CHAPTER 1

Literature Review

1.1. INTRODUCTION

Poliovirus, a member of the Enterovirus genus of the family *Picornaviridae*, is the cause of paralytic poliomyelitis which occurs only in a small proportion (<1%) of poliovirus infections of susceptible individuals (Kew, Sutter et al. 2005). Poliovirus has a predilection for the motor nerve cells of the brain and spinal cord and infection results in their destruction and paralysis of the muscles supplied by the affected cells (Gear 1993). Some infected people experience minor illness of several days and a symptom-free interval of 1 to 3 days, followed by acute onset of flaccid paralysis with fever. Within few days, paralysis which is usually asymmetric, progresses depending upon the sites of virus replication in the central nervous system and may affect skeletal muscles (spinal poliomyelitis), respiratory muscles (bulbar poliomyelitis), or both (bulbo-spinal poliomyelitis) (Racaniello 1996). Polioviruses have been around for a long time in man's history with >99% reduction of cases since 1988 (WHO 2004). Before the development of killed in the 1950s 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 (Melnick 1983). Two different poliovaccines have been developed, a), the inactivated poliovirus vaccine (IPV) of Salk and Youngner (Salk 1954; Plotkin 2004) and b) the live, attenuated oral poliovirus vaccine (OPV) of Sabin (Sutter, Caceres et al.

2004). Both IPV and OPV; have their particular advantages and disadvantages, OPV needs 4 or more doses, while IPV only need 2 or 3 doses. OPV has high intestinal immunity while IPV low intestinal immunity. OPV is cheap compared to IPV (Melnick 1978).

Active vaccination campaigns have been implemented since 1980 with the original aim of eradicating polio by the year 2000. This has led to a reduction in poliovirus cases worldwide with many developed and developing countries being declared polio-free. In parts of the developing world however, certain regions have remained endemic. Major setbacks have been experienced since 2002 with importations and outbreaks especially in Africa.

1.2. POLIOVIRUSES

1.2.1. *Classification*

Polioviruses are members of the Picornaviridae family, a group of non-enveloped positive-strand RNA viruses (Martin, Dunn et al. 2000). They group into three distinct serotypes, type 1, type 2 and type 3, and replicate mainly in the gut. Viruses within a serotype exhibit greater than 70% homology at the nucleotide level and 88% at the amino acid level. Poliovirus has an icosahedral structure, which consists of 60 identical asymmetrical protomers. These protomers are arranged along fivefold, threefold and two fold axes (Rueckert, Dunker et al. 1969). Each protomer is composed of virion proteins: VP1, VP2, VP3 and VP4. VP1 exhibits the greatest sequence variability, and plays a key role in receptor attachment (Rueckert, Dunker et al. 1969). VP1, VP2 and VP3 are very similar in tertiary structure but differ in size and in their amino acid level (Hogle, Chow et al. 1985). VP4 is the smallest structural protein and is located on the

VP3 are very similar in tertiary structure but differ in size and in their amino acid level (Hogle, Chow et al. 1985). VP4 is the smallest structural protein and is located on the inner surface of the capsid (Minor 1992). Each capsid protein present a common structure of an 8-stranded antiparallel β-barrel core but differ both in N- and C- terminals and in their size and loop structure that connect the outer strands of the β-barrels (Figure 1.1). The loops represent the major antigenic sites of the virus. The N- terminal are intertwined internally forming a connected network that contributes to its stable structure (Hogle, Chow et al. 1985).

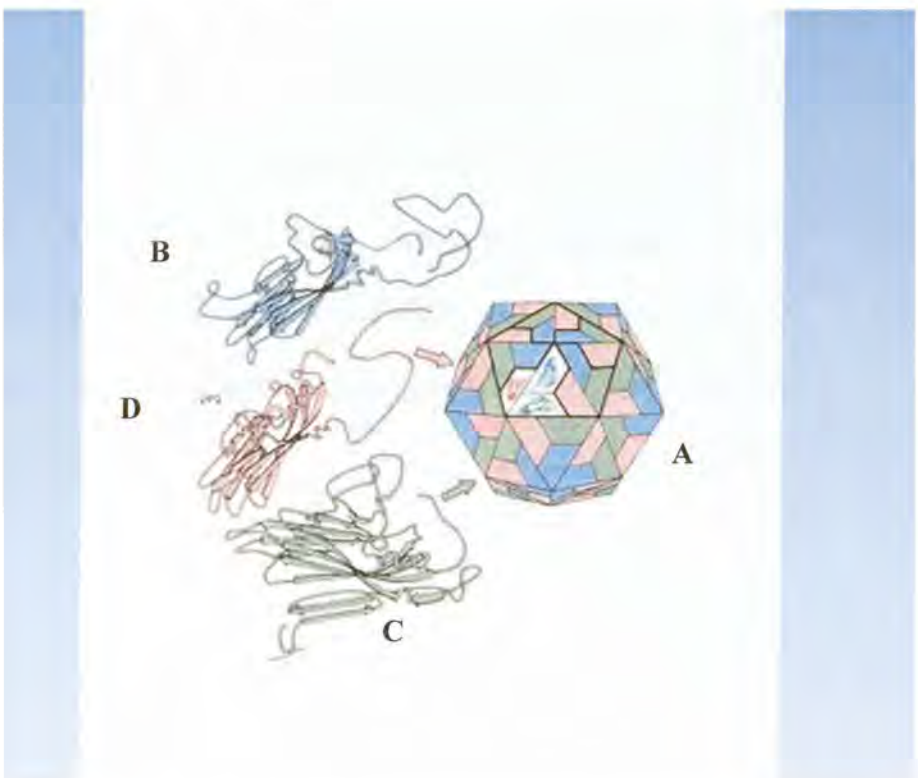

Figure 1.1: (A) Schematic representative of the icosahedral capsid structure of poliovirus, (B), (C) and (D) represent VP1, VP2 and VP3 proteins respectively in their tertiary configuration. (reproduced from http://www.biol.vt.edu/faculty/lederman/biol4664/text/images/).

1.2.2. *Genome organization and Replication*

The genomic RNA is approximately 7500bp long and terminates at the 3'-end in a polyadenylated tail of 70-100 nucleotides, and is covalently bound at the 5'-end to a small protein known as VPg (Figure 1.2). A 5' non-translated region (NTR) of about 740 nucleotides precedes a single large open reading frame. The coding region is translated as a single polyprotein that is post-translationally processed to generate the viral capsid that consist of VP4, VP2, VP3 which are products of proteolytic processing of the precursor, P1. The N- termini of VP0, VP4 and P1 are myristylated. Proteolytic processing of P2 and P3 non-structural proteins give rise to polypeptides $2A^{pro}$, 2B, 2C, 3A, 3B, $3C^{pro}$ and $3D^{pol}$ (Wimmer and Nomoto 1993)). Non-structural proteins have two or more distinct functions. For example, the 3C region code for a protease ($3C^{pro}$) which is responsible for the maturation cleavages in the precursor polyproteins. The 3D regions code for an RNA-dependent RNA polymerase ($3D^{pol}$) which has regions of homology with many DNA and RNA polymerases (Porter, Ansardi et al. 1993) (Figure 1. 2).

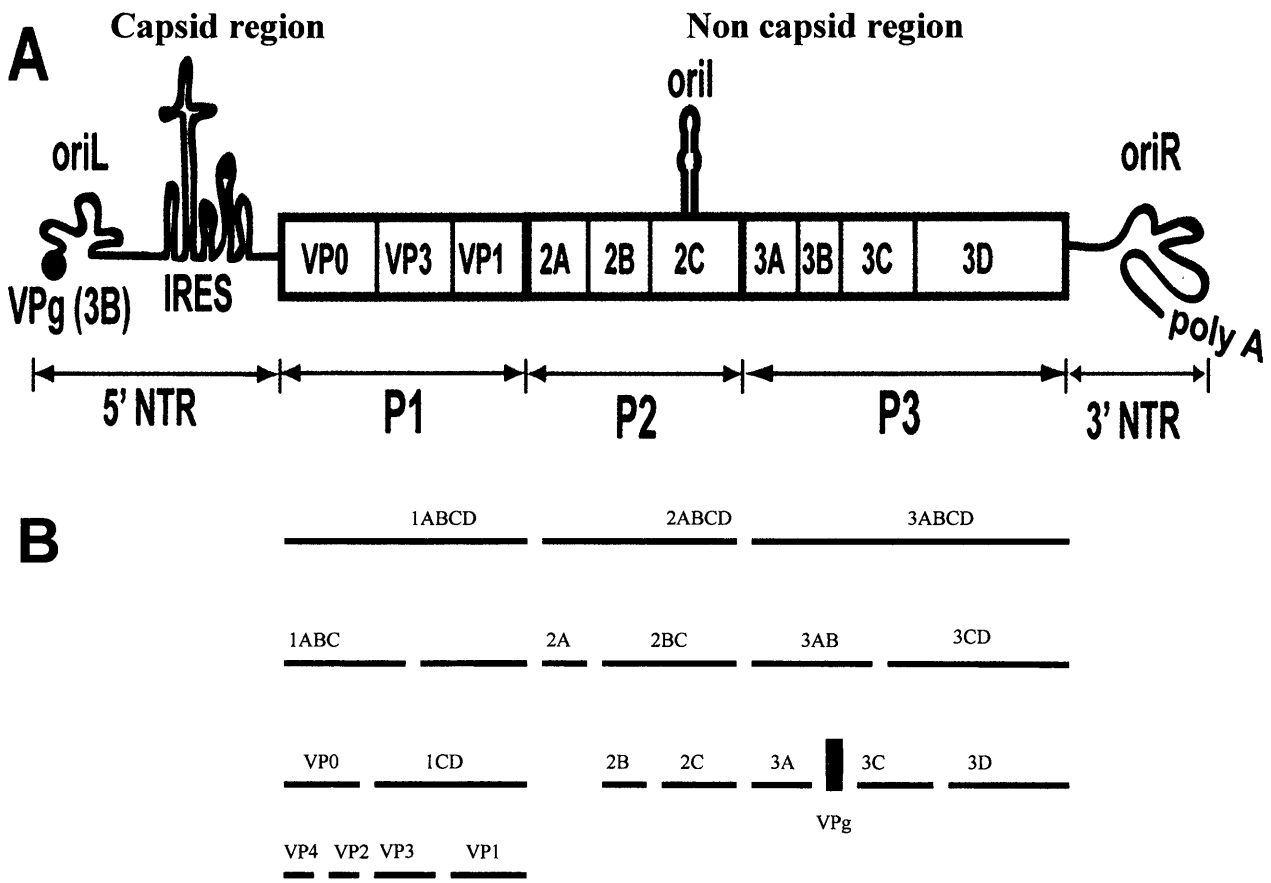

Figure 1.2. (A) Poliovirus genome organization and (B) processing pathway (reproduced from (Oh, Pathak et al. 2009).

The proteolytic processing can be divided into three stages, (i) the cleavage of P1 capsid protein precursor from the nascent polypeptide, at a junction of VP1 and 2A which is catalysed by the viral protease $2A^{pro}$(Toyoda, Nicklin et al. 1986), (ii) $3C^{pro}$ and $3CD^{pro}$ catalyse the processing of the capsid and non-capsid precursors. 3AB gives rise to 3B which represents VPg and thought to be involved in initiation of RNA synthesis (Pallansch, Kew et al. 1980). 3D is responsible for elongating nascent RNA chain from the RNA template (Flanegan, Petterson et al. 1977). 2B and 2C have key support functions in RNA replication (Bernstein, Sarnow et al. 1986);(Li and Baltimore 1988). 2A

is involved with abolishing of cap-dependent translation and also involved with cap-independent translation (Bernstein, Sonenberg et al. 1985);(Macadam, Stone et al. 1994), and (iii) during encapsidation of the viral RNA, VP0 is cleaved into VP4 and VP2 (Rueckert, Dunker et al. 1969).

The non-translated regions on each side of the protein coding region consist of highly conserved sequences. The initiation of translation takes place at the 5' NTR while initiation of RNA synthesis is at the 3' end of the positive and negative sense strand respectively (Rueckert, Dunker et al. 1969). Most eukaryotic mRNAs have a m^7GpppNp cap structure at the 5' nontranslated end, a characteristic that is missing in the poliovirus secondary structure. The 5' NTR is about 740 nucleotides in length (Kitamura, Semler et al. 1981);(Racaniello and Baltimore 1981). The 5' NTR contains a stem loop structure and has an unusually large number (8) of AUGs prior to the authentic start at nucleotide 743 (Figure 1.3) (Rivera, Welsh et al. 1988); (Pilipenko, Blinov et al. 1989); (Skinner, Racaniello et al. 1989). The 5'-end of the genome forms a cloverleaf structure with 90 nucleotides that interacts with VPg protein and viral protein 3CD to form a complex for RNA synthesis (Skinner, Racaniello et al. 1989); (Andino, Rieckhof et al. 1990); (Harris, Xiang et al. 1994); (Xiang, Harris et al. 1995).

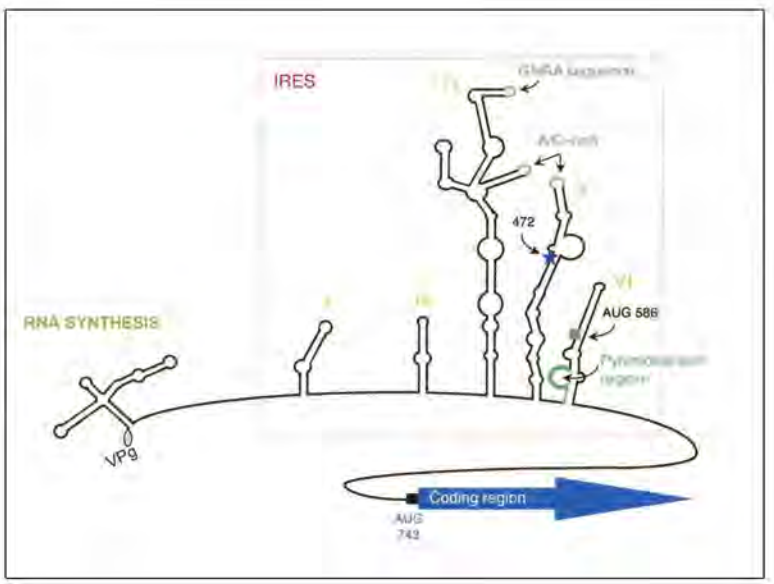

Figure 1.3: Predicted RNA secondary structure of the poliovirus 5′ NTR (Semler 2004). Computer prediction and chemical and enzymatic RNA-structure probing were used to deduce a consensus RNA conformation. Conserved sequences among picornaviruses include a NRA tetraloop (thought to function in tertiary 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)).

For cap-independent initiation of translation, it has been found that 5' NTR acts as a internal ribosomal (IRES) (Pelletier and Sonenberg 1988). Domains III, VI, and V are the main important cis-acting elements in the 5' NTR (Nicholson, Pelletier et al. 1991);(Percy, Belsham et al. 1992) and the AUG triplet which has shown to be complementary to conserved sequences in the 18S ribosomal RNA (Nicholson, Pelletier et al. 1991).

A common feature of all 3 Sabin strains is the specific mutation at the 5' NTR which in Sabin 1 is located at position 480 (Kawamura, Kohara et al. 1989)), 481 for Sabin 2 (Equestre, Genovese et al. 1991); (Macadam, Ferguson et al. 1991); (Ren, Moss et al. 1991) and 472 for Sabin 3 (Cann, Stanway et al. 1984); (Evans, Dunn et al. 1985);(Westrop, Wareham et al. 1989). The attenuation mutations in the IRES of the 5' NTR alter the stem loop structure and reduce the initiation of translation of mutant poliovirus RNA (Svitkin, Pestova et al. 1988); (Ehrenfeld and Gebhard 1994); (Gutierrez, Denova-Ocampo et al. 1997).

1.2.3. ***Poliovirus infection cycle***

The poliovirus replication starts with the interaction of the poliovirus with host cell's surface protein and the poliovirus receptor (PVR) called: CD-155. The PVR has been identified as a member of a family of proteins called the immunoglobulin (Ig) superfamily. The PVR has three loops expressed outside the cell arranged as follows: V-C2-C2, where V is variable and C is constant (Mendelsohn, Wimmer et al. 1989). The protein extends through the bilayer cell membrane as represented in figure 1.4. The initial event involved the attachment of the virion to the PVR (step 1), this triggers the conformational change in the virus capsid that involves the loss of the internally located VP4 protein (De Sena and Mandel 1977), extrusion of the VP1 protein (Fricks and Hogle 1990) and to the delivery of viral RNA across the membrane by the process of endocytosis (step 2) leading to the process of translation (step 3). The viral RNA strand is copied to make the negative strand genomic RNA in order to form the complementary new plus strands (step 4), which takes place in the endoplasmic reticulum (Caliguiri and Tamm 1970). The newly synthesized plus strand recycled over and over in additional

replication centres leading to a higher levels of viral protein production (steps 5, 6, and 7). The newly synthesized RNA molecules are packaged into virions (Baltimore, Jacobson et al. 1969). For virions assembly to take place, coat precursor protein P1 must be cleaved to form immature protomers composed of VP0, VP3 and VP1. With an increasing of proteinase activity, the protomers are assembled to form provirions which are packaged to uninfectious provirions (step 8).

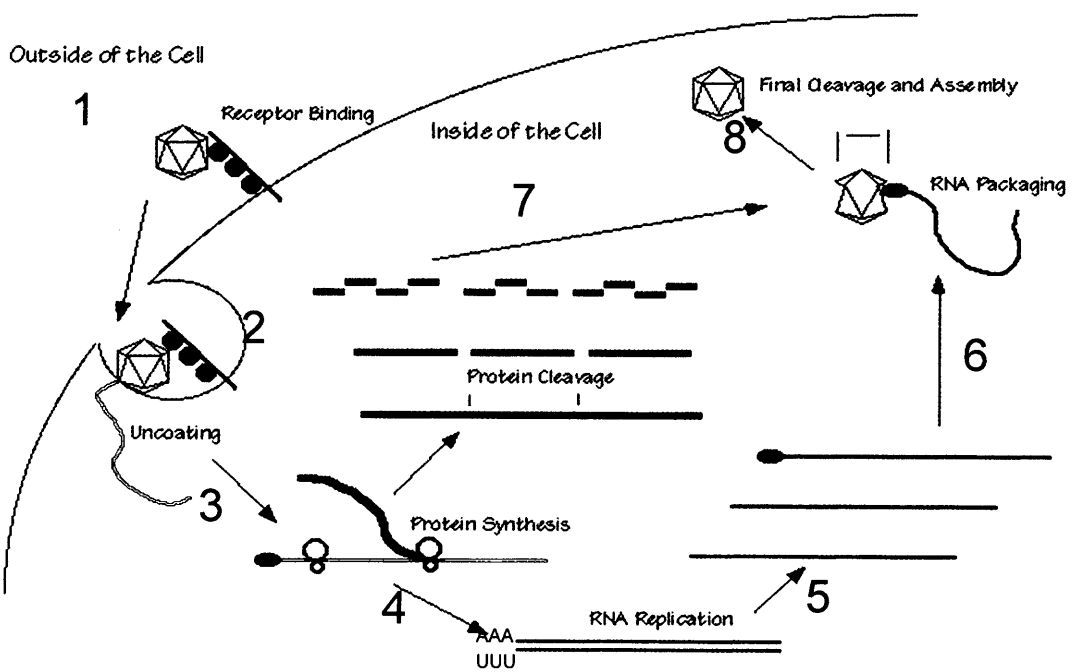

Figure 1.4. Poliovirus infection cycle (reproduced from the Polio Information Center Online, http://microbiology.columbia.edu/pico/Chapters/cellular.html

1.2.4. *Poliovirus vaccine*

Poliovirus vaccination with oral poliovirus vaccine (OPV), consisting of live attenuated strains of each of the three poliovirus serotypes (Nathanson and Fine 2002), has resulted in a worldwide decrease in the circulation of the wild polioviruses (WPV) (Wood, Sutter et al. 2000). The oral vaccine originally developed by Sabin in the 1960's and the inactivated poliovirus vaccine (IPV) developed by Salk in the 1950s (Salk, Krech et al. 1954) have made poliomyelitis a preventable and eradicable disease. Despite the success in the developed world, over 4000 cases were reported in Africa from January 2000 until now. The only remaining endemic country in the region is Nigeria, but additional outbreaks in several countries have resulted from importations from this reservoir and from outside the region (Taren, Nesheim et al. 1987). The advantages of OPV over IPV are that it can be easily administered by mouth, thereby, facilitating its widespread use; it induces intestinal immunity, making recent OPV recipients resistant to infection by polioviruses and effectively blocking wild poliovirus transmission when used in mass campaigns; and it provides long–term protection against polio through durable humoral immunity (Nathanson and Fine 2002; Dowdle, De Gourville et al. 2003). Despite its many advantages, OPV use carries certain risks (Fine and Carneiro 1999). OPV virus can spread from OPV recipients to unvaccinated contacts (Heymann 2004). The appearance of cases of vaccine-associated paralytic poliomyelitis (VAPP) was the evidence of clinical disease following genetic changes of OPV (Kim, Kim et al. 2007).

One of the adverse events associated with OPV use is vaccine-associated paralytic poliomyelitis (VAPP), which is characterized by clinical signs typical of paralytic poliomyelitis and also isolates shed from VAPP cases that typically contain neurovirulent

vaccine-related virus (Sutter, Caceres et al. 2004). Despite the extraordinary safety record of OPV, cases of VAPP demonstrate the clinical consequence of the genetic instability of OPV strains. There are ~250-500 annual VAPP cases worldwide and most of them occur in countries already free of polio (Sutter, Caceres et al. 2004). The incidence of VAPP is highest in immunodeficient people (iVAPP), particularly among B-cell immunodeficiencies. Laboratory findings suggest that Sabin 1 vaccine is rarely associated with VAPP cases among immunocompetent individuals, whereas Sabin 3 and Sabin 2 are more frequently associated to iVAPP cases. Isolates from VAPP cases share the genetic reversion to the neurovirulent phenotype and many of them are vaccine/vaccine recombinants. Regardless of the extent of divergence between VAPP isolates and their parental Sabin vaccine, VAPP isolates are attenuation revertants and VAPP cases are clinically indistinguishable from cases associated with WPV. The minimum difference separating wild polioviruses from Sabin strains is >15% divergence at the VP1 region. Closely related (<1% VP1 divergence) Sabin-like strains are classified as OPV-like strains.

Although cases of VAPP have long been recognized, more recently two additional OPV-related problems have been identified that have a significant impact on polio eradication. These are the long-term excretion of vaccine-derived polioviruses (VDPVs) in persons with primary humoral immunodeficiencies, so-called immunodeficiency vaccine-derived poliovirus (iVDPV) and polio outbreaks associated with circulating vaccine-derived poliovirus (cVDPV) in areas with low rates of OPV coverage(Kew, Sutter et al. 2005). Poliovirus isolates sharing between 1% and 15 % of VP1 divergence from Sabin reference strains are VDPVs.

VDPVs are classified into three categories, namely: 1) the immunodeficiency vaccine-derived polioviruses (iVDPV) obtained from person with defects in antibody production (Bellmunt, May et al. 1999); (Kew, Sutter et al. 1998); (Yoneyama, Hagiwara et al. 1982), 2) circulating vaccine-derived polioviruses (cVDPV) associated with person-to-person transmission (Yang, Naguib et al. 2003) and 3) ambiguous vaccine-derived polioviruses (aVDPVs) isolated from environmental isolates whose ultimate source has not been identified (Kew, Sutter et al. 2005).

The immediate public health importance of cVDPV was underscored by episodes of cVDPV in Egypt (1988-93) (Yang, Naguib et al. 2003), Hispaniola (2000-01) (Kew, Morris-Glasgow et al. 2002) (CDC. 2001d) (CDC. 2001e), Philippines (Kew, Morris-Glasgow et al. 2002), Madagascar (2001-02, 2005) (Rakoto-Andrianarivelo, Gumede et al. 2008) (CDC. 2002b and Nigeria (2005-2011) (CDC.2009b). Recent molecular studies have also detected the likely localized spread of OPV-derived virus in previous decades in Belarus (1965-66) (Korotkova, Park et al. 2003). cVDPVs show a significant sequence drift which indicates a prolonged replication of the vaccine strain and thereby acquiring the phenotypic property of transmissibility and circulation in the community. 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 poliomyelitis (Fine and Carneiro 1999; Wood, Sutter et al. 2000; CID.2002). In addition persons with primarily immunodeficiency are at risk to become chronic excretors of polio vaccine viruses and may represent a potential reservoir for poliovirus reintroduction after polio eradication. Once exposed to OPV, immunocompetent persons excrete polio vaccine viruses for up to 2 – 3 months (Alexander, Gary et al. 1997; Jorba, Campagnoli et al. 2008) although cases of the

prolonged excretion of VDPV by immunodeficient persons have been reported (MacCallum 1971);(Lopez, Biggar et al. 1974);(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). This could also influence the decision on polio vaccination cessation if poliovirus eradication is achieved.

To increase the sensitivity of detecting VDPV's, the Global Polio Laboratory Network (GPLN) implemented additional testing requirements in 2001 for all isolates under investigation (World Health Organization recommendations to Polio Reference Centers, 2002): This includes an ELISA test to identify non-Sabin-like strains and a PCR test to identify Sabin-related strains. Isolates that showed a discrepancy between the two tests are considered to be potential VDPV's. It is known that ELISA does not detect all VDPVs, therefore a new VDPV screening method known as real-time assay has been introduced into the GPLN. All potential VDPV's are therefore also confirmed by sequencing. The potential risk of cVDPV emergence has increased dramatically in recent years as wild poliovirus circulation ceased in most of the world and vaccine coverage dropped in some countries. The risk appears highest for the type 2 OPV strains because of its greater tendency to spread to contacts.

Although the mutations associated with reversion of Sabin to virulent wild virus are known, a predictive scoring system that will allow the identification of high risk Sabin drift towards VDPVs will be invaluable for the early detection and control of VDPV outbreaks. It has been shown that recombination events between circulating enteroviruses and polio vaccine were associated with some of the current VDPVs.

The control of poliovirus in the post-eradication era remains a big challenge. One of the suggested options for the complete eradication of live poliovirus from the world is to stop the use of OPV worldwide at some point after the world has been certified free from circulating wild polioviruses (Cherkasova, Korotkova et al. 2002). This may limit the circulation of derivatives of the Sabin strains, and it is assured that the derivatives will be unable to survive in nature long enough to evolve into highly transmissible neurovirulent strains (Fine and Carneiro 1999); (Wood, Sutter et al. 2000). The long-term persistence of vaccine derivatives in immunocompromised persons and the ability of the evolved variants to cause paralytic disease are well-established phenomena (Bellmunt, May et al. 1999);(Yoneyama, Hagiwara et al. 1982); (Kew, Sutter et al. 1998). This and low vaccine coverage in a population my result in the emergence of a new setback to polio eradication namely, virulent vaccine-derived polioviruses.

1.3. **EPIDEMIOLOGY**

The most neurovirulent of the three serotypes is poliovirus type1 (Salk 1956) which causes most epidemics followed by type 3 and type 2 (Salk 1956). Poliovirus is transmitted through fecal-oral route and the infection remains 7 to 14 days after the onset of paralysis. Poliovirus is excreted in the feces for approximately 3 to 6 weeks (Gelfand, Fox et al. 1957). Susceptible households have high secondary infection rate (Gelfand, Fox et al. 1957). The period of 3 to 6 days is enough for incubation between infection and first symptoms of minor illnesses, and from infection to the date of onset of paralysis usually 7 to 21 days (Horstmann and Paul 1947). Previously, an immunocompetent person was thought to excrete poliovirus for a period of 4 to 6 weeks

and an immunocompromised person can excrete from 3 years to 7 years as reported for a patient with common variable immunodeficiency disorder (CVID) who acquired vaccine-associated paralytic poliomyelitis (CDC. 1997a); (Kew, Sutter et al. 1998).

Forty-eight previously reported outbreaks occurred between 1976 and 1995 involving approximately 17000 unvaccinated and inadequately vaccinated cases, caused by poliovirus type 1 (Patriarca, Sutter et al. 1997). In the developing countries these cases were amongst children of 0 - 2 years and in industrialised countries occurred in older people (Sutter, Jafari et al. 2008). In developing countries, lower socioeconomic conditions have been shown to be one of the risk factors for paralytic poliomyelitis due to vaccine failure after OPV administration because of concurrent enterovirus infections (Bernkopf, Medalie et al. 1957).

Wild animals such as great apes, chimpanzees, gorillas and orangutans can experience paralytic disease after poliovirus infection, and such outbreaks had been reported in literatures by Allmond et al. and Dowdle et al.(Allmond, Froeschle et al. 1967; Dowdle and Birmingham 1997). In contrast, monkeys cannot be infected by OPV and cannot participate in the chain of transmission; therefore animals are not thought to be reservoir for poliovirus (Dowdle and Birmingham 1997).

The epidemiology of poliomyelitis has been observed in three patterns; 1) endemic, 2) epidemic and 3) vaccine era (Kew, Ryves et al. 1997; Sutter, Jafari et al. 2008). In the 19th century and the beginning of the 20th century, a change from endemic to epidemic was observed in the countries such as United States, Norway and Sweden. In developing countries such as Nigeria, Pakistan, Afghanistan and India an endemic

pattern is dominating while transmission of indigenous wild poliovirus was interrupted in other countries by year 2006 (CDC. 2010a) .

In 2011, reported polio cases amounted to ~618 (only from serotypes 1 and 3), a >99% decline since 1988 in which the number of polio cases reached an estimated number of ~350,000 (from all three serotypes) while the number of endemic countries declined from 125 to 4 during the same period of time (Figures 1.5 and 1.6). Three WHO Regions have already been declared polio free: 1) the Americas Region in 1994, 2) the Western Pacific Region in 2000, and 3) the European Region in 2002. However, there are several immediate challenges facing the GPEI, among them: (a) the re-introduction of wild polioviruses in polio free countries like Angola, Chad, Democratic Republic of the Congo and Sudan (CDC. 2009b) and (b) appearance of polio outbreaks due to the transmission of VDPVs (Kew, Sutter et al. 2005).

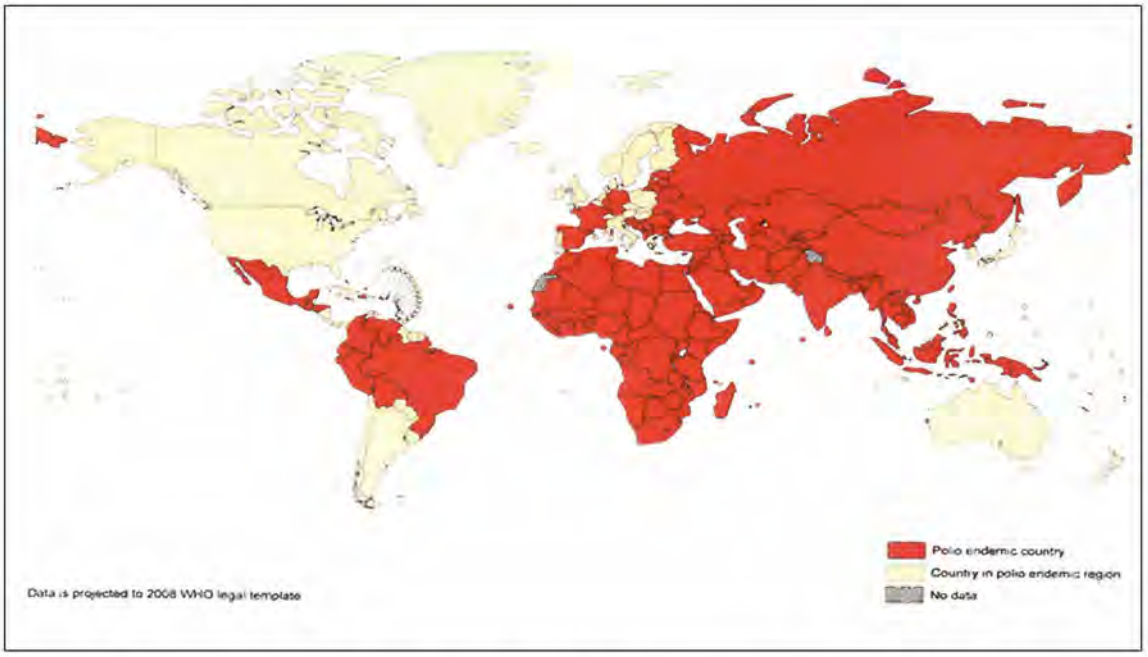

Figure 1.5: Countries in red with reported cases of poliomyelitis in 1988. Source: WHO

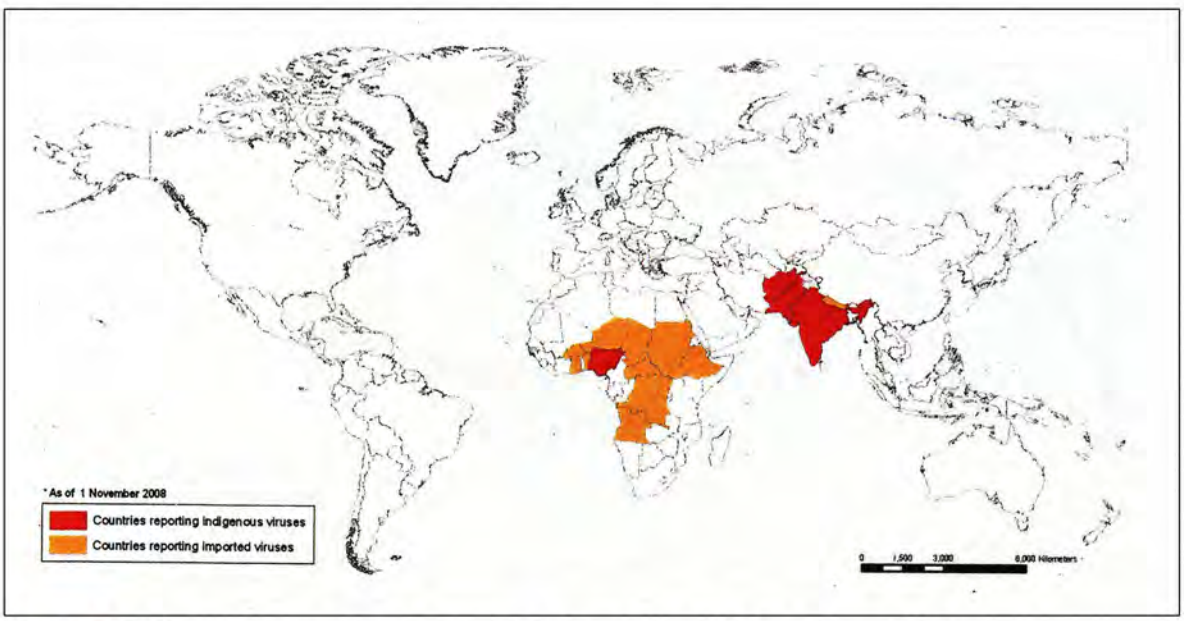

Figure 1.6: Countries with reported cases of poliomyelitis in 2010. Source: WHO

The recurring polio outbreaks especially in sub-Saharan Africa have been a setback in the eradication campaign. Many of these are re-infected countries, particularly in sub-Saharan Africa, at risk due to low routine immunization coverage (<80%), suboptimal outbreak response, weak health systems, and importations originating from the West Africa, into central Africa and to the Horn of Africa. By mid-2009, Angola, Chad, the DRC and southern Sudan had persistent for >12 months leading to their designation as having re-established transmission while other countries suffered new importations (Figure 1.7) (Jenkins, Aylward et al.).

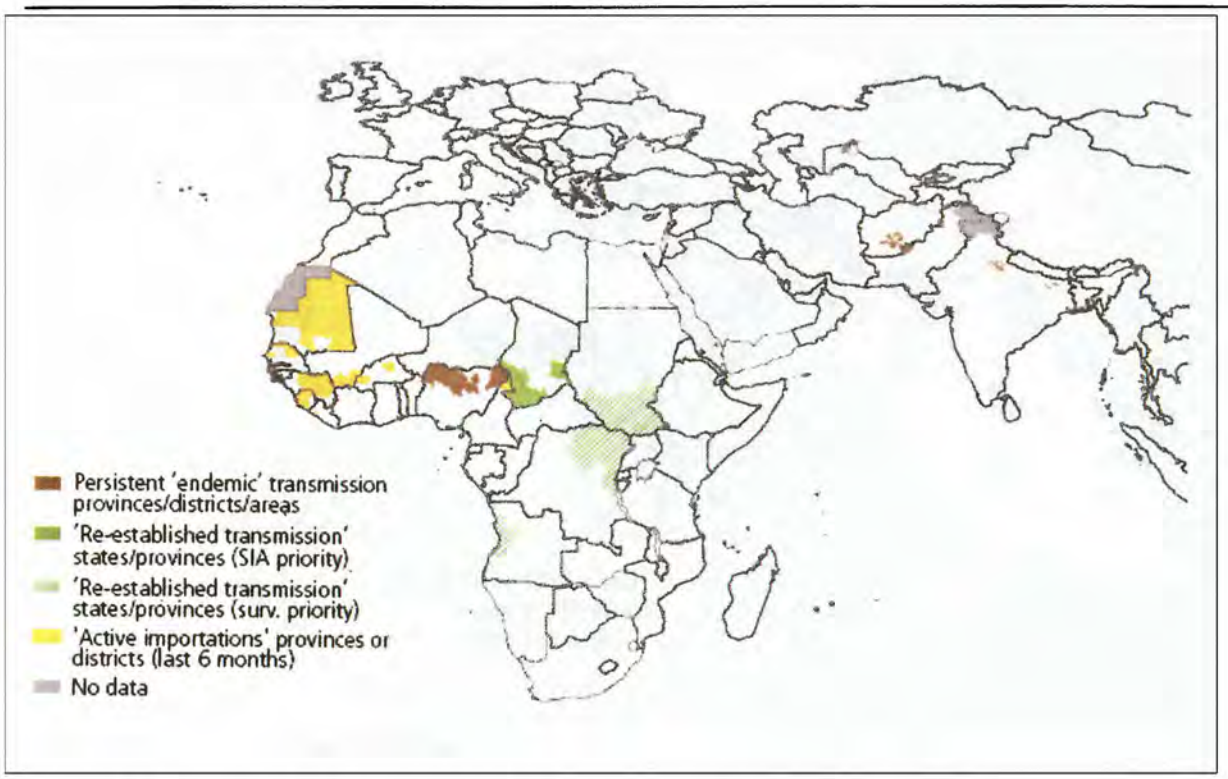

Figure 1.7: Countries with reported cases of poliomyelitis in 2010. Source: WHO

Over 500 AFP cases associated with VDPVs have been reported globally (Figure 1.8). Nigeria had the largest outbreak affecting neighbouring countries like Niger and Chad. The first VDPV case was identified from a child in 2002 (Adu, Iber et al. 2007). In Nigeria, the outbreak continued into 2011 and a total of 355 cases were reported (CDC. 2011).

As of July 2011, new outbreaks of VDPVs were identified in Afghanistan and India ((CDC. 2011). Ethiopia and the DRC also experienced emerging of VDPVs as described in Chapter 5 and Chapter 6.

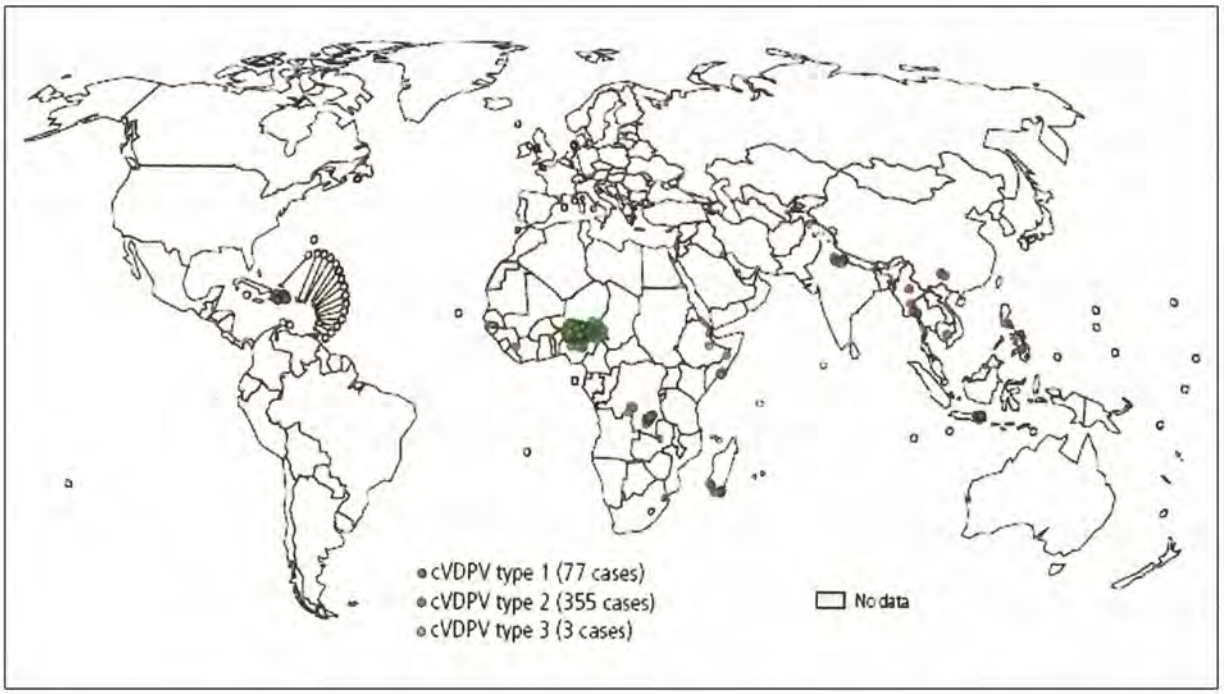

Figure 1.8: Countries with reported cases of vaccine derived polioviruses as of 2010. Source: WHO

Exposure to poliovirus occurred early in life but earlier theories suggested that the poliomyelitis was not a health burden as infants are protected from paralysis by maternal antibodies but this has been recently reviewed (Sutter, Jafari et al. 2008).

In the absence of vaccination, there would be more cases of paralysis per year according to WHO estimation and the vaccine coverage has resulted in some countries from the developing world to shift from endemic to epidemic pattern (Patriarca, Sutter et al. 1997), (Otten, Deming et al. 1992),(Afif, Sutter et al. 1997) (Reichler, Abbas et al. 1997).

National immunization campaigns were not developed in many developing countries until 1970s and 1980s and in 1990s the global OPV coverage with three doses reached 80% in children of 1 year old

(www.who.int/vaccines.../GlobalSummary/GlobalSummary.pdf.WHO/IVB/2006).

In order to achieve high vaccine coverage and control of poliomyelitis, supplemental doses of OPV in addition to routine coverage are needed (www.who.int/vaccines.../GlobalSummary/GlobalSummary.pdf.WHO/IVB/2006).

1.4. MOLECULAR EPIDEMIOLOGY

The Global Poliomyelitis Eradication Initiative (GPEI) was initiated in 1988, after the declaration of the eradication of smallpox in 1980 by the World Health Assembly, to eradicate poliomyelitis globally by 2000 (CDC. 2009a). The GPEI has been successful in reducing cases and by the end of 2010, cases had been reduced from 350,000 cases in 1988 to fewer than 1000 cases by the end of 2011, and Pakistan, Afghanistan and Nigeria the only remain endemic countries (CDC.2006c) .

Any case of new onset of hypotonic weakness in a child aged less than 15 years of age is defined as acute flaccid or floppy paralysis (AFP) which include Guillian-Barre' syndrome, transverse myelitis, enterovirus infections and traumatic neuritis. To make sure not to miss any polio case, AFP surveillance is targeting an AFP symptom rather that a specific disease (CDC. 2000a). AFP surveillance is a GPEI strategy to detect poliovirus circulation, re-importation of wild poliovirus into polio free-areas or regions and emerging VDPVs. As polio eradication is approaching, it is crucial to maintain high quality AFP surveillance

(www.hpsc.ie/hpsc/A-Z/VaccinePreventable/Polio/.../File,2461,en.pdf).

In 1996, National Immunization Days (NIDs) and AFP surveillance was implemented by the African Region (AFR) (CDC.2000c)(. WHO indicator of AFP surveillance, which is a measure of surveillance system sensitivity, is targeted at >/= 2 nonpolio AFP case per 100,000 population aged <15 years (CDC. 2000c). By 1999, AFP surveillance had improved and as a result of adequate surveillance, AFP cases were 4999 compared to 1754 reported in 1998 (CDC. 2000c). Wild poliovirus surveillance is based on AFP case investigation and virological studies (Kew, Sutter et al. 2005), as was the case of this study. There are different etiologies associated with AFP, hence a need of combining AFP surveillance with virological studies to investigate the role of wild polioviruses (Kew, Sutter et al. 2005), VDPVs and VAPP. In support of GPEI, a Global Polio Laboratory Network has been established by WHO to apply virological tools of poliovirus surveillance (Kew, Sutter et al. 2005). Methods of isolating virus from stool specimen (www.who.int/vaccines/en/poliolab/WHO-Polio-Manual-9.pdf.WHO/IVB/04.10); intratypic differentiation of vaccine from a wild poliovirus by the use of PCR (Kilpatrick, Nottay et al. 1996; Kilpatrick, Nottay et al. 1998) and ELISA (van der Avoort, Hull et al. 1995), and by real-time (Kilpatrick, Yang et al. 2009) and the molecular techniques were developed and implemented in the GPLN.

Polioviruses recombine with each other (Cooper 1968); (Cammack, Phillips et al. 1988); (Kilpatrick, Ching et al. 2004) and with human enteroviruses of species C (Guillot, Caro et al. 2000); (Liu, Zheng et al. 2003); (Shimizu, Thorley et al. 2004). For closely related isolates the nature and recombination junction provide markers for the resolution of poliovirus lineages (Liu, Zheng et al. 2003);(Cherkasova, Yakovenko et al. 2005).

The finding that polioviruses evolve at a rate of approximately 1% per year(Jorba, Campagnoli et al. 2008), has meant that the sequence analysis provides the resolving power for molecular epidemiological studies that has confirmed the existence of different genotypes or lineages within each outbreak (Liu, Zheng et al. 2000). Genotypes are found in different regions world-wide and are distributed geographically (Rico-Hesse, Pallansch et al. 1987) (Figure 1.9 and Figure 1.10). For WPV1, 10 genotypes were circulating worldwide between 1999 and 2004 with 8 out of 10 affecting the East, North and West African region. The remaining two affecting India, Pakistan and Afghanistan which are still endemic countries. For the same period, WPV3 had 5 genotypes that affected Africa and Asia, with 4 out of these circulating in the African region.

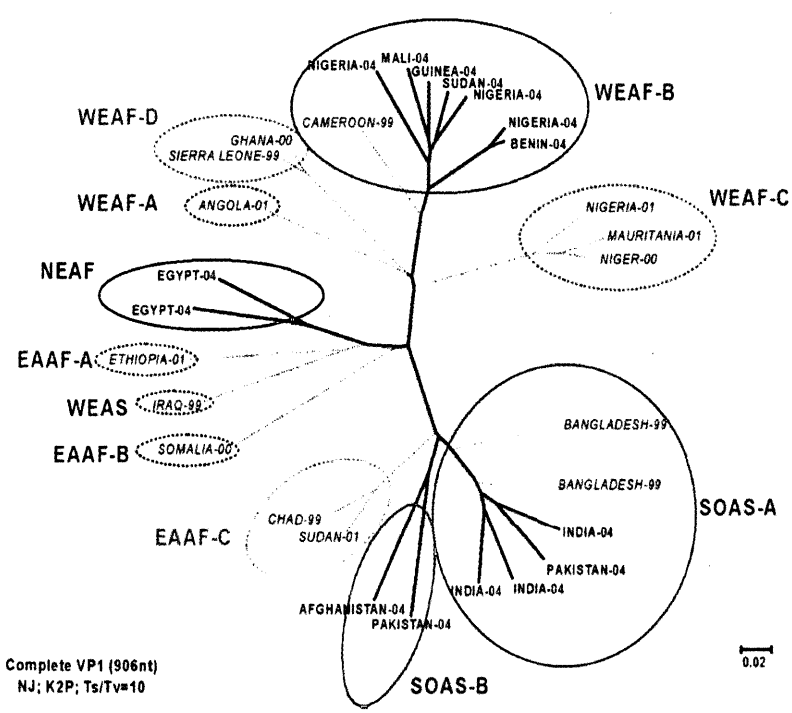

Figure 1.9: Neighbour-joining tree of WPV1 showing different genotypes from different regions between 1999 – 2004. Source: CDC.

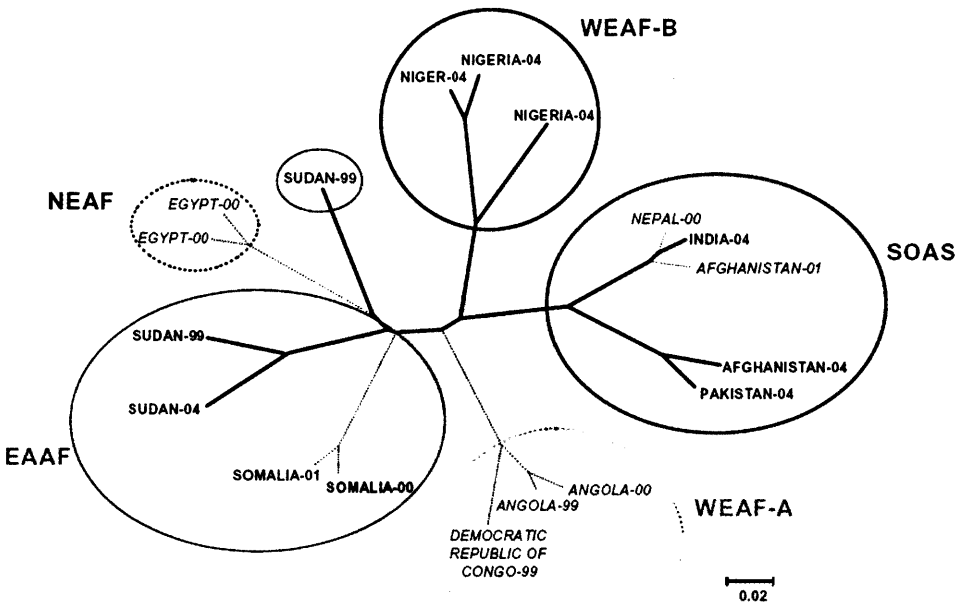

Figure 1.10: Neighbour-joining tree of WPV3 showing different genotypes from different regions between. 1999 – 2004. Source: CDC

Molecular epidemiology has been used to identify the poliovirus reservoirs, the pathway of poliovirus transmission, the tracing the sourcing of importation, the development of new molecular techniques and finally the progress towards the eradication of polioviruses (Kew and Nottay 1984).

Genomic sequencing and phylogenetic analysis of polioviruses are powerful molecular tools that help to understand the epidemiology of poliomyelitis disease, therefore poliovirus transmission and the characterisation of importation from endemic countries can be identified. These molecular methods have managed to identify and distinguish between different poliovirus endemic genotypes in outbreaks and locate them to their indigenous location (Shulman, Handsher et al. 2000; Kew, Morris-Glasgow et al. 2002).

They also help to identify different outbreaks of VDPVs where recombination with other enteroviruses have been identified.

Different genotypes of poliovirus can co circulate and cause poliomyelitis in the same region (Afif, Sutter et al. 1997). There are only two remaining genotypes circulating worldwide and have been localized in areas with low OPV coverage. These genotypes are SOAS and WEAF-B and both for serotype 1 and serotype 3 respectively.

Although the initial target date for polio eradication in the world was the year 2000, several factors and polio vaccine coverage has been a challenge globally over the past years and wild polioviruses have reemerged and VDPV has emerged in several countries. Therefore, evaluation of this situation using molecular and bioinformatics techniques remains a very important goal.

1.5. AIM AND STUDY OBJECTIVES

Aim:

Identification and molecular characterisation of wild-type, Sabin-like and vaccine derived polioviruses from the African Region.

To achieve this aim the study was divided into different sections and the following objectives set:

1) To investigate wild polio outbreaks in the African region that occurred after 2000 with specific reference to:

- Molecular epidemiological investigation of poliovirus outbreaks in Namibia and Angola in 2005-2008 through phylogenetic analysis of isolates. (Chapter 2)
- To identify the poliovirus genotype in Angola and Namibia. (Chapter 2)
- To determine the epidemiological pattern of poliovirus strain the Democratic Republic of Congo (DRC). (Chapter 3).
- To analyse the distribution of strains in different countries. (Chapter 2 and 3)

2) To investigate Vaccine-derived poliovirus (VDPV) outbreaks in the African region that occurred after 2000 with specific reference to:

- Identification and molecular epidemiological investigation of VDPV outbreaks in Africa:
    - Phylogenetic analysis of VDPVs outbreaks in Madagascar. (Chapter 4)
    - Phylogenetic analysis of VDPVs outbreaks in the DRC. (Chapter 5)
    - Phylogenetic analysis of VDPVs outbreaks in Ethiopia. (Chapter 6)

3) To investigate Vaccine-associated paralytic poliomyelitis (VAPP) outbreaks in South Africa.

- Identification of cases that fit the clinical criteria of VAPP from Southern Africa since 2000 and PCR amplification of the 5'-NTR region of Sabin-like strains that fit the VAPP criteria to identify loss of attenuation mutations by sequencing. (Chapter 7)
- Identification of recombination events across the genome of Sabin-like strains that fit VAPP criteria by both real-time PCR and sequencing. (Chapter 7).