaccinology Congress, 31 October – 01 November 2009, Hermanus, South Africa.
- HN Gumede. "Screening of known VDPVs and SL isolates from 2007 and 2008". Informal consultation on Global polio laboratory network, 23-25 June 2008, Geneva, Switzerland.
- HN Gumede. "Update on poliovirus circulation in Africa". FIDSSA 2nd Joint Congress 2007, 28-31 October 2007, , Cape Town.
- HN Gumede. "Circulation of vaccine derived polioviruses". Vaccinology Congress, 22-23 October 2007, Hermanus, South Africa.
- HN Gumede, Alfred Mawela, Mashudu Rampilo, Olivia Lentsoane, and Mbavhalelo Denga. "Wild polio detection and molecular epidemiology". 9th Meeting of laboratory Directors, July 2006, Pretoria, South Africa,.

- 
- HN Gumede. "Circulation of vaccine derived polioviruses". Vaccinology Congress, 31 October–01 November 2005, Hermanus, South Africa.
  - HN Gumede. "Viruses from Botswana and countries of west Africa- tracing the transmission pathways". Informal consultation on Global polio laboratory network, September 2005, Buckhead, Atlanta, USA.
  - HN Gumede. "Update on poliovirus circulation in Africa". Informal consultation on Global polio laboratory network, September 2004, Geneva, Switzerland.
  - HN Gumede. "Vaccine-Derived Polioviruses (VDPV) Issues in Africa Vaccinology Congress, 12-13 October 2004, Hermanus, South Africa.
  - HN Gumede. "Molecular epidemiology of polioviruses in sub-Saharan Africa". Medical Virology Congress of South Africa, 18-21 May 2003, Berg-en-Dal, Kruger National Park, South Africa.
  - HN Gumede. "Update on poliovirus circulation in Africa". Informal consultation on Global polio laboratory network, September 2003, Geneva, Switzerland.
  - HN Gumede. "Update on poliovirus circulation in Africa". 6th meeting of Laboratory Directors, October 2003, Antananarivo, Madagascar.
  - HN Gumede. " Update on containment in South Africa". Containment of laboratory stocks of wild poliovirus meeting, 3 April 2001, Decatur.
  - HN Gumede. "Molecular epidemiology of polioviruses in sub-Saharan Africa". Coordination & further development of the Global polio laboratory network, 4 – 5 April 2001, Decatur, USA.
  - HN Gumede. "Viruses from Africa- tracing the transmission links among viruses from Nigeria, Niger, Chad, Cameroon, Somalia and Sudan". 5th meeting of Data Managers and Directors, October 2001, Pretoria, RSA.

- HN Gumede. “Update on poliovirus circulation in Africa” Informal consultation on Global polio laboratory network, October 2001, Pretoria, RSA.
- HN Gumede. “Update on poliovirus circulation in Africa”. 4th meeting of Data Managers and Directors, 16 – 21 October 2000, Lusaka, Zambia.
- HN Gumede. “Update on poliovirus circulation in Africa”. Informal consultation on Global polio laboratory network, October 2000, Geneva, Switzerland

## **(Posters)**

- 14th Bioinformatics Workshop, University of Western Cape, 1-5 September 2008
- NICD Academic Day November 2008
- NICD Academic Day, 22-23 November 2010.

## **Acknowledgements**

All the experimental work was conducted at the Molecular Polio Unit of the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa.

### **NICD and University of Pretoria**

My Sincere thanks to my supervisors, Prof Marietjie Venter at the NICD and at the Department of Medical Virology, University of Pretoria and Prof Barry Schoub at the NICD for their guidance, and interest throughout my studies. Dr Martin Coetzee at the Forestry and Agriculture Biotechnology Institute (FABI), University of Pretoria for the advance phylogenetic workshop during the data analysis of this thesis.

### **Centres for Disease Control and Prevention (CDC)**

My co-supervisor, Dr. Mark Pallansch from the Division of Viral Diseases, National Centre for immunization and respiratory Diseases, CDC, Atlanta, Georgia for revision of



this thesis. Dr Cara Burns from the Division of Viral and Diseases, National Centre for immunization and respiratory Diseases, CDC, Atlanta, Georgia for excellent technical guidance and mentorship throughout my study. Dr Jorba Jauma from the Division of Viral and Diseases, National Centre for immunization and respiratory Diseases, CDC, Atlanta, Georgia for teaching me molecular evolution and helping me with Bioinformatics tools.

### **Polio Laboratory Network**

African polio lab network for providing most of isolates used in this study. The staff members of the Molecular polio unit at NICD for their interest and support. The Polio diagnostic staff at NICD for regrowing some of the isolates used in this study.

### **Funding**

The University of Pretoria, Centres for Disease Control and Prevention (CDC), Atlanta, Georgia, National Health Laboratory Services (NHLS) and World Health Organization (WHO) for financial support.

### **My family**

To my husband, Joe, and my sons Lesego and Thato, thank you for understanding my journey, encouraging me and allowing me to travel to CDC, US to collaborate some of the chapters described in this thesis, without your support finishing this project was not going to be possible.

Last but not least, through God all things are possible and I give Him all the Glory.

---

## TABLE OF CONTENTS

CHAPTER 1.....	- 1 -
LITERATURE REVIEW.....	- 1 -
1.1. INTRODUCTION .....	- 1 -
1.2. POLIOVIRUSES .....	- 2 -
1.2.1. Classification .....	- 2 -
1.2.2. Genome organization and Replication.....	- 4 -
1.2.3. Poliovirus infection cycle .....	- 8 -
1.2.4. Poliovirus vaccine.....	- 10 -
1.3. EPIDEMIOLOGY .....	- 14 -
1.4. MOLECULAR EPIDEMIOLOGY .....	- 20 -
1.5. AIM AND STUDY OBJECTIVES.....	- 24 -
CHAPTER 2.....	- 27 -
Identification and molecular characterisation of an Indian genotype of poliovirus type 1 isolated during consecutive outbreaks of poliomyelitis in Angola and Namibia from 2005- 2009.....	- 27 -
2.1 INTRODUCTION .....	- 27 -
2.2 MATERIAL AND METHODS.....	- 29 -
2.2.1 Viruses .....	- 29 -
2.2.2 Laboratory Diagnosis.....	- 29 -
2.3 RESULTS .....	- 36 -
2.3.1 Identification of wild poliovirus 1: .....	- 36 -
2.3.2 Outbreak description: .....	- 36 -

---

2.3.3	Phylogenetic and P-distance analysis .....	- 37 -
2.3.4	Calculation of circulation time of poliovirus strains in Africa .....	- 40 -
2.3.5	Amino acid substitutions in the VP1 region .....	- 41 -
2.4	DISCUSSION.....	- 44 -
CHAPTER 3.....		- 47 -
Identification and molecular characterisation of an Indian genotype of poliovirus type 1 isolated during outbreaks of poliomyelitis in the Democratic Republic of Congo (DRC) from 2006-2008.....		- 47 -
3.1	INTRODUCTION .....	- 47 -
3.2	MATERIAL AND METHODS.....	- 50 -
3.2.1	Viruses. ....	- 50 -
3.2.2	Laboratory Diagnosis.....	- 50 -
3.3	RESULTS .....	- 53 -
3.3.1	Outbreak description: .....	- 53 -
3.3.2	Prevalence and properties of the India strain in Africa: .....	- 54 -
3.3.3	Phylogenetic analysis.....	- 54 -
3.4	DISCUSSION.....	- 60 -
CHAPTER 4.....		- 63 -
Characterisation of type 2 and type 3 circulating vaccine derived polioviruses isolated during an outbreak of Poliomyelitis in Madagascar in 2005 .....		- 63 -
4.1	INTRODUCTION .....	- 63 -
4.2	MATERIALS AND METHODS .....	- 65 -
4.2.1	Viruses. ....	- 65 -
4.2.2	Epidemiological investigation .....	- 65 -
4.2.3	Virus isolation and intratypic differentiation .....	- 66 -



---

4.2.4	Reverse Transcription PCR for sequence analysis. ....	- 66 -
4.2.5	Sequence Analysis. ....	- 66 -
4.2.6	Phylogenetic Analysis. ....	- 67 -
4.3	RESULTS .....	- 69 -
4.3.1	Prevalence and properties of circulating vaccine derived polioviruses from AFP cases in Madagascar. ....	- 69 -
4.3.2	Phylogenetic analysis of the different regions of the cVDPV .....	- 71 -
4.3.3	Recombinant features of VDPVs .....	- 74 -
4.4	DISCUSSION.....	- 77 -
CHAPTER 5	.....	- 80 -
Vaccine Derived Polioviruses (VDPV) threatening eradication of polio in the Democratic Republic of Congo (DRC) .....		- 80 -
5.1	INTRODUCTION .....	- 80 -
5.2	MATERIALS AND METHODS .....	- 83 -
5.2.1	Viruses. ....	- 83 -
5.2.2	Diagnosis.....	- 83 -
5.3	RESULTS .....	- 86 -
5.3.1	Identification of circulating vaccine derived polioviruses from AFP cases in the DRC .....	- 86 -
5.3.2	Phylogenetic analysis of the VP1 region of the VDPVs .....	- 87 -
5.4	DISCUSSION.....	- 90 -
CHAPTER 6	.....	- 93 -
Identification of type 2 and type 3 circulating vaccine derived polioviruses isolated during an outbreak of Poliomyelitis in Ethiopia in 2008-2010.....		- 93 -
6.1	INTRODUCTION .....	- 93 -

---

6.2	MATERIALS AND METHODS .....	- 97 -
6.2.1	Diagnostic reverse-transcription polymerase chain reaction (RT-PCR) for poliovirus.....	- 97 -
6.2.2	ELISA for poliovirus .....	- 97 -
6.2.3	RNA Extraction.....	- 97 -
6.2.4	Reverse Transcription PCR for sequence analysis. ....	- 97 -
6.2.5	RT-PCR product analysis .....	- 97 -
6.2.6	PCR product purification.....	- 98 -
6.2.7	Cycle sequencing .....	- 98 -
6.2.8	Sequence Analysis .....	- 98 -
6.2.9	Phylogenetic Analysis.....	- 98 -
6.3	RESULTS .....	- 99 -
6.3.1	Prevalence and properties of circulating vaccine-derived polioviruses from AFP cases in Ethiopia.....	- 99 -
6.3.2	Amino acid substitutions in the VP1 region .....	- 100 -
6.3.3	VDPV Distribution in Ethiopia, 2008 – 2010 .....	- 102 -
6.4	DISCUSSION.....	- 104 -
CHAPTER 7	.....	- 108 -
	Identification of and molecular characterisation of Vaccine Associated Paralytic Polioviruses (VAPP) in South Africa.....	- 108 -
7.1	INTRODUCTION .....	- 108 -
7.2	MATERIALS AND METHODS .....	- 111 -
7.2.1	Case history .....	- 111 -
7.2.2	Virus Isolation.....	- 111 -



---

7.2.3	Real time reverse-transcription polymerase chain reaction for intratypic differentiation (rRT-PCR ITD) and for vaccine derived polioviruses (rRT-PCRVDPV) for poliovirus.....	- 112 -
7.2.4	RNA Extraction .....	- 113 -
7.2.5	Reverse Transcription PCR for sequence analysis .....	- 113 -
7.2.6	RT-PCR product analysis .....	- 114 -
7.2.7	PCR product purification.....	- 114 -
7.2.8	Cycle sequencing .....	- 114 -
7.2.9	Sequence Analysis .....	- 114 -
7.2.10	Phylogenetic Analysis.....	- 115 -
7.3	RESULTS .....	- 116 -
7.3.1	Prevalence and properties of vaccine associated paralytic polioviruses from AFP cases in South Africa .....	- 116 -
7.3.2	RT-PCR.....	- 119 -
7.3.3	Nucleotide sequence analysis .....	- 120 -
7.3.4	Pattern of recombination .....	- 122 -
7.4	DISCUSSION.....	- 125 -
CHAPTER 8	.....	- 127 -
CONCLUDING REMARKS	.....	- 127 -
REFERENCES	.....	- 133 -
APPENDIX A	.....	- 154 -
Summary of AFP surveillance performance indicators, Ethiopia, 2006 -2010.....		- 154 -
APPENDIX B	.....	- 155 -
Poliovirus sequences submitted to the GenBank.....		- 155 -
APPENDIX C	.....	- 160 -



---

ETHICS APPROVAL.....- 160 -

---

# MOLECULAR CHARACTERISATION OF WILD AND SABIN-LIKE POLIOVIRUSES CIRCULATING IN AFRICA AFTER 2000

by

**HERONYMA NELISIWE GUMEDE-MOELETSI**

**PROMOTER:** Prof M Venter (University of Pretoria/NHLS and NICD)

**CO-PROMOTER:** Prof B Schoub (NICD)

**CO-PROMOTER:** Dr M Pallansch (CDC)

**DEPARTMENT:** Medical Virology, Faculty of Health Sciences

**DEGREE:** PhD (Medical Virology)

---

## SUMMARY

Polioviruses have been around for a long time in man's history. Before the development of killed and live virus vaccines in the 1960's, poliomyelitis was a serious problem in public health. Since then paralytic poliomyelitis remains a threat in certain underdeveloped countries but has been considered a conquered disease in the developed world.

The molecular epidemiology of wild-type 1 polioviruses (WPV1) isolated in Angola in 2005, the Democratic Republic of the Congo (DRC) in 2006-2008 and Namibia in 2006 were investigated by sequence analysis of the complete VP1 gene of all isolates. All outbreak viruses clustered with the Indian type 1 genotype (SOAS) which was unique to India circulating endemically in the Uttar Pradesh (UP) and Bihar provinces in Northern



India. Epidemiological and virological analyses suggested that the Namibia outbreak virus had been circulating without detection for at least one year in Angola.

Four cases of acute flaccid paralysis (AFP) occurred in children in Madagascar in 2005. Molecular analysis confirmed cVDPV type 2 and 3 in affected patients. The first case, occurred in Toliara II district, on 09 April 2005. The last two cases were in the Toliara I and Beloha districts and paralysis onset on 26 June and 13 July 2005 respectively. Partial genomic sequencing of the poliovirus isolates revealed considerable divergence from the prototype Sabin strain in all cases. This is the second time that type 2 cVDPV is associated with an outbreak of AFP in Madagascar, and to our knowledge the first time that a type 3 cVDPV is identified in Madagascar.

A total of fifty-six children with AFP were found to excrete VDPVs of serotype 2 in the DRC between 2005 and 2010. These viruses represent at least three emergences and at least two outbreaks. Partial genomic sequencing of the poliovirus isolates revealed considerable nucleotide sequence divergence of between 1.1% to 2% from the prototype Sabin strain in the VP1 region of the viral genome. This was the first time that a type 2 cVDPV outbreak was detected in the DRC.

In total, 89 viral isolates obtained from Ethiopia during 2007 to 2010 and partial sequencing analysis confirmed that 13 isolates were VDPV's. Seven AFP cases were type 3, 4 AFP cases were type 2 and 2 contacts for type 3. Partial genomic sequencing of the poliovirus isolates revealed considerable divergence from the prototype Sabin strain in all cases.

Finally, cases of AFP where only Sabin-like viruses were identified were investigated in South Africa with 11 possible VAPP cases identified with recombinant events in the 3D region and also revealing a mutation that restore the original stem-loop structure in the internal ribosomal entry site (IRES) in the 5' Non-Translated Region (NTR).

In this study, the molecular epidemiology of poliovirus outbreaks that occurred in Angola, Namibia, and the DRC is described that were associated with wild polio 1 and 3. Investigation of Sabin-like vaccine strains in the DRC, Madagascar and Ethiopia identified vaccine-derived polioviruses in AFP cases as well as possible vaccine-associated paralytic poliovirus in South Africa.

## LIST OF FIGURES

**Figure 1.1:** (A) Schematic representative of the icosahedral capsid structure of poliovirus, (B), (C) and (D) represent VP1, VP2 and VP3 proteins respectively in their tertiary configuration.....-3-

**Figure 1.2:** (A) Poliovirus genome organization and (B) processing pathway (reproduced from Oh, Pathak et al. 2009).....-5-

**Figure 1.3:** Predicted RNA secondary structure of the poliovirus 5' NTR (Semler 2004). Computer prediction and chemical and enzymatic RNA-structure probing were used to deduce a consensus RNA conformation. Conserved sequences among picornaviruses include a NRA tetraloop (thought to function in tertiary interactions of RNAs and in protein binding), A/C-rich loops, and a pyrimidine-rich region just upstream of the conserved AUG codon. The IRES domain is boxed by red lines. (reproduced from (Semler 2004)). .....-7-

**Figure 1.4:** Poliovirus infection cycle (reproduced from The Polio Information Center Online, <http://microbiology.columbia.edu/pico/Chapters/cellular.html>).....-9-

**Figure 1.5:** Countries in red with reported cases of poliomyelitis in 1988. Source: WHO.....-16-

**Figure 1.6:** Countries with reported cases of poliomyelitis in 2010. Source: WHO.....-17-

**Figure 1.7:** Countries with reported cases of poliomyelitis in 2010. Source: WHO.....-18-

**Figure 1.8:** Countries with reported cases of vaccine-derived polioviruses as of 2010 Source: WHO.....-19-

**Figure 1.9:** Neighbour joining tree of WPV1 showing different genotypes from different regions between 1999 – 2004. Source: CDC.....-22-

**Figure 1.10:** Neighbour joining tree of PVW1 showing different genotypes from different regions between 1999 – 2004. Source: CDC.....-23-

---

**Figure 2.1:** An unrooted tree showing the close relationship of the current the DRC isolates to Sabin strain.....-39-

**Figure 2.2:** A Bayesian tree of wild poliovirus VP1 gene nucleotide sequences (906 bp):.....-40-

**Figure 2.3:** Amino acids comparison of VP1 region of the genome for wild-type polioviruses from Africa. Only variable positions are shown. The countries/states are abbreviated as follows: ANG, Angola; UP, Uttar Pradesh; NAM, Namibia .....-43-

**Figure 3.1:** A Bayesian tree with an estimated root demonstrating the evolution over time. The scale indicate the time of first isolation (2005) to the last (2008) at a rate of 1% per year as demonstrated for poliovirus.....-57-

**Figure 3.2:** A map showing a geographical distribution of selected viruses from the DRC during 2006-2008 outbreak.....-58-

**Figure 4.1:** A Madagascar map showing geographical distribution of the cases.....-71-

**Figure 4.2:** Neighbour-joining phylogenetic tree of circulating vaccine-derived polioviruses VP1 gene nucleotide sequences (903 bp). Numbers at branches nodes refer to the number of bootstrap repetitions (of 1000) at which the distal sequences grouped together. Closed diamonds represent AFP cases, open diamonds represent contacts. Sabin type 2 virus was used as an out-group. Genetic clusters of strains that were isolated from 3 years are indicated.....-73-

**Figure 4.3:** Neighbour-joining phylogenetic tree of circulating vaccine-derived polioviruses VP1 gene nucleotide sequences (900 bp). Numbers at branches nodes refer to the number of bootstrap repetitions (of 1000) at which the distal sequences grouped together. Closed diamonds represent AFP cases, open diamonds represent contacts. Sabin type 3 virus was used as an out-group.....-74-

**Figure 4.4. A.** The unrooted neighbour-joining tree of the 5' NTR region (nt 207-586) with reference to Sabin 2 demonstrating the relationships between circulating vaccine-derived (VDPV) Madagascar. Madagascar VDPV identified in 2001 and 2002 and bold and underlined (GenBank accession Number: AM084225 and AM084223). Sequence of isolates related to Sabin 3, are coloured orange and VDPV type 2 cases and contacts are coloured blue and are related to non OPV viruses.....-75-

**Figure 4.5. B.** The unrooted neighbour-joining tree of the 3D region (nt 6145 – 6507) demonstrating relationship with unknown human enterovirus C species (HEV-C). GenBank accession numbers HEV-C used are: AF499635=CA1; AF499636=CA11; AF499637=CA13; AF499638=15; AF499639=CA17; AF499640=CA18; AF499641=CA19; AF499642=CA20; AF546702=CA21; AF499643=CA22 and D90457=CA24. All cases are bolded.....-76-

**Figure 5.1:** A map showing a geographical distribution of VDPV from the DRC.....-87-

**Figure 5.2:** A neighbour-joining tree rooted at the Sabin 2 reference strain closest to the VDPV type 2 strain isolated in the DRC.....-88-

**Figure 6.1:** Amino Acids comparison of VP1 region of the genome for Sabin 2, wild type 2 and VDPVs type 2 from Africa. W2\_AY082676, MEF\_AY082677, Laderle\_AY082678 and Lansing\_AY082680 are from the GenBank. RDC strains are from the DRC (detailed in Chapter 4) and ETH strains are from Ethiopia.....-101-

**Figure 6.2:** Amino Acids comparison of VP1 region of the genome for Sabin 3, wild type 3 and VDPVs type 3 from Africa. NIE strains are from the Nigeria and ETH strains are from Ethiopia.....-101-

**Figure 6.3:** Geographical distribution of VDPV cases in Ethiopia in 2008 to 2010...-103-

**Figure 6.4:** The graph showing outbreak response in Ethiopia.....-107-

**Figure 7.1.** Ethidium bromide-stained 1.5% agarose gel showing amplification of the (A) 5' NTR and (B) VP1 regions of poliovirus type 3 strains. Sabin 3 used as a positive controls (+ve) and negative controls (-ve) are reactions without template. Lanes: 1. PMOL070911, 2. PMOL100360, 3. PMOL080728, 4. PMOL070899, 5. PMOL070805, 6. PMOL070678, 7. PMOL070769, 8. PMOL070798, 9. PMOL070521, 10. PMOL040953, 11. PMOL080391 in panel A and negative control in panel B. Lane 12 and 13, negative control in panel A and Molecular weight marker in panel B. Last lane is a molecular weight marker for panel A.....-119-

**Figure 7.2:** (A,B,C,D,E and F): BootScan results for South Africa viruses with strong evidence of recombination. The percentage of permuted trees is shown along y axis and the position is shown along the length of the sequence (x axis). The initial bootscan results were obtained using Sabin 3 reference strain. The bootscan was redone using the Coxsackie A24 (CAV24) and Sabin 2 (used as a control)..... 122- to-124-

**Figure 7.3:** Maximum-likelihood (PHYML) phylogenetic tree based on the 3D gene sequence of South African Sabin-like strains with recombination. Type of recombination is indicated on the right hand side. Coxsackie A 24 (CVA24) strain is highlighted in red and the Sabin reference strains are highlighted in blue.....-124-

## LIST OF TABLES

<b>Table 2.1:</b> Selected wild poliovirus type 1 and type 3 isolated in Angola and Namibia between 2005 and 2009.....	-35-
<b>Table 2. 2:</b> The nucleotide pairwise distance of the SOAS (Indian) genotype in Africa. The P-distance range within each country is bold and the difference between each country are below.....	-41-
<b>Table 3.1:</b> Laboratory data of wild poliovirus strains detected in acute flaccid paralysis (AFP) from the DRC. Epid Number indicates Epidemiological number (EPID Number).....	-55-
<b>Table 4.1:</b> Laboratory data of circulating vaccine-derived polioviruses and Sabin-like strains detected in acute flaccid paralysis (AFP) and contacts samples from Madagascar.....	-70-
<b>Table 6.1:</b> List of selected VDPV cases detected in Ethiopia since 2008. Epid Number is an epidemiology number given to a case or contact.....	-99-
<b>Table 7.1:</b> Prime pair used for amplification of and sequencing of the different regions of the poliovirus genome.....	-113-
<b>Table 7.2:</b> AFP Poliovirus Sabin-like strains selected for screening of VAPP's in South Africa.....	-116-to-118-
<b>Table 7.3:</b> Nucleotide changes between South African Sabin-like viruses and Sabin reference strains.....	-121-

---

## SELECTED ABBREVIATIONS USED IN THE TEXT

AFP	Acute flaccid paralysis
BDD	Bandundu
CDC	Centers for Disease Control and Prevention
cVDPV	Circulating vaccine-derived polioviruses
CVID	Common variable immunodeficiency disorder
CPE	Cytopathic effect
DNA	Deoxyribose nucleic acid
EQT	Equateur
GPLN	Global Polio Laboratory Network
RD	Human Rhabdomyosarcoma
iVDPV	Immunodeficiency vaccine-derived polioviruses
IPV	Inactivated poliovirus vaccine
IRES	Internal Ribosomal Entry Site
SOAS	South Asia genotype (Indian genotype)
ITD	Intratypic Differentiation
KOC	Kasai Occidental
KAT	Katanga
mOPV	Monovalent Oral Poliovirus Vaccine
NIDs	National Immunization Days
NICD	National Institute for Communicable Diseases
NTR	Non-Translated Region
OPV	Oral Poliovirus Vaccine strains
ORT	Orientale



---

PCR	Polymerase chain reaction
PEI	Polio Eradication Initiative
PV	Polio Virus
PVR	Polio Virus Receptor
RT-PCR	Reverse transcription PCR
RNA	Ribose nucleic acid
SNNPR	Southern Nations Nationalities people Region
SNIDs	Sub-National Immunization Days
SKV	Sud- Kivu
DRC	the Democratic Republic of the Congo
P-distance	The nucleotide pairwise distance
tOPV	Trivalent Oral Poliovirus Vaccine
UP	Uttar Pradesh
VAPP	Vaccine-associated paralytic poliomyelitis
VDPV	Vaccine-derived Polioviruses
WEAF-A	Western African A or African genotype
WEAF-B	Western African B or African genotype
WPV	Wild poliovirus
WPV1	Wild poliovirus 1