CHAPTER 6

Identification of type 2 and type 3 circulating vaccine-derived polioviruses isolated during an outbreak of poliomyelitis in Ethiopia in 2008-2010

6.1 INTRODUCTION

Poliomyelitis is highly contagious, and wild polioviruses in endemic areas infect virtually the entire population (Gear 1993). Polio is a seasonal disease, with peak transmission in the summer and autumn and reduced transmission during winter. Seasonal differences in transmission are far less pronounced in tropical areas (Nathanson and Martin 1979; Sabin 1985). Paralytic attack rates vary by serotype, with the highest rates (~0.5%) associated with poliovirus type 1 and the lowest rates (<0.05%) associated with poliovirus type 2 (Nathanson and Martin 1979). Poliovirus is transmitted person to person by the fecal-oral route and also by the oral-oral route, especially in areas with good sanitation and hygiene. Major risk factors for poliovirus transmission include poor sanitation and hygiene, tropical and subtropical conditions, arge birth cohorts, and high population densities (Nathanson and Martin 1979); (Sabin 1985). In Ethiopia, as in other developing countries, poliomyelitis was a major public health problem affecting mainly preschool children (Cockburn and Drozdov 1970). A study conducted at a major pediatric hospital in Addis Ababa demonstrated an increase in new cases between 1971 and 1981. Eighty-nine per cent of the cases with residual paralysis were 2-year-old

and 1981. Eighty-nine per cent of the cases with residual paralysis were 2-year-old children. There was no seasonal variation. A study in 1979 involving over a quarter of a million school age children in Addis Ababa indicating a prevalence of 2.7 per 1,000, with an estimated annual incidence of 7.8 per 100,000 (Maru, Getahun et al. 1988). Another study, conducted from February to July in 1993, indicated that the prevalence among children aged 5-9 years was 7.3 per 1,000 (Maru, Getahun et al. 1988). Other school and institutional based studies revealed a higher prevalence (Hull, Ward et al. 1994)

In 1988, the World Health Assembly resolved to eradicate poliomyelitis worldwide. Subsequently, the Global Polio Eradication Initiative (GPEI) of the World Health Organization (WHO) reduced the global incidence of polio associated with wild polioviruses (WPVs) from an estimated 350,000 cases in 125 countries in 1988 to 1,651 reported cases in 2008 and reduced the number of countries that have never interrupted WPV transmission to four endemic countries (Afghanistan, India, Nigeria, and Pakistan) (Maru, Getahun et al. 1988). However, because vaccine-derived polioviruses (VDPVs) can produce polio outbreaks in areas with low rates of oral poliovirus vaccine (OPV) coverage and can replicate for years in immunodeficient persons, enhanced strategies are needed to limit emergence of VDPVs and stop all use of OPV once WPV transmission is eliminated.

The polio eradication initiative in Ethiopia started in 1996 following the signing of the Yaoundé Declaration on Polio Eradication in Africa in 1996. Since then, Ethiopia has accelerated implementation of polio eradication strategies. Acute Flaccid Paralysis (AFP) surveillance, the detection and reporting cases of AFP in children less than 15

means for detecting circulating wild poliovirus in a population and therefore is crucial to the global polio eradication effect.

Ethiopia conducted the first sub-National Immunization Days (sNIDs) in 1996 and then two rounds of National Immunization Days (NIDs) every year thereafter. House-to-house immunization started in 1999 and contributed to the increased immunization coverage and AFP surveillance rates in parallel.

The surveillance quality at National level continues to show progressive improvement from year to year. Achieving uniform quality of surveillance at the lower level of the administration system in the country remains a great challenge. Uniform nationwide surveillance is crucial for ruling out undetected transmission of poliovirus. The key to evaluating the status of polio eradication is combining epidemiological and laboratory investigation of AFP among infants and children (Hull, Ward et al. 1994). An AFP surveillance indicator is detection of two AFP case per 100,000 children under 15 years of age. From 1997 to 2001, Ethiopia made remarkable progression on the rate of collection of two stool specimen within 14 days after onset of paralysis cases and met the surveillance indicator of WHO (Appendix A).

During this period five indigenous cases of WPV1 were isolated. The first isolate was in 1999 from Oromia Region East Shewa zone. In the year 2000 two cases from Southern Nations Nationalities people region (SNNPR) and one case from North Gonder zone in Amhara region were isolated. Three of these patients had not received OPV. Residual paralysis was observed in four of the five patients. Genetic sequencing revealed that the wild isolates identified in 1999-2001 were not related to other viruses isolated in neighboring countries or elsewhere. It is unlikely that the virus was imported from

outside. The last indigenous of WPV1 case in 2001 was in SNNPR which is southern part of Ethiopia (Mentaye, Tomori et al. 2002).

Ethiopia has achieved tremendous progress in its Polio Eradication Initiative activities since it commenced in 1996. The AFP surveillance system proved to be sensitive enough to detect imported WPV outbreaks and OPV coverage rates have increased from less than 400,000 children in 1996 to more than 14 million in 2001 leading to reduced transmission of the virus. From January 2002 to 2003 no WPV was detected in Ethiopia and the country was categorized as an area with low transmission. However, following a massive polio outbreak in Sudan that paralyzed 132 Sudanese children in May 2004, the virus spread to Ethiopia. The first two confirmed cases of WPV1 were isolated in the Tigray region and genetic sequencing data confirmed that the two cases are linked to polio circulating in Sudan.

In this report, we describe the detection of type 2 and type 3 circulating VDPVs from 2007 to 2010. Like other outbreaks in Hispaniola (Kew, Morris-Glasgow et al. 2002), the Philippines and Madagascar (Rakoto-Andrianarivelo, Gumede et al. 2008) the circulation of VDPVs occurred after the elimination of the corresponding serotype of wild poliovirus at a time when the rate of vaccine coverage was insufficient.

6.2 MATERIALS AND METHODS

6.2.1 Diagnostic reverse-transcription polymerase chain reaction (RT-PCR) for poliovirus

Refer to 2.2.2.1

6.2.2 ELISA for poliovirus

Refer to 2.2.2.2

6.2.3 RNA Extraction

Refer to 2.2.2.3

6.2.4 Reverse Transcription PCR for sequence analysis.

Reverse transcription PCR (RT-PCR) was performed in a single step as described previously (Yang, De et al. 1991; Chezzi and Schoub 1996; Kilpatrick, Nottay et al. 1996; Kilpatrick, Nottay et al. 1998) and as explained in chapter 2.

6.2.5 RT-PCR product analysis

PCR products were analysed by agarose gel electrophoresis. The DNA was visualised by addition of 6 µl of 10 mg/ml ethidium Bromide. A 100 bp DNA ladder was used as the molecular weight marker (DNA molecular marker XIV, Roche diagnostics, Mannheim Germany). The gels were visualized and photographed on UVP trans-illuminator using Grab IT annotating Grabber 2. 51 software (UVP (Inc), USA).

6.2.6 PCR product purification

PCR products were purified with the Qiaquick PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations. The Qiaquick columns have a silica-gel membrane with selective binding properties. In short, five volumes of PB buffer (guanidine hydrochloride, isopropanol) were added to 1 volume of PCR reaction. Primers and impurities are removed by washing with 750 µl PE buffer containing 88% ethanol. PCR product was eluted with distilled water.

6.2.7 Cycle sequencing

As described in 2.2.2.5

6.2.8 Sequence Analysis

Refer to Chapter 2.

6.2.9 Phylogenetic Analysis

The alignments (nucleotide and translated amino acid sequences) were analyzed with Genedoc version 2. 6001 (www. psc. edu/biomed/genedoc) and MEGA version 4 to identify specific mutations and positive selection (Kumar, Nei et al. 2008).

6.3 RESULTS

6.3.1 Prevalence and properties of circulating vaccine-derived polioviruses from AFP cases in Ethiopia

In total, 89 viral isolates obtained from Ethiopia during 2007 to 2010 were tested by intratypic differentiation tests to screen out Sabin-like strains from wild type strains using both RT-PCR and ELISA. All confirmed Sabin-like strains were further analysed by real-time assay. The genetic variability of all isolates was further investigated by nucleotide sequence analysis of the VP1 region. Partial sequencing and P-distance analysis confirmed that 13 isolates were VDPV's with > 1% divergence from the parental Sabin strain (Table 6.1).

Table .6.1: List of selected VDPV cases detected in Ethiopia since 2008. Epid Number is an epidemiology number given to a case or contact.

Epid Number	OPV doses given	Date Of Onset of paralysis	Serotype	Nucleotide difference to Sabin reference strain
ETH-ORO-BAL-10-034	1	06/01/2010	3	2.33%
ETH-ORO-BAL-10-136	1	17/02/2010	3	2.57%
ETH-ORO-BAL-10-330	3	08/04/2010	3	2.56%
ETH-ORO-BAL-10-430	3	17/05/2010	3	2.78%
ETH-ORO-BAL-10-C30	3	Not available	3	2.78
ETH-ORO-EHA-09-258	3	27/04/2009	3	1.33%
ETH-ORO-EHA-09-108	2	06/02/2009	2	1.11%
ETH-ORO-EHA-08-1033	1	14/12/2008	2	1.11%
ETH-ORO-EHA-08-727	0	04/10/2008	2	1.22%
ETH-ORO-EHA-08-925	3	17/11/2008	2	1.22%
ETH-SOM-DEG-10-251	2	05/02/2010	3	2.56%
ETH-SOU-BEN-10-937	1	11/04/2010	3	2.12%

In total, seven AFP cases for type 3, 4 AFP cases for type 2 and 2 contacts for type 3 were detected. The four AFP cases of type 2 had 98.9% - 98.78% sequence identity in the VP1 gene to the Sabin reference strain and 99.78% - 99.10% sequence identity to each other. The VP1 sequence of the cVDPV type 3 had 98.70% – 96.90% sequence identity to the Sabin reference strain and 99.00% to 95.60% to each other.

6.3.2 Amino acid substitutions in the VP1 region

Comparisons of the VP1 amino acids sequences of the VDPV type 2 from Ethiopia relative to Sabin 2 are shown in the alignment in Figure 6.1. Most substitutions resulted in silent mutations and occurred on the 5' end of the VP1 region. A proline at position 21 of Sabin 3 is replaced by a leucine, indicated as P21L; as well as I143T. T143I encoded by C2909U nucleotide appear to be responsible for the attenuated phenotype of Sabin 2 (Ren, Moss et al. 1991; Macadam, Pollard et al. 1993). Any back mutation may cause neurovirulence (Sabin 1985) as in the case of Ethiopia strains which demonstrated the same base as it is with wild type 2 strains.

The VDPV type 3 had three substitutions that were observed at positions, 12, 53 and 106, i.e., G12S, A53V and M106T (Figure 6.2). ETH-10-10-251 had an A to S substitution at position 144 (A144S) and D24M which was not observed in other Ethiopia strains which indicates further genetic drift from the original virus from Sabin reference strain. One strain from Ethiopia (ETH-10-10-258) contained an additional substitution of E8D. Most of Ethiopia strains share the same substitutions relative to Sabin 2 and 3.

STRAINS **VARIABLE POSITIONS**

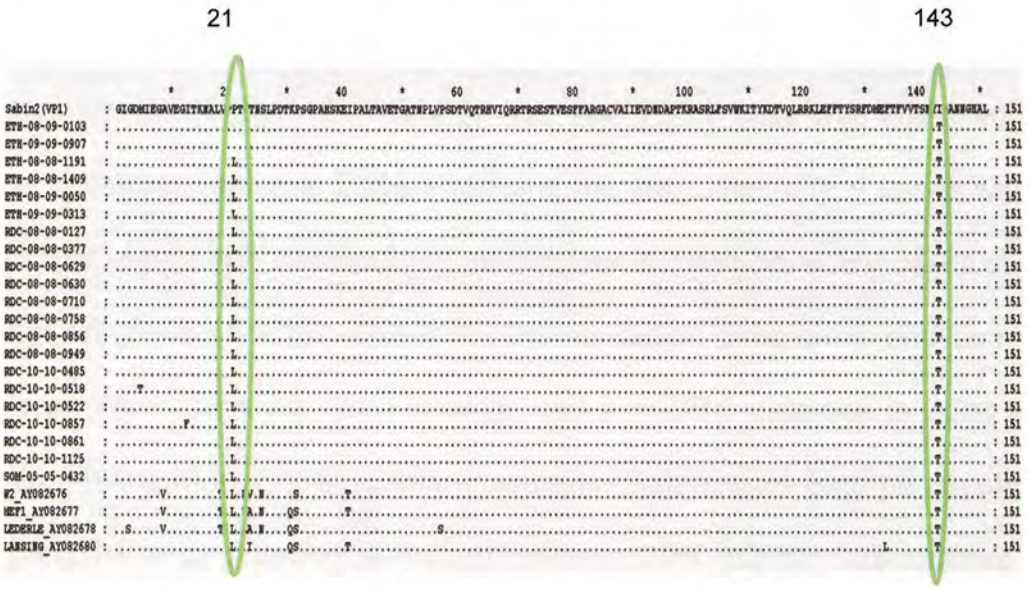


Figure 6.1 Amino Acids comparison of VP1 region of the genome for Sabin 2, wild type 2 and VDPVs type 2 from Africa. W2_AY082676, MEF_AY082677, Laderle_AY082678 and Lansing_AY082680 are from the genebank. RDC strains are from the DRC (detailed in Chapter 4) and ETH strains are from Ethiopia.

STRAINS **VARIABLE POSITIONS**

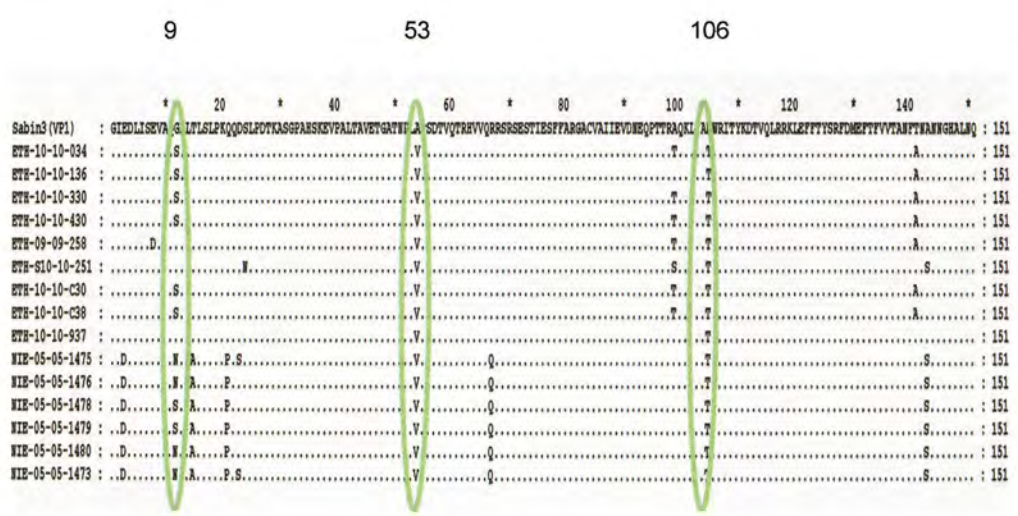


Figure 6.2. Amino Acids comparison of VP1 region of the genome for Sabin 3, wild type 3 and VDPVs type 3 from Africa. NIE strains are from the Nigeria and ETH strains are from Ethiopia.

6.3.3 VDPV Distribution in Ethiopia, 2008 – 2010

A total of nine VDPV cases were identified from two regions Oromia and one from Somali from 2008-2010 (Figure 6.3). The first VDPV case was identified in East Harge zone in Oromia region in 2008. Following this two other VDPV cases were identified in the same region and zone in 2009. In the year 2010 three VDPV cases one from Somali region Degehabour zone, one from Oromia region Bale zone and another from East Harerge were identified. East Hararge had a cVDPV outbreak in 2008 and 2009 with similar finding of low immunization coverage. Data shows that the circulation has been ongoing for a long period. Although trivalent OPV (tOPV) Sub-national Immunization Activities (SIAs) were conducted right around the identification of the first case in E. Hararge, the virus may have already entered Bale. The case in Somali Region is right at the border with Somalia.

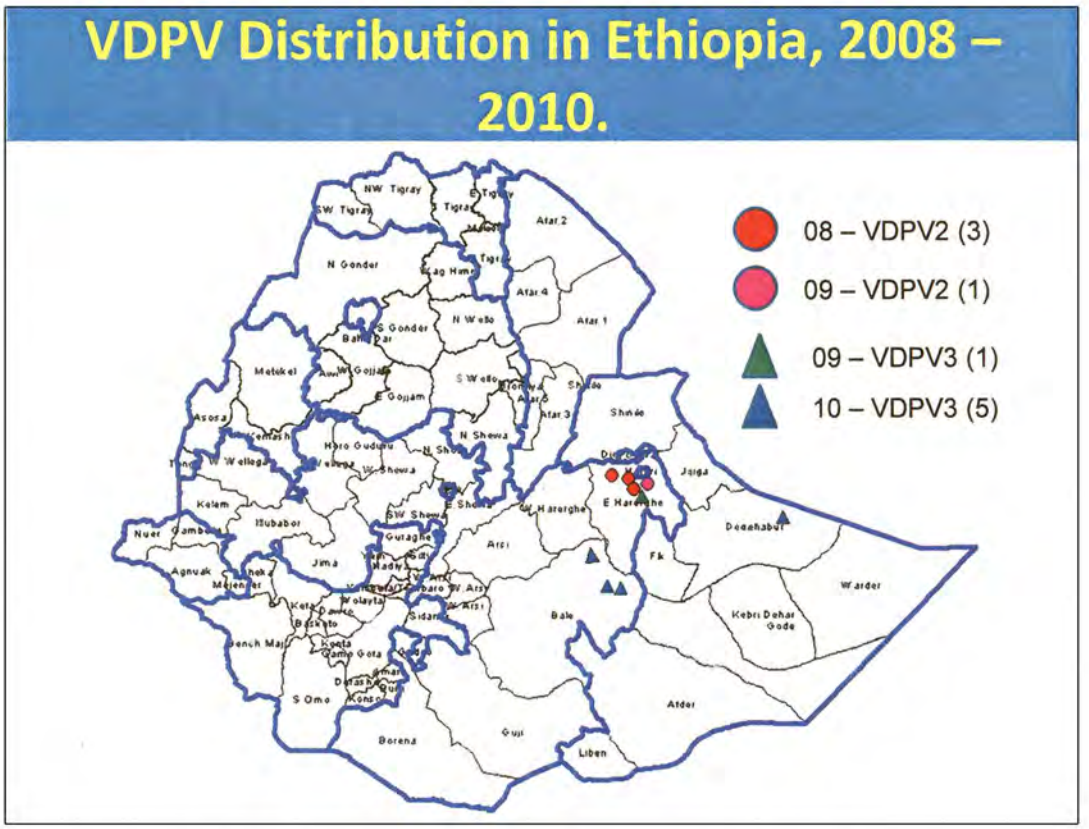


Figure 6. 3.: Geographical distribution of VDVP cases in Ethiopia in 2008 to 2010

6.4 DISCUSSION

VDPVs can cause paralytic polio in humans and has the potential for sustained circulation. VDPVs resemble WPVs biologically and differ from the majority of Sabin vaccine-related poliovirus isolates by having genetic properties consistent with prolonged replication or transmission. Because poliovirus genomes evolve at a rate of approximately 1% per year (Jorba, Campagnoli et al. 2008), Sabin vaccine-related isolates that differ from the corresponding OPV strain by more than 1% of nucleotide positions (usually determined by sequencing the genomic region encoding the major viral surface protein, VP1) are estimated to have replicated for at least 1 year after administration of an OPV dose. This is substantially longer than the normal period of vaccine virus excretion of 4-6 weeks.

The transmission of vaccine-related virus from vaccinees to susceptible close contacts was recognized prior to the introduction of oral polio vaccine. Vaccine viruses were transmitted from person to person in families, but less readily than wild poliovirus strains (Hull, Ward et al. 1994). The overriding factor for the emergence of all VDPVs is the same as for WPV circulation, low immunity levels in a population. Where routine coverage with OPV is poor or where there is an absence of high quality supplementary immunization activities, a population can become susceptible to the emergence of VDPV. This is because the virus has time to change, replicate and exchange genetic material with other enteroviruses, while spreading through a population (Rakoto-Andrianarivelo, Gumede et al. 2008). Importantly, if a population is fully immunized

against polio, it will be protected against the spread of both wild and vaccine strains of poliovirus (Rakoto-Andrianarivelo, Gumede et al. 2008).

Studying each outbreak has shown that most were either self-limiting or rapidly stopped with OPV campaigns. As long as wild poliovirus circulates anywhere in the world, it is vital to maintain high population immunity levels. Maintaining high population immunity will also minimize the risk of cVDPVs occurring.

A vaccine-derived poliovirus is a rare strain of poliovirus, genetically mutated from the reference Sabin strain. More cases have been reported since the start of the VDPV2 outbreak in Madagascar in 2002 and 2005 (Rakoto-Andrianarivelo, Gumede et al. 2008), 58 detected in the DRC (chapter 5), and 11 from Ethiopia reported in here (Gumede et al, in preparation).

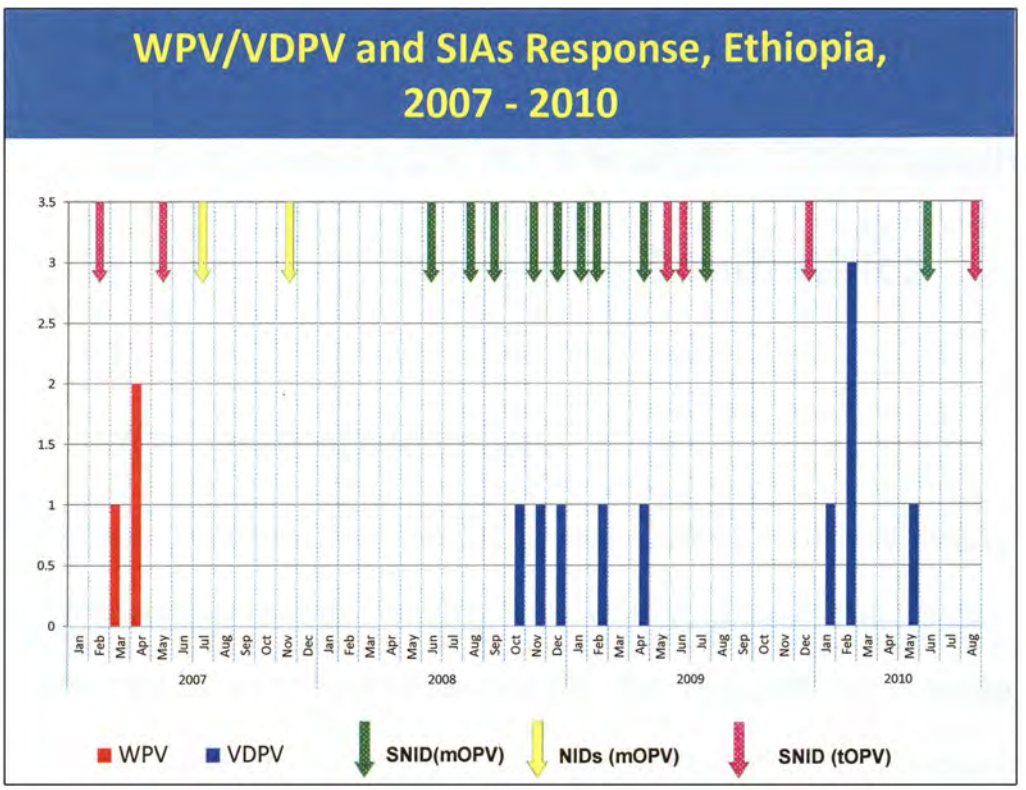
Amino acid changes in the VP1 gene that were similar to wild-type strains but different to Sabin could be identified which may be associated with the reversion to virulence, as demonstrated in Figure 6.1 and Figure 6.2. The origin of cVDPV2 in Ethiopia was probably the result of low population immunity to poliovirus, due to a combination of low OPV coverage in some communities and the prior elimination of the indigenous wild poliovirus of the same serotype. Such risk factors are same as for wild poliovirus (WPV) circulation, and imported wild polioviruses of circulated in the same country during the period 2005 to 2008 (Nathanson and Martin 1979; Fine and Carneiro 1999). Poor hygiene and sanitation and tropical climate were probably additional factors that facilitated circulation of both WPV and VDPVs in some communities.

The management of cVDPVs is a necessary part of the global polio eradication effort, and is similar to management of wild poliovirus outbreaks; i.e., by rapid implementation of large-scale, high-quality SIAs. OPV is the polio vaccine by which to achieve the eradication of wild polioviruses worldwide. Once wild poliovirus transmission has been interrupted globally, however, OPV use in routine immunization programmes will eventually be stopped, in order to eliminate also the rare risks posed by VDPVs and as recommended by the Advisory Committee on Polio Eradication.

Studies have also been proposed to measure the prevalence and duration of poliovirus excretion among human immunodeficiency virus-infected children in developing countries (Ochoa and Lago 1987).

The emergence of a cVDPVs in Ethiopia reaffirms that not enough children are protected from poliovirus (wild or vaccine-derived) and that much more must be done to reach all children with vaccine.

In response to recent outbreak in Bale Zone of Oromia and Degahabour Zone of Somali two rounds of campaign using mOPV3 and tOPV was conducted in June and August 2010 during SNIDs (Figure 6.4) (personal communication with Epidemiology group from Ethiopia).



Key

WPV:	Wild poliovirus
VDPV:	Vaccine-derived poliovirus
SNID:	Sub-National Immunization Day
mOPV:	Monovalent Oral Poliovirus Vaccine
NIDs:	National Immunization Days
tOPV:	Trivalent Oral Poliovirus Vaccine

Figure 6. 4: The graph showing outbreak response in Ethiopia.