CHAPTER 5

Vaccine-derived Polioviruses (VDPV) threatening eradication of polio in the Democratic Republic of Congo (DRC)

5.1 INTRODUCTION

Four countries remain endemic for indigenous wild type poliovirus namely, Nigeria, India, Afghanistan and Pakistan (CDC. 2006b). Several African countries have experienced transmission in 2010 following an importation of poliovirus. Worldwide, a 19% decrease of cases was reported in 2010 compared to 2009 (Zheng, Zhang et al. 1993). Live, attenuated oral poliovirus vaccine (OPV) is still the vaccine of choice for developing countries. However, reversion to virulence may occur during OPV replication in humans and may result in the rare cases of vaccine-associated paralytic poliomyelitis (VAPP) in OPV recipients and their close contacts. Although cases of VAPP have long been recognized, two additional OPV-related problems that may have a significant impact on polio eradication have been identified. These are long-term persistent infection with OPV-derived viruses in persons with primary humoral immunodeficiencies (so-called immunodeficiency-associated vaccine-derived polioviruses, iVDPVs) and polio outbreaks caused by person-to-person spread and circulation of vaccine-derived polioviruses (cVDPV) in areas with low rates of vaccine coverage (Kew, Sutter et al.

2005). VDPV strains are defined as follows: a) VDPV strains of types 1 and 3 have less than 99% nucleotide sequence identity to the capsid VP1 coding region to Sabin reference strain, and b) VDPV strains of type 2 have < 99.4% sequence identity to the Sabin reference VP1 (Kew, Sutter et al. 2005) and http://www.polioeradication.org/GPLN_meeting_recommendation_2010.pdf).

cVDPVs show significant sequence drift, indicating prolonged replication of the vaccine strain in human hosts and consequent changes in phenotypic properties of neurovirulence and transmissibility. Poliomyelitis outbreaks associated with cVDPVs have been reported in several countries, such as Egypt (retrospectively detected for the period 1982-1993); Haiti (2000-2001); Dominican Republic (2000-2001) (Kew, Morris-Glasgow et al. 2002); Philippines (2001) (Kew, Morris-Glasgow et al. 2002); China (2004) (Tong, Zhang et al. 2005; Liang, Zhang et al. 2006); Cambodia (2005-2006); Indonesia (2005) (Estivariz, Watkins et al. 2008); Madagascar (2002 and 2005) (Rakoto-Andrianarivelo, Gumede et al. 2008); and Nigeria 2005-2010 (Jenkins, Aylward et al.; Wassilak, Pate et al.). As a result of the accumulating evidence about the emergence and spread of cVDPV, there are plans for synchronized cessation of the use of OPV and implementation of more widespread use of IPV (Kew, Morris-Glasgow et al. 2002; Rousset D 2003; Yang, Naguib et al. 2003; Jarzabek 2005).

In addition, some persons with primary immunodeficiency have been found to be persistently infected with VDPV. Once exposed to OPV, immunocompetent persons usually excrete polio vaccine viruses for up to 2 – 3 months (Alexander, Gary et al. 1997). In contrast, a few cases of prolonged excretion of VDPV for 6 months to more

than 10 years have been found in some immunodeficient persons (MacCallum 1971; Lopez, Biggar et al. 1974; Davis, Bodian et al. 1977; Hara, Saito et al. 1981; Misbah, Lawrence et al. 1991; Kew, Sutter et al. 1998; Bellmunt, May et al. 1999; Martin, Dunn et al. 2000). A better understanding of VDPV persistence and circulation is very important for decision making about when and how to stop immunization with oral poliovirus vaccine (OPV) after the global eradication of wild polioviruses (Fine and Carneiro 1999; Wood, Sutter et al. 2000).

This chapter presents the genetic characterization of the first cVDPV outbreaks detected in DRC. A total of 58 children with AFP were found to excrete VDPVs of serotype 2 in the DRC between 2004 and 2010. This is the first description of AFP cases associated with VDPVs of serotype 2 identified in the DRC.

5.2 MATERIALS AND METHODS

5.2.1 Viruses.

Virus isolates from faecal specimens of AFP cases were referred as described in Chapter 2 (van der Avoort, Hull et al. 1995). The original stool specimens from which these isolates were obtained were also referred to NICD for confirmation of virus isolation results using methods recommended by the World Health Organization (http://www.who.int/immunization_monitoring/Supplement_polio_lab_manual.pdf).

5.2.2 Diagnosis

Polio Virus (PVs) isolates were determined to be Sabin-like or wild-type strains by Intratypic differentiation (ITD) using a PCR-based technique (Yang, De et al. 1991; Kilpatrick, Nottay et al. 1996; Kilpatrick, Nottay et al. 1998), and ELISA as recommended by the World Health Organization (WHO) (Yang, De et al. 1991; Chezzi 1996; Kilpatrick, Nottay et al. 1996; Kilpatrick, Nottay et al. 1998).

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

Refer to Chapter 2

5.2.2.2 ELISA for poliovirus

Refer to Chapter 2

5.2.2.3 Real-Time PCR for Sabin-like strains

A real-time assay developed by CDC, Atlanta was used for screening Sabin and wild-type strains, (Kilpatrick, Yang et al. 2009) and for screening VDPV strains (David Kilpatrick, personal communication).

5.2.2.4 RNA Extraction

Refer to Chapter 2

5.2.2.5 Reverse Transcription PCR for sequence analysis

Refer to Chapter 2

5.2.2.6 Sequence Analysis

Before sequencing, the RT-PCR products were purified with the QIAquick PCR purification kit (Qiagen). The complete VP1 gene (nucleotides 2480 to 3385) was sequenced as described in Chapter 2. In 2010, the Global Polio Laboratory Network (GPLN) established a new definition for VDPVs of type 2, based on accumulating data that showed the inadequacy of the current definition. The current definition is based on the detection of more than 1 AFP case with genetically related viruses showing > 10 nucleotide (nt) changes from VP1 nucleotide sequence of the Sabin reference strain of the same serotype. However the accumulated data from several countries point to situations that have programmatic implications, yet do not meet the current definition for circulation, including amongst others: Sabin-like viruses from different individuals that cluster by serotype, time or place and have VP1 sequences suggestive of a common evolutionary pathway (lineage) based on accumulation of mutations (some of which are

shared) yet sequence divergence from Sabin does not exceed 1%. This situation has been seen most frequently with serotype 2 viruses. Therefore, inadequacies of the current cVDPV definition used in the Polio Eradication Initiative programme (PEI) may delay the characterization or underestimate the scope of some outbreaks. According to the new definition, VDPVs of type 2 are those that have more than 5 nucleotide differences from Sabin 2 in the VP1 coding region.

(http://www.polioeradication.org/GPLN_meeting_recommendation_2010.pdf).

5.2.2.7 Phylogenetic Analysis

Phylogenetic analysis was carried out on the complete VP1 gene,(903bp). To determine VP1 nucleotide diversity we compared all complete VP1 sequences of isolates from AFP cases and their contacts with that of the Sabin type 2 OPV reference strain. Nucleotide sequences were aligned with Clustal X (Thompson, Gibson et al. 1997). A Neighbour-joining was constructed with MEGA version 4.0 (Kumar, Tamura et al. 2004; Tamura, Dudley et al. 2007; Kumar, Nei et al. 2008) rooted at the Sabin reference strain to demonstrate the evolution since the introduction of the VDPV into Africa. 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 (Tamura, Dudley et al. 2007).

5.3 RESULTS

5.3.1 Identification of circulating vaccine-derived polioviruses from AFP cases in the DRC

In total, over 600 viral isolates obtained from the DRC from 2003 to 2010 were tested by RT-PCR using pan-enterovirus, pan-poliovirus, serotype-specific, and Sabin type 1, 2 and 3 virus specific primers. Some isolates were further tested by ELISA, and both techniques identified the isolates as Sabin-like poliovirus. All isolates were further screened by Real-Time RT-PCR (rRT-PCR), which included a screen for VDPV as has been implemented in the GPLN (Taren, Nesheim et al. 1987; CDC. 2009a) . The genetic variability of virus isolates from 58 AFP cases was further investigated by performing nucleotide sequence analysis of the VP1 coding region. Partial genomic sequencing confirmed that 58 cases had $\leq$ 99.4% VP1 sequence identity to the parental type 2 Sabin strain, and the isolates were classified as VDPVs.

The polio cases were found in several regions of DRC during this time period (Figure 5.1). Altogether 27 cases occurred in Katanga (KAT) province, 5 cases in Kasai Occidental (KOC), 2 cases in Bandundu (BDD), 1 case in Sud Kivu (SKV), 6 case in Orientale (ORT), 11 cases in Maniema (MAN) and 4 cases in Équateur (EQT). The first case occurred in Inongo district of Bandundu province in September of 2005 and the last case was in the Tshikapa (TKP) district of KOC province and paralysis set in on 24th of September 2010.

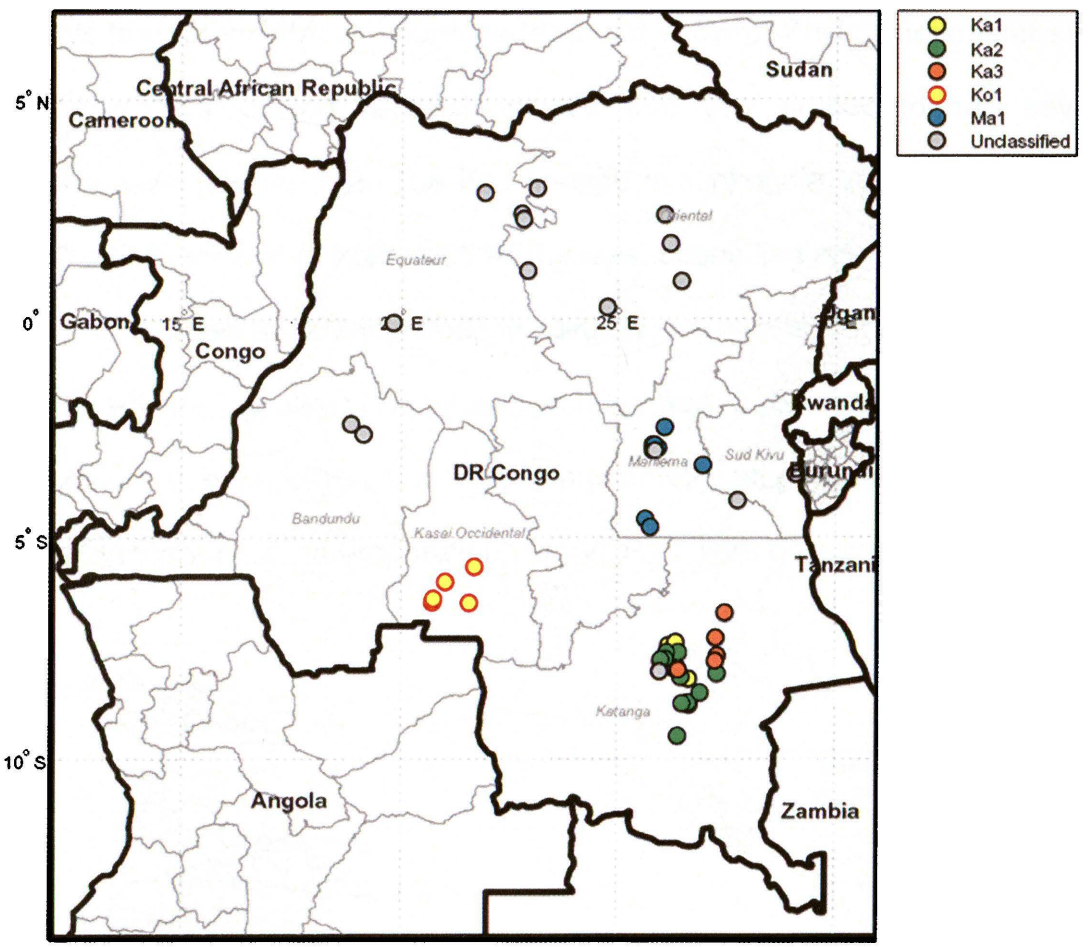
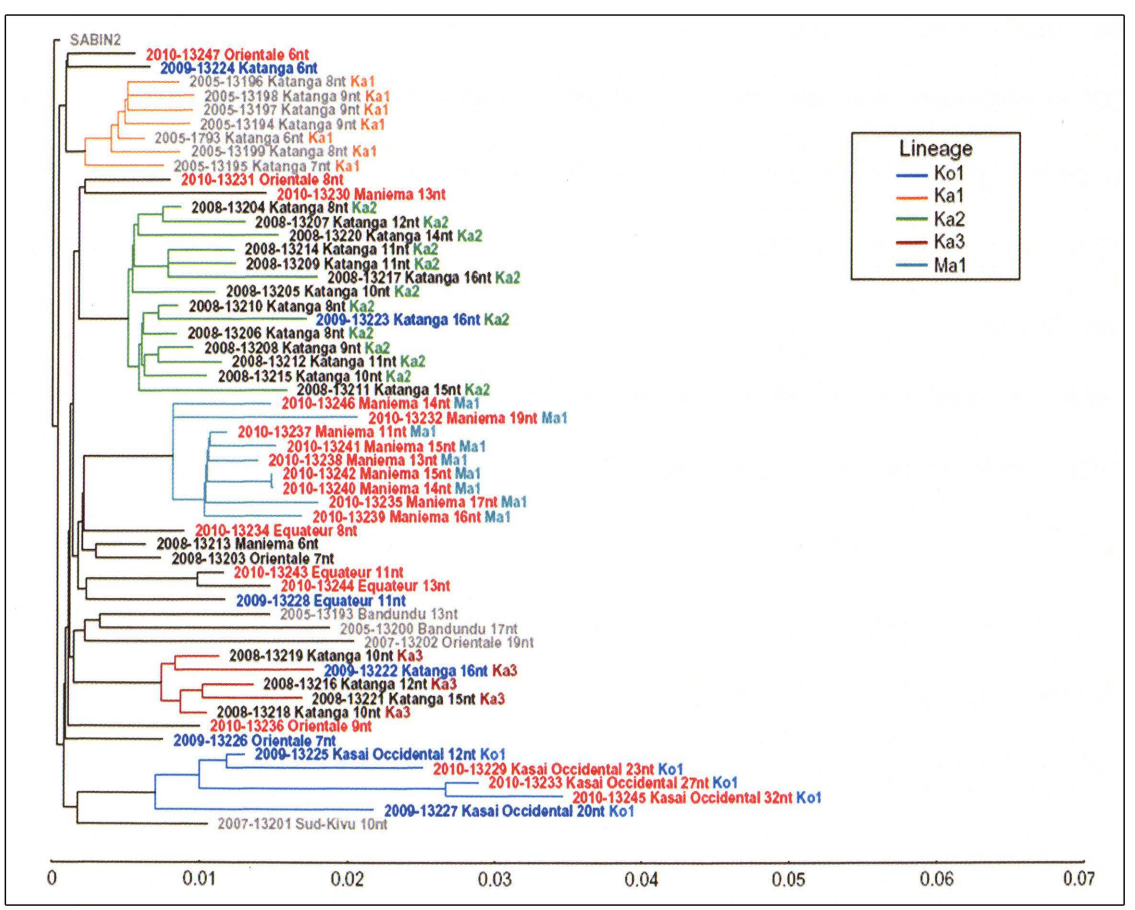


Figure 5.1: A map showing a geographical distribution of VDPV from the DRC

5.3.2 Phylogenetic analysis of the VP1 region of the VDPVs

The DRC type 2 cVDPVs characterized in this study were found in the southern part of the DRC and classified into 5 lineages (Ka1, Ka2, Ka3, Ko1 and Ma1) and several were referred as unclassified group identified in Bandundu, Equateur and Oriental provinces (Figure 5.1). We found that the DRC VDPVs were not closely related to previously isolated VDPVs from other African countries (data not shown). Phylogenetic analysis of the DRC capsid VP1 coding regions showed that the viruses formed several

(Figure 5.1). We found that the DRC VDPVs were not closely related to previously isolated VDPVs from other African countries (data not shown). Phylogenetic analysis of the DRC capsid VP1 coding regions showed that the viruses formed several independent lineages (Figure 5.2). The Ka1 lineage in Kinkondja and Malemba-Nkulu consisted of 7 cVDPVs isolated from 2005 AFP cases, circulating primarily in Kinkondja. Circulation of this lineage apparently stopped abruptly in 2005, as no additional related viruses were detected. The other four viruses isolated from 2005 and 2007 cases were not closely related to each other, and they represented independent emergences of VDPV from OPV (Shaw et al., in preparation). No VDPVs were detected in 2006.



In 2008, two concurrent cVDPV2 outbreaks occurred in Katanga. Lineages Ka2 and Ka3 were represented by 15 and 5 viruses, respectively. Both Ka2 and Ka3 outbreaks continued into 2009. Ka3 circulated primarily in Mulongo and Manono districts, whereas the Ka2 lineage circulated primarily in Kinkondja, Mukanga and Butumba. Viruses from these lineages were not detected after 2009. These two outbreaks accounted for 20 cVDPVs, almost one third of the total number of VDPVs detected. Those in the KAT province showed 99.20% to 98.2% similarity to Sabin type 2.

In 2008 and 2009, several independent emergences of VDPVs occurred in the provinces of MAN, ORT, and EQT. These sporadic detections were scattered in districts such as Kabondon-Dianda, Yahuma, Yambuku, Bumba, Monieka, Aketi, Salamabila, Banalia throughout the 3 provinces. In 2009, an outbreak was detected in KOC, and it continued into 2010 (Ko1 lineage). In 2010, an outbreak (Ma1 lineage) occurred in the MAN province in several districts (last case = RDC-MAN-KAM-10-008 onset 26 October 2010). The virus from this case had accumulated up to 14 nucleotide differences from Sabin type 2 in VP1 region. The viruse from MAN province showed 99.34% to 97.9% similarity to Sabin type 2. The 0.66-3.5% VP1 sequence divergence of the DRC lineages indicates circulation of approximately 6 months to 3.5 years, based on the previous published estimates of a rate of 1% per year for PV nucleotide sequence evolution (Alexander, Gary et al. 1997; Jorba, Campagnoli et al. 2008). The Ko1 lineage exhibited the highest sequence divergence, with the VP1 sequences showing 98.67% to 96.5% similarity to Sabin type 2. The sequence with the highest divergence from Sabin type 2 was RDC-KOC-TKP-10-008, with 32 nucleotide differences. Based on the rate of nucleotide evolution, it is likely that this lineage emerged and started to circulate in 2008.

5.4 DISCUSSION

In this study, we identified 58 AFP cases associated with type 2 VDPVs in the DRC in the period 2004 to 2010. Sequence analysis of the VP1 coding region showed that 41 of these isolates represented 5 outbreaks of cVDPV's, and 17 others represented several separate independent VDPV emergences. Although one third of the VDPVs were isolated from cases in a single province in DRC, favorable conditions for VDPV emergence existed throughout much of DRC.

The VDPV2 definition was changed recently in response to mounting evidence that viruses with less than 10 nucleotide differences were circulating and causing paralysis in human populations. In order to detect cVDPV and respond rapidly by immunization, it was necessary to develop a new definition for type 2 VDPV. For example, viruses in the Ka1 lineage had 6 – 9 nt. differences in VP1 and would not have been considered to be VDPV according to the old VDPV2 definition. The need to classify these programmatically relevant polioviruses as VDPV was shown by the genetic evidence of circulation, their ability to cause paralysis, and loss of the genetic markers of attenuation. Likewise, four of the viruses in the Ka2 lineage have 7 - 8 nt. differences from Sabin and are closely related to viruses that have more than 9 nt. differences from Sabin 2.

AFP surveillance has been a key strategy used to achieve eradication of poliomyelitis world-wide. The poliomyelitis outbreaks in the DRC demonstrated sub-optimal vaccine coverage, however, which led to outbreaks caused by both imported wild-type poliovirus and emergence and circulation of VDPVs during the period discussed. The Katanga province had more VDPV cases than the other provinces; this could be due to sub-

optimal vaccine coverage or better AFP surveillance than elsewhere in the country. Follow up investigations revealed that immunization coverage had been low (43%) at the beginning of the study conducted by Odusanya et al in 2006 (Grais, X et al. 2006). Vaccine coverage was low nationwide in the DRC, and wild type poliovirus is still circulating in the DRC (CDC. 2009a). The cVDPV2 outbreaks in MAN and KOC might still be ongoing.

A high level of geographic clustering was observed for viruses within a lineage. For example, the 9 AFP cases in the Ma1 lineage were found in one province, in 7 districts. Likewise, six of the seven viruses in the Ka1 lineage were from the Kinkondja district; the remaining virus was from nearby district Malemba-Nkulu, all in Katanga province. The extent of geographic clustering varies by serotype for poliovirus, although this has been studied primarily for types 1 and 3 poliovirus.

The detection of several distinct cVDPV lineages in the DRC suggest that cVDPVs can emerge independently in locations where there is inadequate immunity to poliovirus (Kew, Wright et al. 2004); (Wassilak, Pate et al.). The cVDPVs recovered the two most important biological properties of wild polioviruses, namely, (i) the capacity to cause paralytic disease in humans and (ii) the capacity for continuous person-to-person transmission. VP1 amino acid changes in position 143 associated with the reversion to virulence were identified. The origin of cVDPV2 in the DRC was probably the result of low population immunity to poliovirus, due to a combination of low vaccine coverage in some communities and the prior elimination of the indigenous wild poliovirus of the same serotype. Such risk factors are the same as for wild-type poliovirus (WPV) circulation, (Nathanson and Martin 1979; Fine and Carneiro 1999) and imported wild

polioviruses circulated in RDC during this time period. Poor hygiene and sanitation and tropical climate were probably additional factors that facilitated circulation of both WPV and VDPVs in some communities. The occurrence of WPV outbreaks during the same period emphasizes the need to maintain high vaccine coverage and AFP surveillance to minimize the risk of emergence of VDPVs or circulation of imported WPVs. Because similar conditions may exist elsewhere in Africa, ongoing high-quality surveillance will be essential for the achievement of polio eradication in Africa.