CHAPTER 2

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

2.1 INTRODUCTION

Great progress has been made in the World Health Organization's (WHO's) program for the eradication of poliomyelitis with only four countries still remaining endemic for wild-type poliovirus namely; Nigeria, India, Afghanistan and Pakistan (CDC. 2006b). Most countries in the WHO southern Africa region have been reporting a high vaccine coverage with three doses of oral poliovirus vaccine (OPV) in children under one year and were expected to be certified as a polio-free region (CDC. 1994). Unfortunately, outbreaks of poliomyelitis continue to be reported from hitherto polio-free regions as a result of importation from endemic countries (van Niekerk, Vries et al. 1994). If the elimination of poliomyelitis is to be achieved in Africa, there is a need for characterizing the patterns of spread of wild-type viruses to improve strategies of interrupting virus transmission (Chezzi, Blackburn et al. 1997; Chezzi, Blackburn et al. 1997). A major component of the WHO strategy of eradicating poliomyelitis is laboratory surveillance of Acute Flaccid Paralysis (AFP) cases in order to identify the presence of wild-type virus infections (Chezzi, Blackburn et al. 1997; Chezzi, Blackburn et al. 1997).

The rate of nucleotide sequence evolution in poliovirus is approximately 1% per year (Alexander, Gary et al. 1997); (Jorba, Campagnoli et al. 2008) and this has given rise to different genetic strains of wild-type polioviruses, termed genotypes which can be linked to specific geographic regions (Chezzi, Blackburn et al. 1997; Chezzi, Blackburn et al. 1997). Sequence analysis of the full VP1 900 nucleotides (nt) gene and molecular epidemiological comparisons of isolates in outbreaks can be used to answer epidemiological questions regarding the likely location of endemic virus reservoirs, patterns of virus transmission or source of imported strains.

Over 10000 AFP cases of both poliovirus type 1 and 3 were reported in Africa from January 2000 until 2010. Although most cases were concentrated in Nigeria, importation has resulted in outbreaks in a number of neighbouring and distant countries in West, East and Central Africa (CDC. 2002d). In 2005, ten cases of AFP associated with type 1 poliovirus and one contact were reported from various districts of Angola. The last wild-type poliovirus 1 (PV1) case in Angola was reported in 2001 in the Province of Lunda Sul. Following the outbreak in Angola in 2005, Namibia also experienced outbreaks by the same strain. The last cases of wild-type polio occurred in Namibia in 1993 following a wild-type PV1 outbreak with 53 cases (van Niekerk, Vries et al. 1994). Vaccine coverage in both countries has been inadequate with routine coverage of only approximately 50% (CDC. 2000a), especially in the regions affected by the outbreak.

Here the genetic characterization of the wild-type PV1 isolated during these outbreaks of poliomyelitis in Angola and Namibia is reported.

2.2 MATERIAL AND METHODS

2.2.1 Viruses

Virus Isolates from original stool specimen of suspected poliovirus AFP cases were submitted to the National Institute for Communicable Diseases (NICD) in South Africa for diagnostic reverse transcription polymerase chain reaction (RT-PCR), ELISA and partial genomic sequencing from Angola and Namibia as part of the poliovirus WHO Network (Table 2.1). The original stool specimen of suspected poliovirus were also sent to the NICD for confirmation by virus culture and micro-neutralization with antiserum pools from the National Institute for Public Health and Environment Protection (RIVM), Bilthoven, The Netherlands (van der Avoort, Hull et al. 1995). Virological investigation identified polioviruses of serotype 1.

2.2.2 Laboratory Diagnosis

Intratypic differentiation of vaccine and wild-type strains was carried out by RT-PCR as described previously (Yang, De et al. 1991); (Chezzi and Schoub 1996); (Kilpatrick, Nottay et al. 1996);(Kilpatrick, Nottay et al. 1998), and ELISA (van der Avoort, Hull et al. 1995)as recommended by the World Health Organization (WHO) .

2.2.2.1 Diagnostic RT-PCR

For the intratypic differentiation, all viral cultures that showed cytopathic effect (CPE) were tested for poliovirus using an RT-PCR kit supplied by the Centres for Disease Control and Prevention, Atlanta, USA (CDC, Atlanta) that included separate reactions with primers for pan-enterovirus, pan-poliovirus, serotype-specific (Kilpatrick, Nottay et

al. 1996); (Kilpatrick, Nottay et al. 1998), and specific for the Sabin type 1, 2 and 3 viruses (Yang, De et al. 1991). In brief, 1ul of the 1:4 diluted virus isolates was added to the master mix containing 19µl of primer and 5 µl of buffer B (containing 2.8ul of 1M Dithiothreitol (Roche Diagnostic GmbH, Mannheim, Germany), 14.4ul of 5U/ul myeloblastosis virus Reverse Transcriptase (Roche Diagnostic GmbH, Mannheim, Germany), 27.6ul of 40U/ul Rnase Inhibitor (Roche Diagnostic GmbH, Mannheim, Germany) and 54.8ul of 5U/ul Taq Polymerase (Roche Diagnostic GmbH, Mannheim, Germany)). Reverse transcription was carried out at 42°C for 20 minutes in a GeneAmp 9700 thermocycler (Applied Biosystem, Foster City, CA), followed by 95°C for 3 minutes (95°C for 45 seconds, 42°C for 45 seconds and 60°C 45 seconds) x30 cycle. Amplicons were separated on a 10% polyacrylamide gel and visualized by ethidium bromide staining.

2.2.2.2 ELISA

An ELISA developed by National Institute of Public Health and the Environment, Bilthoven, Netherlands, was used for identification of Sabin-like and wildtype strains using highly specific cross-adsorbed antisera (van der Avoort, Hull et al. 1995). In brief, wells of microtitre plates are coated with bovine IgG antibodies to poiliovirus type 1, type 2 and type 3 and incubated with the identified and typed poliovirus strain to be tested. Incubation is then carried out with the type specific, cross-adsorbed rabbit antisera. After washing off any unbound rabbit sera, a peroxidase-labelled anti-rabbit IgG antibody is added to detect bound rabbit sera. The OD readings which are measured at 450nm by

loading the plate on a spectrophotometer are used to calculate a ratio which is in turn used to determine whether the virus is either Sabin-like strain or Non-Sabin–like strain.

2.2.2.3 RNA Extraction

RNA was extracted from 140µl of clarified stool or cell culture supernatant using QIAamp Viral RNA extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations. In brief, 140µl of tissue culture supernatant was added to 560µl of prepared Buffer AVL and incubated at room temperature for 10 minutes. At the end of incubation 560µl of ethanol (96 – 100%) was added to the sample mix. The mixture was transferred to the QIAamp spin column and centrifuged at 8000rpm for 1 minute. The spin column was washed twice by adding 500µl of Buffer AW1 followed by adding 500µl of Buffer AW2. RNA was eluted by adding 60µl of Buffer AVE.

2.2.2.4 RT-PCR for sequence analysis

RT-PCR was performed in a single step as described before (Yang, De et al. 1991); (Chezzi and Schoub 1996; Kilpatrick, Nottay et al. 1996; Kilpatrick, Nottay et al. 1998). Briefly, the extracted RNA (10 µl) was added to 90 µl of the amplification mixture containing 10 µl standard 10 × reaction buffer, 100 µM of each dNTP (Roche Diagnostics GmbH, Mannheim, Germany), 10 mM dithiothreitol, 80 pmol of each primer sets ((Y7R: (5'GGTTTTGTGTCAGCITGYAAYGA3') and Q8: (5'AAGAGGTCTCTRTTCCACAT3')) (Manor, Handsher et al. 1999), 20 U of placental RNase inhibitor (Roche Diagnostics GmbH, Mannheim, Germany), 12. 5 U of myeloblastosis

virus reverse transcriptase (Roche Diagnostics GmbH, Mannheim, Germany), 5U of Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany). Reverse transcription was carried out at 42°C for 60 min in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA) followed by 95°C for 3 min (95°C for 30 sec, 42°C for 30 sec, and 60°C for 2 min) x30 cycles

2.2.2.5 Cycle sequencing

The complete VP1 gene (nucleotides 2480 to 3385) was sequenced. The primers used for sequencing are specific to the 5' ends of the VP1 region and were as follows:

Y7R (5'GGTTTTGTGTCAGCITGYAAYGA3'); PV1A TTIAIIGCRTGICCRTTRTT3');

PV2S (CITAITCIMGITTYGAYATG 3') and Q8 (5'AAGAGGTCTCTRTTCCACAT3').

Nucleotide sequencing was carried out on the sense and anti-sense strands using the ABI Prism^{R}BigDye^{TM} Terminator Cycle Ready Reaction kit v3. 1 (PE Biosystems, Foster City, USÁ) according to the manufacturer's recommendations. The reaction was carried out in a 10 µl reaction: In brief, 4 µl of double distilled water was mixed with approximately 1 µl of 5–20 ng of PCR product; 2.0 µl Terminator Ready Reaction Mix (A, C, G and T-big dye terminators; (PE Biosystems, Foster City, USA), 2 µl of 5X Sequencing Buffer and 1 µl of 3.2 pmol primer. Cycle sequencing was performed according to the protocol specified for the GeneAmp 2400 thermocycler: 94°C for 1 minute; (94°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes) for 25 cycles; and finally 4°C infinity.

2.2.2.6 *Sequence Analysis*

The complete VP1 gene (nucleotides 2480 to 3385) was sequenced as described (Liu, Zheng et al. 2000) using cycle sequencing with the Big Dye Terminator Cycle sequencing kit version 3. 1 (Applied Biosystems, Foster City, USA) on the Gene Amp 9700 (Applied Biosystems). The DNA sequence was determined using the ABI 3100 GeneticAnalyzer, version 3. 1 (Perkin Elmer Applied Biosystems, Foster City, USA). Raw data was edited using the Sequencher™ software package version 4. 1. 4.

2.2.2.7 *Phylogenetic Analysis*

Phylogenetic analysis was carried out on the complete VP1 gene, which corresponds to 906 nt for all isolates from patients with AFP. Sequences were aligned with Clustal X (Thompson, Gibson et al. 1997). Neighbour-joining tree was constructed with MEGA version 4.0 (Kumar, Tamura et al. 2004; Tamura, Dudley et al. 2007; Kumar, Nei et al. 2008). Bayesian analysis was carried out with the programme BEAST version 1.4 using the actual sampling date of each specimen to estimate the rates of evolution (Drummond and Rambaut 2007). The GTR + G substitution model was used to allow different sites in the alignment to evolve at different rates and the substitution rate calculated from the data assuming a molecular clock. The Markov chain Monte Carlo search was set at a chain length of 10 000 000. The log and tree files were analysed with Logcombiner and the Treeannotator programmes and visualised with Figtree version 1. 1. 2 (http://tree. bio. ed. ac. uk/software/figtree))(Drummond and Rambaut 2007).

The alignments (nucleotide and translated amino acid sequences) were analysed with Genedoc version 2. 6001 (www. psc. edu/biomed/genedoc) to identify specific mutations and positive selection.

Table 2.1: Selected wild poliovirus type 1 isolated in Angola and Namibia between 2005 and 2009.

Epidemiological Number (EPID)	Country	Year of onset of paralysis
ANG-BEN-LOB-05-003	ANGOLA	2005
ANG-BEN-LOB-05-008	ANGOLA	2005
ANG-LSL-SAU-05-001	ANGOLA	2005
ANG-LSL-SAU-05-002	ANGOLA	2005
ANG-LUA-CAC-05-003	ANGOLA	2005
ANG-MOX-MOX-05-001	ANGOLA	2005
NAM-HAR-ARA-06-015	NAMIBIA	2006
NAM-KHO-WHK-06-018	NAMIBIA	2006
NAM-KHO-WHK-06-020	NAMIBIA	2006
NAM-KHO-WHK-06-022	NAMIBIA	2006
NAM-KHO-WHK-06-024	NAMIBIA	2006
NAM-KHO-WHK-06-027	NAMIBIA	2006
NAM-KHO-WHK-06-029	NAMIBIA	2006
NAM-KHO-WHK-06-031	NAMIBIA	2006
NAM-KHO-WHK-06-036	NAMIBIA	2006
NAM-KHO-WHK-06-039	NAMIBIA	2006
NAM-KHO-WHK-06-062	NAMIBIA	2006
NAM-KHO-WHK-06-067	NAMIBIA	2006
NAM-KHO-WHK-06-123	NAMIBIA	2006
NAM-KHO-WHK-06-171	NAMIBIA	2006
NAM-KHO-WHK-06-194	NAMIBIA	2006
NAM-OHA-ENG-06-054	NAMIBIA	2006
NAM-OHN-OSH-06-102	NAMIBIA	2006
NAM-OHN-OSH-06-137	NAMIBIA	2006
NAM-OMU-OKA-06-059	NAMIBIA	2006
ANG-LUA-CAZ-07-004	ANGOLA	2007
ANG-LUA-CAZ-07-005	ANGOLA	2007
ANG-LUA-KIL-07-003	ANGOLA	2007

2.3 RESULTS

2.3.1 Identification of wild poliovirus 1:

Viral isolates obtained from Angola, and Namibia in 2005, 2006 and 2007 were tested by RT-PCR using pan-enterovirus, pan-poliovirus, serotype-specific, and Sabin types 1, 2 and 3 virus specific primers. All isolates were positive for poliovirus type 1. The RT-PCR test was confirmed by ELISA and both techniques identified the isolates as wild-type poliovirus. These isolates were characterised by sequencing of 906 nt representative of the complete VP1 gene and all strains were found to be wild-type 1.

2.3.2 Outbreak description:

From 2005-2007 of AFP cases associated with poliovirus 1 have been reported from 2 countries in southern Africa.

2.3.2.1 Angola 2005-2007

In 2005, ten cases of AFP associated with type 1 poliovirus and one contact were reported from a number of districts of Angola. The first case (onset in April 2005) was a 17-month old girl who had a previous history of oral polio vaccine from the urban district of Cacuaco. She developed fever and paralysis on the 25 April 2005, in Luanda province. The patient and her family had no travel history. The Cacuaco district reports routine polio vaccine coverage of 50%. Two further cases were reported in Angola in 2006 with paralysis setting on the 14 November 2006 in the final case. In 2007, 8 cases

had been reported with the first onset in May while the last case developed paralysis on the 8th of July 2007.

2.3.2.2 Namibia 2006

In 2006 the first polio outbreak in more than 10 years was reported in Namibia affecting mainly adults. The index case was a 39 year old man from the Hardap region, south-east of the capital Windhoek, who had an onset of AFP on the 6th of May 2006 (CDC. 2006a). As of October 2, 2006, 19 AFP cases were reported, and confirmed as wild-type poliovirus type 1 and six of the confirmed cases died. The last case had an onset in June 2006. Wild-type polio virus was also identified in AFP cases in Windhoek in the Khomas region and in a northern area bordering Angola as well as with three regions adjacent to the border of Angola, Omusati, Oshana and Ohangwena. These are the most populated areas in the country.

2.3.3 Phylogenetic and P-distance analysis:

Blast search analysis of all polioviruses isolated in the current investigation identified the Indian genotype (SOAS) as being the most closely related genotype to strains identified in Angola and Namibia. Initial analysis of all wild-type PV1 genotypes facilitated the classification of the Angola and Namibia strains to the SAOS genotype. Figure 2.1 illustrates the relationship between selected representative wild-type PV1 isolates from southern Africa and India. In order to investigate this relationship and evolution over time unrooted tree was constructed using Bayesian analysis on all Angola and Namibian

isolates from the current outbreak as well as the African strains belonging to the African genotype (WEAF-B) isolated in the last prior outbreak, and Indian strains isolated in 2004 belonging to the SOAS genotype. Strains from India that were identified by BLAST seem to be the closest to the described outbreaks were included in the analysis. All polioviruses investigated fell within the same genotypes, i.e. SOAS (India genotype). Figure 2.2 shows the Bayesian tree rooted at the closest related Indian strain demonstrating the evolution that has occurred since the introduction of this genotype into Africa using Bayesian analysis with the assumption of a molecular clock at a set rate for poliovirus of 1 substitution per year.

Since all previous poliomyelitis cases in both countries were associated with the African genotype (WEAF-B), the homology of all isolates identified in the outbreaks in Angola and Namibia was investigated by full VP1 gene sequence analysis and included in these phylogenetic trees.

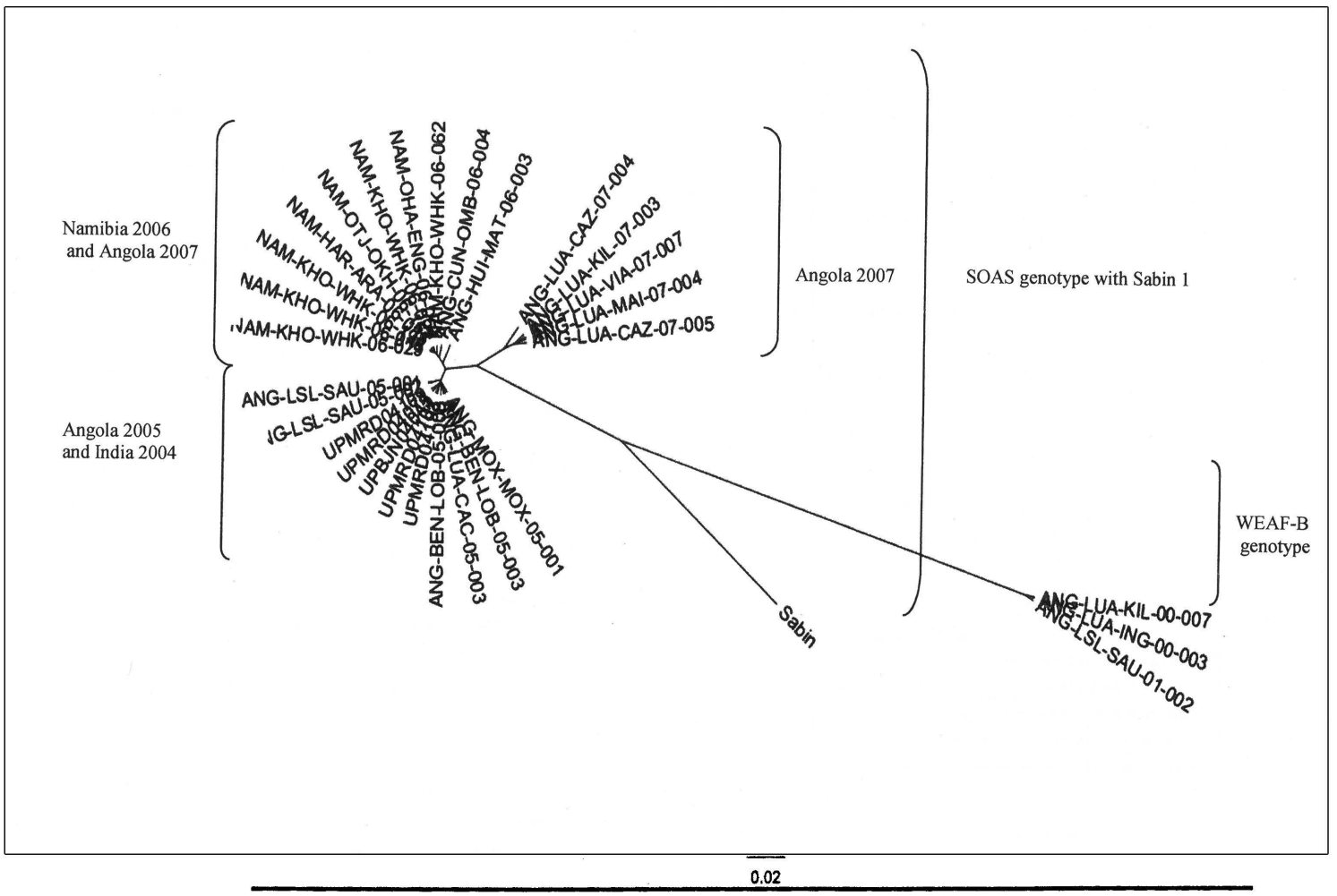

Figure 2.1. An unrooted tree showing the close relationship of the Angola and Namibia isolates to Indian strains. Sabin 1 reference strain also added

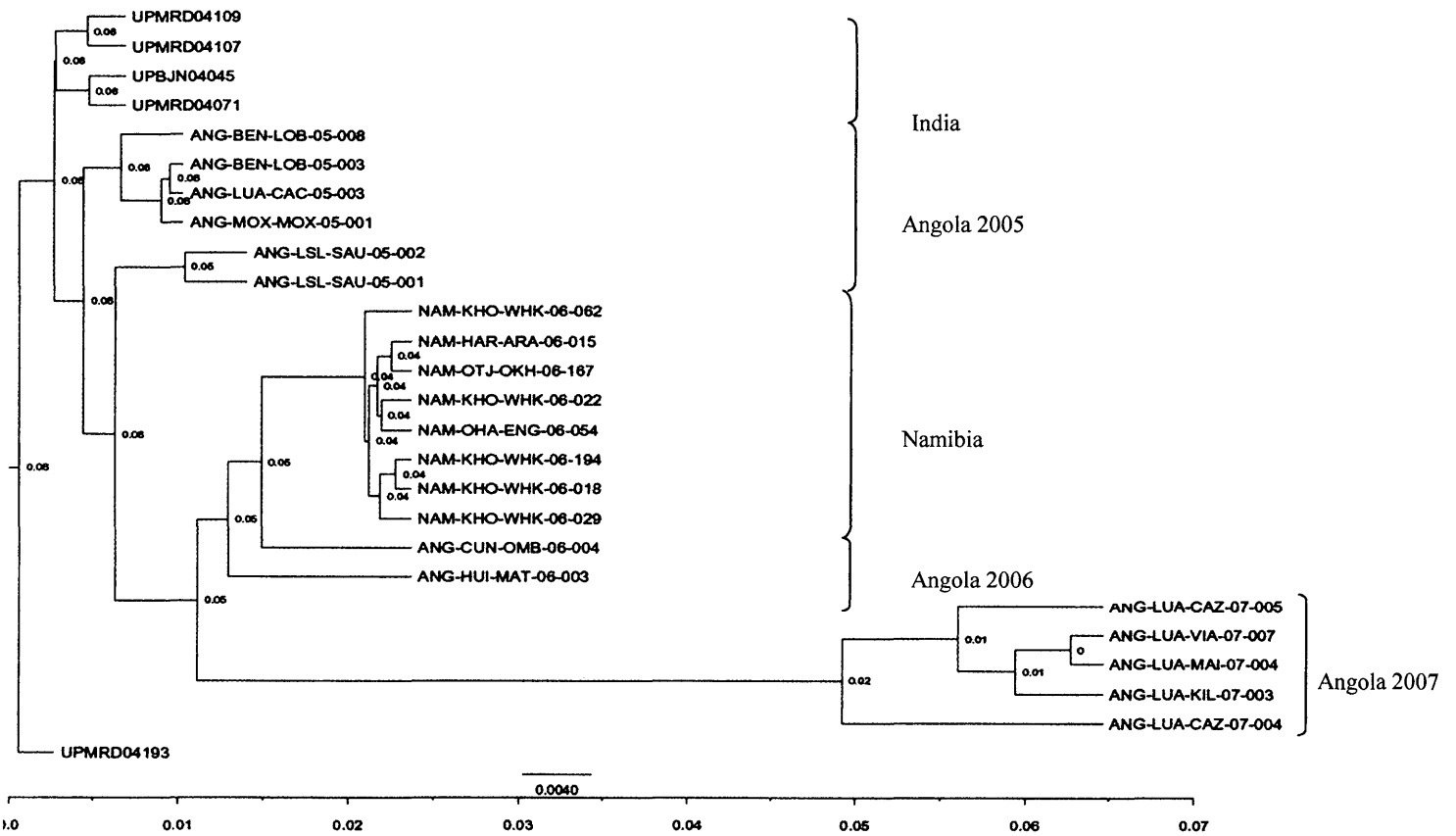

Figure 2.2: A Bayesian tree of wild poliovirus VP1 gene rooted at India strain demonstrating the evolution that has occurred since introduction of India genotype into Africa at a set rate for poliovirus of 1% per year.

2.3.4 Calculation of circulation time of poliovirus strains in Africa

The nucleotide pairwise distance (P-distances) of the sequenced VP1 region within India strain ranged from 0% to 1%, Angola strains from 0% to 2% and within the Namibia strains ranged from 0% to 1%. The calculated P-distance between the closest branched

strain isolated in Angola in 2005 and the Namibian strains was 2%. The rate of nucleotide sequence evolution in poliovirus is approximately 1% per year (Alexander, Gary et al. 1997; Jorba, Campagnoli et al. 2008). This suggests that the virus that caused the outbreak in Namibia had been circulating in Angola for at least a year undetected. The calculated P-distance between the Namibian, Angola and Indian strains are listed in Table 2.2.

Table 2. 2: The nucleotide pairwise distance of the SOAS (Indian) genotype in Africa. The P-distance range within each country is bold and the differences between each country are below.

COUNTRY	INDIA	NAMIBIA	ANGOLA
INDIA	**(0-1%)**		
NAMIBIA	(2-3%)	**(0-1%)**	
ANGOLA	(0-6%)	(2-7%)	**(0-7%)**

2.3.5 Amino acid substitutions in the VP1 region

Comparisons of the VP1 amino acids sequences relative to Sabin 1 are shown in the alignment in Figure 2.3. Most substitutions resulted in silent mutations. Both genotypes (WEAF-B and SOAS) have 10 identical substitutions relative to Sabin 1: An alanine at

position 21 of Sabin is replaced by a serine, indicated as A21S; as well as a A32S; V67I; A88T; I90M; K99T; N100S; T106S; V138I and T292A.

The first outbreak in Angola in 2005 was caused by a strain that had an identical amino acid sequence to a strain from India, suggesting the origin to be by importation from India. Angola 2006 strains maintained the same identity with the exception of a change at position 168 (E168G). The Angola strains for 2007 have a unique substitution at position 77 relative to Sabin 1 (I77V) which was not present in the other groups.

Namibia strains of 2006 had additional changes which were not observed in previous SOAS strains. Changes in positions 17 and 215 (T17A and V215I) indicates further genetic drift from the original virus from India which were not observed in the Angola strains. One strain from Namibia contained an additional substitution of A222V.

Genotype WEAF-B displayed its own distinctive changes relative to the Namibia isolates at position 215 (V215I). All SOAS strains share the same six substitutions relative to Sabin 1 that is not present in some of WEAF-B strains.

STRAINS		VARIABLE POSITIONS																	
		17		21	32	67	77	88	90	99	100	106	110	138	168	169	215	222	223	292
	Sabin1(VP1)	T	G	A	A	V	I	A	I	K	N	T	I		E	K	V	A		T
India	UPBJN04045																			
	UPMRD04071			S	S	I		T	M	T	S	S		I					E	A
	UPMRD04107			S	S	I		T	M	T	S	S		I					E	A
	UPMRD04109			S	S	I		T	M	T	S	S		I					E	A
	UPMRD04193			S	S	I		T	M	M	S	S		I					E	A
Angola	ANG-LSL-SAU-05-001			S	S	I		T	M	T	S	S		I					E	A
	ANG-BEN-LOB-05-008			S	S	I		T	M	T	S	S		I					E	A
	ANG-LUA-CAC-05-003			S	S	I		T	M	T	S	S		I	G				E	A
	ANG-LSL-SAU-05-002			S	S	I		T	M	T	S	S		I					E	A
	ANG-MOX-MOX-05-001			S	S	I		T	M	T	S	S		I					E	A
	ANG-BEN-LOB-05-003			S	S	I		T	M	T	S	S		I					E	A
	ANG-CUN-OMB-06-004			S	P	I		T	M	T	S	S		I	G				E	A
	ANG-HUI-MAT-06-003			S	S	I		T	M	T	S	S		I	G				E	A
	ANG-LUA-CAZ-07-005			S	S	I	V	T	M	T	S	S		I					E	A
	ANG-LUA-VIA-07-007			S	S	I	V	T	M	T	S	S		I					E	A
	ANG-LUA-CAZ-07-004			S	S	I	V	T	M	T	S	S		I					E	A
	ANG-LUA-KIL-07-003			S	S	I	V	T	M	T	S	S		I					E	A
	ANG-LUA-MAI-07-004			S	S	I	V	T	M	T	S	S		I					E	A
Namibia	NAM-HAR-ARA-06-015	A		S	S	I			M	T	S	S		I	G		I		E	A
	NAM-KHO-WHK-06-018	A		S	S	I		T	M	T	S	S		I	G		I		E	A
	NAM-KHO-WHK-06-022	A		S	S	I		T	M	T	S	S		I	G		I		E	A
	NAM-KHO-WHK-06-029	A		S	S	I		T	M	T	S	S		I	G		I		E	A
	NAM-KHO-WHK-06-062	A		S	S	I		T	M	T	S	S		I	G		I		E	A
	NAM-KHO-WHK-06-194	A		S	S	I		T	M	T	S	S		I	G		I	V	E	A
	NAM-OHA-ENG-06-054	A		S	S	I		T	M	T	S	S		I	G		I		E	A
	NAM-OTJ-OKH-06-167	A		S	S	I		T	M	T	S	S		I	G		I		E	A
WEAF-B	ANG-LUA-ING-00-003			S	S	I		T	M	T	S	S		I			I			A
	ANG-LUA-KIL-00-007			S	S	I		T	M	T	S	S		I			I			A
	ANG-LSL-SAU-01-002			S	S	I		T	M	T	S	S		I	G	E	I			A

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 are abbreviated as follows: ANG, Angola; UP, Uttar Pradesh, province of India and NAM, Namibia. The previous African genotype WEAF-B is shown at the bottom for Angola.

2.4 DISCUSSION

In this study the complete VP1 gene of wild-type polio strains causing AFP during outbreaks in Angola and Namibia was sequenced to investigate the molecular epidemiology of these strains in Africa. In the past, the VP1/2A junction of 150 nt was used to study the epidemiology and circulation of wild-type polioviruses worldwide (Zheng, Zhang et al. 1993); (Huovilainen, Mulders et al. 1995);(Lipskaya, Chervonskaya et al. 1995);(Mulders, Lipskaya et al. 1995). The complete VP1 region has since been chosen for molecular epidemiological investigations as it exhibits the greatest sequence variability, and plays a key role in receptor attachment (Wimmer, Hellen et al. 1993).

The first AFP cases associated with wild-type polio virus were identified in April 2005 in Angola along the coast of the Luanda province and had a 99.34% identify to isolates from Uttar Pradesh, in northern India. Isolates from Angola and Namibia grouped with isolates from India, (Figure 2.1). The 2005 index strain (ANG-LUA-CAC-05-003) from Angola was most closely related to the Indian genotype with a P-distance (P) of 0.01. The Namibia strains were closest to India strain by 0.02 – 0.03 P distance range. The Bayesian tree (Figure 2.2) which assumes a molecular clock demonstrates the drift that had occurred from the introduction of the Indian strains in Angola in 2005 to 2007 suggests that the Angola 2006 strain was most probable the source for the 2006 outbreak in Namibia. All 2006 strains from Angola grouped with isolates from the border of Namibia that separate the two countries suggesting the possible route of introduction

into Namibia. The similarity in nucleotide sequences of the Namibia strains suggests rapid epidemic transmission amongst inadequately immunized individuals.

P-distances and substitutions over time of the Angola strains relative to those of India suggest a time of circulation of 2 to 3 years. This is consistent with the recent undetected circulation and surveillance gaps in Angola in 2006 and 2007. Surveillance of AFP has been a key factor in efforts to achieve eradication of poliomyelitis worldwide. The outbreaks in Angola and Namibia demonstrated that low levels of vaccines coverage, in combination with poor surveillance of AFP, could result in undetected and prolonged circulation of poliovirus in a community before cases of poliomyelitis are evident (Kew, Morris-Glasgow et al. 2002). Low vaccine coverage may have allowed the transmission of the imported strain amongst pockets of unvaccinated individuals.

Displacement of an endemic circulating genotype by an imported genotype has also been reported in South America (Rico-Hesse, Pallansch et al. 1987). In Africa, the WEAF-B genotype which was last detected in September 2001 in Angola was replaced by the imported SOAS genotype in 2005. This is the first time that AFP cases associated with the Indian genotype were identified in Africa. The 2005 importation in Angola lasted until 2007 and led to outbreaks in Namibia, the DRC, Central African Republic and Burundi (CDC. 2006e). Re-established transmission in Angola has continued into 2011. This emphasises the vulnerability of regions with suboptimal vaccination coverage for importation and reintroduction of wild-type polio virus from the remaining endemic countries. Namibia has been free of polio more than 11 years before

the 2006 Indian genotype outbreak. Until the establishment of the Namibian Expanded Program on Immunization in 1990, Namibia public health services had been disrupted by conflict during 1966 to 1989 (CDC. 2006d) . Since 1990, vaccine increased from 37% in 1989 to 76% in 2000. During 2006 outbreak, all confirmed WPV1 cases occurred in persons aged >14 who either had not been vaccinated for polio or had incomplete polio vaccine schedule (CDC. 2006d) . In response to the outbreak, the Namibia Ministry of Health and Social Services (MoHSS) activated the National Health Emergency Committee to coordinate activities and several SIAs were conducted (CDC. 2006d) .

The Indian genotype has been associated with several outbreaks and has circulated for years in India. This genotype has also been associated with cases in Bangladesh (CDC. 2002c). The urgency of reaching the goal of global polio eradication had been underscored by the importation of wild polioviruses into these countries (Angola and Namibia). This can only be achieved by the elimination of remaining reservoirs of wild polioviruses endemicity in South Asia and Sub-Saharan Africa (CDC. 2002c; CDC. 2002d; CID. 2002).