POLIOVIRUS SHEDDING AMONG CHILDREN AGED BELOW FIVE YEARS VACCINATED WITH TRIVALENT POLIOVIRUS VACCINE AT COTTOLENGO CHILDREN'S HOME

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Poliovirus Shedding among Children Aged below Five Years Vaccinated with Trivalent Poliovirus Vaccine at Cottolengo Children's Home

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

“To my Husband Allan and my Son Elly without whom this thesis would have not been completed, and to the under-fives of Cottolengo children home who offered an insight to the future generations on issues Polio”.

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ABBREVIATIONS AND ACRONYMS

µg	Microgram
µl	Microlitre
cDNA	Complementary Deoxyribonucleic Acid
CPE	Cytopathic Effect
cVDPV	Circulating Vaccine-Derived Polioviruses
CVID	Common Variable Immunodeficiency
EPI	Expanded Programme on Immunization
EPID number	Epidemiological Number Assigned to AFP Case
G	Relative Centrifugal Force
g	Gram
GM	Growth Medium
HIV	Human Immunodeficiency Virus
IPV	Inactivated Polio Vaccine (Salk)
ITD	Intratypic Differentiation
IU	International Units
iVDPVs	Immunodeficiency- Related VDPV
kPa	Kilopascal
L	Litre
L20B	Mouse Cell Line Expressing the Gene for the Human Cellular Receptor for Poliovirus
MEM	Minimal Essential Medium
Mg	Milligram
Min	Minute
Ml	Milliliter
MM	Maintenance Medium
MW	Molecular Weight
NASCOP	National AIDS and STI Control Programme
NIDs	National Immunization Days
nm	Nanometer

NPEV	Non-Polio Enterovirus
OPV	Oral Poliovirus Vaccine
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PV	Poliovirus
RD	Cell Line Derived from Human Rhabdomyosarcoma
RNA	Ribonucleic Acid
RRL	Regional Reference Laboratory
rRT-PCR	Real Time Reverse Transcriptase- Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulfate
tOPV	Trivalent Oral Poliovirus Vaccine
U	Unit
VAPP	Vaccine-Associated Paralytic Poliomyelitis
Vdpv	Vaccine-Derived Polioviruses
WHO	World Health Organization
XLA	X-Linked a Gamma-Globulinaemia.

ABSTRACT

One of the key tools in the fight against polio has been the live, attenuated oral poliovirus vaccine (OPV. Although OPV may require multiple doses to establish immunity, it ultimately offers long-term protection against paralytic disease through lasting humoral immunity. In rare instances, vaccine-associated paralytic poliomyelitis can occur in immunologically normal recipients of OPV, their contacts, or individuals with immunodeficiency. Immunodeficient persons with persistent vaccine-related poliovirus infection may serve as a potential reservoir for reintroduction of polio after wild poliovirus eradication, posing a risk of their further circulation in inadequately immunized populations. Attenuated polioviruses used in oral polio vaccine (OPV) can mutate into vaccine-derived poliovirus (VDPV) and cause poliomyelitis outbreaks. The aim of the study was to determine the duration of vaccine shedding in stool specimens of children <5 years vaccinated with tOPV. The study monitored poliovirus excretion following vaccination among children at an orphanage in Kenya. After national immunization days, serial collection of stool specimens was done from orphanage residents aged <5 years at enrollment until the participants shopped shedding. Data recorded included their HIV status and demographic, clinical, immunological, and immunization data. To detect and characterize isolated polioviruses and non-polio enteroviruses (NPEV), we used viral culture and intratypic differentiation of isolates by real time PCR. Long-term persistence was defined as shedding for >or= 6 months. Twenty-six children (10 HIV-infected, 16 HIV-uninfected) were enrolled, and 80 specimens (21 from HIV-infected, 59 from HIV-uninfected) were collected. All HIV-infected children had CD4 Range of 500-1500 cells/mm³. All participants shed vaccine-related polioviruses at week 2 and 4 and 1 shed NPEV at some point during the study period. Of 54 poliovirus-positive specimens, 20 were from HIV-infected, and 32 from HIV-uninfected children. No participant shed polioviruses for >or= 6 months. The results indicate that both HIV-infected and un-infected children retain the ability to clear enteroviruses, including vaccine-related poliovirus. Larger studies are needed to confirm and generalize these findings. Understanding how mucosal immunity influences shedding patterns in a larger population will help optimize vaccination strategies.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

After the establishment of Global Polio Eradication Initiative (GPEI) which seeks to ensure that all polioviruses are eradicated from circulation in the general population, there has been more than 99.99% decline in the global incidence of wild-polioviruses from 350,000 cases in the year 1988 to only 32 cases in the year 2018. (Bandyopadhyay *et al.*, 2021) Additionally, this led to the eradication of wild polio type 2 while incidence of wild polio type 3 has not been reported since 2012. However, wild polio type 1 is still being transmitted in some regions of Nigeria, Pakistan and Afghanistan. The success in the polio eradication strategy can be attributed to Oral Polio Vaccine (OPV) which is inexpensive, easy to administer and readily available. However, after the eradication of wild polioviruses, there is a risk of emergence of vaccine-derived polioviruses (VDPV) and vaccine-associated paralytic poliomyelitis (VAPP) as a result of the continued use of the live oral poliovirus vaccine (OPV). This necessitated the switch from trivalent OPV (tOPV) which had serotypes 1, 2, and 3 to bivalent OPV (bOPV) which has serotypes 1 and 3 in April 2016 (Jorba *et al.,* 2017). OPV has live attenuated polioviruses. In situations where there is an immunodeficient host or a community which is under immunized, there can be prolonged replication of the attenuated virus and subsequent regaining of its neurovirulence and transmission capabilities. These are called VDPVs and are able to circulate and cause paralytic polio with similar clinical manifestation as the wild polioviruses. Those VDPVs that are able to establish a community transmission are circulating vaccine-derived polioviruses (cVDPVs) while those that emerge from patients with primary immunodeficiencies (PID) due to prolonged intestinal replication are immunodeficiency-associated vaccine-derived polioviruses (iVDPVs). VDPVs also have the potential to cause outbreaks in under-immunized populations (Platt *et al*., 2014). Therefore, immunodeficient persons with persistent vaccine-related poliovirus infection may serve as a potential reservoir for reintroduction of polioviruses into the general population after wild poliovirus

eradication. The surveillance of acute flaccid paralysis (AFP) cases is key to surveillance of polioviruses globally. Its backbone is timely identification of paralyzed patients and stool testing for polioviruses (Li *et al.,* 2014). A further risk of circulation of poliovirus in inadequately immunized populations arises. Chronic enterovirus persistence and increased risk of VAPP among immunodeficient persons with B-cell and combined deficiencies are well documented. Chronic VDPV persistence has been show to affect Persons with certain defects of antibody production (Diop, 2015).

Studies have shown that HIV infected children vaccinated with poliovirus vaccine do not excrete poliovirus for more than 4 weeks and maintain their ability to develop immunity after vaccination. Even after deterioration of CD4 cell counts, these children retained sufficient immunologic memory to prevent persistent infections from repeated exposures to poliovirus. Therefore, HIV positive children are not a high-risk population for long term poliovirus excretion (Manirakiza *et al*., 2010). Prolonged enterovirus shedding might be a biomarker for defective immune defenses against enteroviruses. It would be of interest if HIV-positive individuals infected with enterovirus had prolonged shedding of the same serotype in consecutive stool samples (Di Cristanziano *et al*., 2020).

OPV protects vaccinated individuals against viremia, but not against infection with the same OPV. In normal individuals, the vaccine strains replicate in the pharynx and intestine for 2 to 8 weeks, inducing humoral and mucosal immunity. However, in a small number of vaccinated individuals, OPV may mutate and evolve into vaccine-derived poliovirus (VDPV) during replication (Pöyhönen *et al*., 2019). VDPV can reacquire neurovirulence, leading to outbreaks of disease in regions with low levels of OPV coverage. Most of the reported outbreaks are associated with type 2 viruses, the wild-type counterpart of which has been eradicated worldwide. Thus, VDPV has replaced the wild-type virus as a major threat to global heath ((Burns *et al*., 2014).

The criteria for polioviruses genotypes has been defined as groups of strains that show more than 85% sequence homology in the VP1/2A region and a cluster is defined as a group of isolates showing ≥ 95% sequence similarity. A strain represents

new cluster if it has > 5% nucleotide sequence divergence with previous ones (Angez *et al*., 2012).

1.2 Statement of the Problem

Poliovirus transmission remains a significant challenge due to the presence of three distinct serotypes, various strains of live polioviruses (LPVs), wild polioviruses (WPVs), live attenuated oral poliovirus vaccines (OPVs), and OPV-related strains. OPV-related strains encompass vaccine-derived polioviruses (VDPVs), which can lead to outbreaks of circulating VDPVs (cVDPVs, also known as "variant" polioviruses) if these viruses spread in populations with low vaccination coverage. (Thompson *et al*., 2020) The Global Polio Eradication Initiative (GPEI) reports weekly cases of WPVs and cVDPVs, but not other OPV-related strains. However, other OPV-related strains, such as immunodeficiency-associated VDPVs (iVDPVs), which occasionally occur in individuals with primary immunodeficiencies, pose a significant challenge for eradication efforts. Immunodeficient individuals can become prolonged or chronic poliovirus excreters, potentially serving as a local source for reintroducing the virus in otherwise polio-free areas. Additionally, OPV-related risks include the rare occurrence of vaccine-associated paralytic polio (VAPP) in OPV recipients or their close contacts (Kalkowska *et al.,* 2021).

The highest priority of the polio eradication initiative is its primary objective of interrupting transmission of wild-type poliovirus and VDPVs. This remains to be a challenge for inadequately vaccinated populations like those in Kenya. There are threats of poliovirus importations from the neighboring countries that have not achieved a wild poliovirus free status. Low level of immunity in the population, low levels of sanitation and overcrowding are the risk factors for the spread of poliovirus. Many studies have linked cVDPV to low immunity in the population. The probability of VDPV emerging in a population depends on complex interaction of many factors that include but not limited to; the prevalence of other enteroviruses in the population, the total number of people infected with Sabin poliovirus, the duration of virus shedding among the infected people and the number of people who come in contact with the infected persons (Pons-Salort *et al*., 2016).

In a study done in India on natural clearance of prolonged VDPV infection in immunodeficient children it was suggested that all the patients at risk of immunodeficiency need to be monitored for poliovirus excretion (Mohanty *et al*., 2019). A study done in Zimbabwe found that HIV infection is associated with decrease in mucosal and Humoral immune response to OPV but not prolonged viral shedding that can result to iVDPV (Troy *et al.*, 2013). In Kenya results found that mildly to moderately symptomatic HIV-infected children retain the ability to clear enteroviruses, including vaccine-related poliovirus (Khetsuriani *et al.,* 2009). A small increase in duration of viral shedding of several weeks to months may enhance the evolution and spread of vaccine viruses. Patients who shed the poliovirus for long periods of times (over 6 months) after vaccination developed paralytic polio, demonstrating that prolonged replication even within a single individual increases the virulence of vaccine related viruses(Macklin *et al.,* 2017).

1.3 Justification of the Study

Poliovirus eradication efforts have primarily relied on the administration of oral polio vaccines (OPVs). The trivalent oral polio vaccine (tOPV), which targets all three serotypes of poliovirus, has been instrumental in reducing the global incidence of polio. It contains live, attenuated virus, which can be shed in the feces of vaccinated individuals. However, the effectiveness and safety of tOPV can be influenced by several factors, including mucosal immunity, seroconversion rates, and the health status of the child, particularly in populations with a high prevalence of HIV infection (Macklin *et al.*,2019).

OPVs Shedding can lead to secondary transmission of the vaccine virus, which in rare cases can mutate and lead to vaccine-derived poliovirus outbreaks. Monitoring shedding is crucial to understand the dynamics of viral transmission and mitigate risks associated with VDPV. (Li *et al*., 2014).

Shedding patterns can provide insights into the replication and immune response elicited by the vaccine. Effective mucosal immunity, often indicated by reduced shedding, is critical for preventing both polio transmission and infection. (Diop*et al.,* 2015).

Mucosal immunity in the gut plays a key role in preventing poliovirus replication and shedding. The mucosal lining of the gut is the primary site of entry for poliovirus, and antibodies in the mucosa can neutralize the virus before it can establish an infection. Therefore, a mucosal immunity study could help to better understand the role of mucosal immunity in protecting against poliovirus infection and the potential impact of low mucosal immunity on the spread of cVDPVs. (Connor *et al*., 2022).

The efficacy of tOPV in inducing mucosal immunity can vary among HIV infected and un-infected populations and age groups. Understanding how mucosal immunity influences shedding patterns can help optimize vaccination strategies.

The rate of vaccine shedding can have implications for public health policy, vaccination strategies, and recommendations. High shedding rates may lead to recommendations for alternative vaccines or vaccination strategies in specific populations, whereas low rates may support the continued use of the vaccine. The dependent variable "Rate of Vaccine Shedding among Vaccinated Children" is a crucial element in studies assessing the safety and effectiveness of vaccines. It involves measuring the proportion of vaccinated children who excrete the vaccine virus and provides valuable information for public health decision-making and vaccination program management

1.4 Research Questions

1. What is the duration of poliovirus shedding in children under five years old who have been vaccinated with the Trivalent Polio Vaccine at Cottolengo Children's Home?

2. How does poliovirus shedding vary among children under five years old at Cottolengo Children's Home vaccinated with the Trivalent Polio Vaccine based on Sex, age and other demographic factors?

3. What types of vaccine-derived polioviruses are isolated from children under five years old who have been vaccinated with the Trivalent Polio Vaccine at Cottolengo Children's Home?

1.5 Objectives

1.5.1 Broad Objective

- To determine the rate of poliovirus shedding among children aged 5 years and below vaccinated with trivalent poliovirus vaccine at Cottolengo children's home.

1.5.2 Specific Objectives

1. To determine the duration of poliovirus shedding among children aged below five years vaccinated with Trivalent Polio Vaccine at Cottolengo children's home.
2. To investigate whether there are statistically significant differences in shedding rates between males and females.
3. To identify types of vaccine derived polioviruses being isolated from children aged below five years vaccinated with Trivalent Polio Vaccine at Cottolengo children's home

CHAPTER TWO

LITERATURE REVIEW

2.1 Poliovirus Molecular Analysis

Polioviruses are small viruses, 30 nm in diameter and are non-enveloped. They belong to the Picornavirus family and genus Enterovirus. The genome of Poliovirus is a 7.5 kb positive sense single stranded RNA surrounded by capsid protein. When the PV genome is translated, a glycoprotein is generated which is then cleaved by viral proteases to form four capsid proteins, P1–VP4, and several non-structural proteins. Poliovirus can be categorized into three different genotypes: 1, 2 and 3 all of which cause disease. The genome of each genotype contains a single positive-stranded RNA with a size of approximately 6 kb consisting of a single large open reading frame (ORF) flanked by 5' and 3' untranslated region. Immunity against one of the three PV serotypes does not protect against the other two, and therefore effective polio vaccines contain 3strains of attenuated or inactivated PV (van den Pol, 2013).

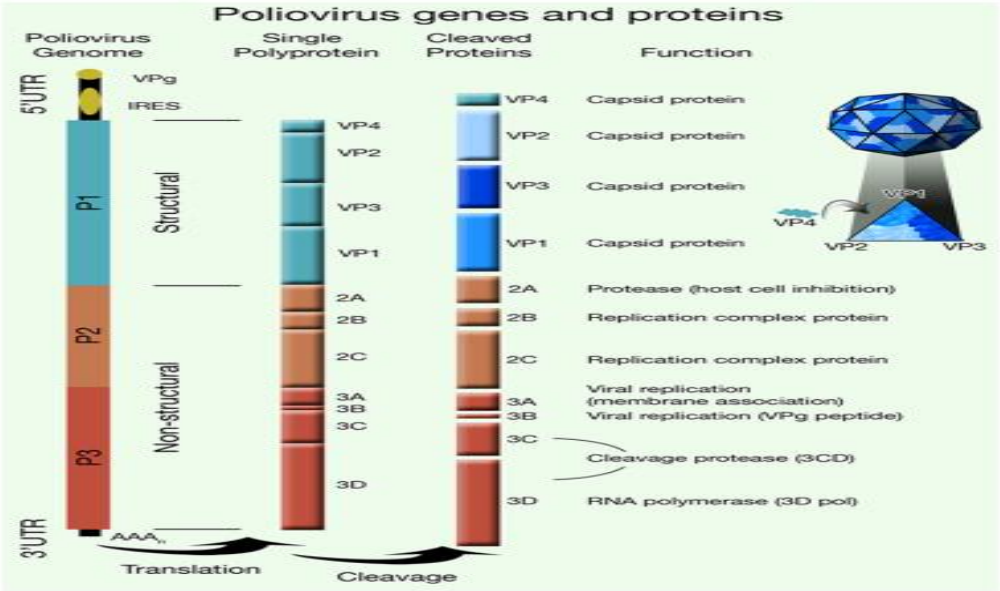

Figure 2.1: Schematic Representation of Poliovirus Genome

Courtesy of European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. alfredo.castello@embl.de

Translation into a single polyprotein, and then cleavage into a number of poliovirus proteins. In the upper right, a model of poliovirus is seen, with the four capsid proteins combining to form the elements of the virus capsid (van den Pol, 2013).

2.2 Poliovirus Genome

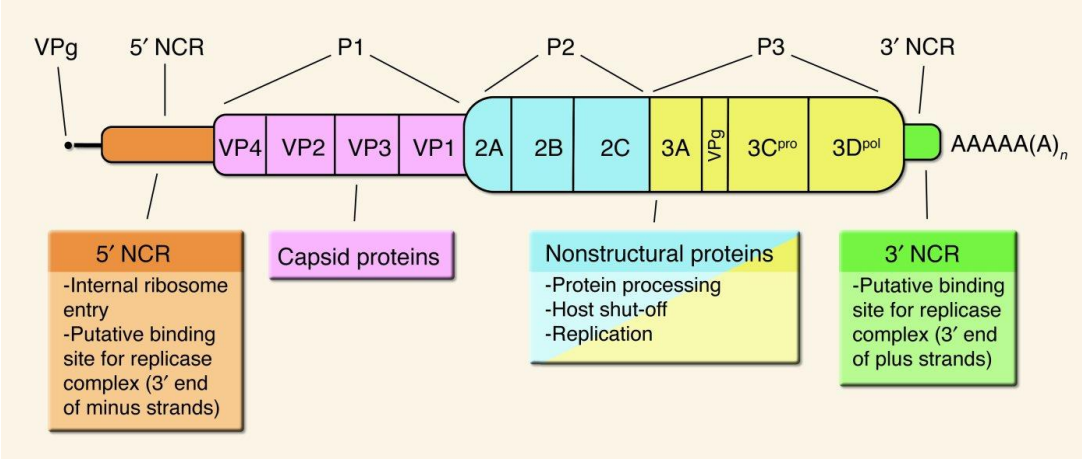

Figure 2.2: Map of the Poliovirus RNA Genome. (J Clin Invest. 2004; 113(12), 1678-1681. https://doi.org/10.1172/JCI22139.)

Genomic RNA is linked to a genetically coded poly (A) tract at the 3′ end and a virus-encoded peptide (VPg) at the 5′ end. The coding region of the virus is divided into three sections, referred to as P1, P2, and P3. The P1 region encodes the structural (capsid) proteins. The P2 region encodes proteins required for RNA replication and one of the viral proteinases responsible for host cell shut-off of cap-dependent translation. The P3 region encodes the major viral proteinase ($3C^{pro}$), the viral RNA dependent RNA polymerase ($3D^{pol}$), and other proteins required for RNA replication. The coding region is preceded by an unusually long 5′ NCR, which directs translation initiation by internal ribosome entry in the absence of cap-dependent functions. The viral genome also contains a short 3′ NCR, which presumably contains cis-acting sequences involved in template recognition by the viral-replication initiation complex (Semler *et al.*, 2018).

2.3 Vaccine-Induced Polio

The global polio eradication initiative relys majorly on Live attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV). Despite OPV being inexpensive, having superior ability to induce intestinal immunity, easy mode of administration and ability to confer passive immunity to unvaccinated individuals, OPV can revert to neurovirulence and cause paralysis. However; this is a rare event and the rate of vaccine-associated paralytic poliomyelitis (VAPP) varies by region but is about 1 case per 750,000 vaccine recipients. Hence, IPV has replaced OPV in the eradication of all polioviruses (Bandyopadhyay *et al.*, 2015). Due to the progress achieved in interruption of WPV, current polioviruses cases are linked to VAPP and cVDPV. Polio type 2 accounts for more than 95% of cVDPV and nearly 30% of VAPP. This requires change from trivalent OPV (tOPV), which protects against types 1, 2 and 3, to bivalent OPV (bOPV), which protects against types 1 and 3. Additionally, one dose of IPV which offers immunity to all polio serotypes has been included in routine immunization. Given that many high-income countries have replaced OPV with IPV, the VAPP burden is concentrated in lower-income countries. (Shutter *et al*., 2014).

2.4 Prolonged Excretion of Vaccine-Derived Virus in Individuals

After exposure to OPV, immunocompetent individuals usually excrete the vaccine virus for 4–8 weeks. However, in immunodeficient individuals; an inability to mount an adequate immune response can result in persistence of the intestinal infection with poliovirus and prolonged viral shedding. In the process, the virus can mutate to re-acquire the neurovirulence and transmissibility characteristics of wild poliovirus. Such individuals constitute the only remaining reservoir of poliovirus after eradication and withdrawal of oral poliovirus vaccine. In populations with high immunity, VDPVs rarely emerge and cause outbreaks. However, in areas with low population immunity, these viruses could potentially re-establish endemic transmission (Macklin *et al*., 2017). The major disadvantage of OPV is that they can in rare cases cause paralytic disease. They cause VAPP in vaccine recipients and close contacts at an estimated rate of about 4.7 per million births. The greatest risk of

VAPP is among immunocompromised people with B-cell deficiencies as they carry greater than 3200-times the risk of VAPP than the general population .Wild poliovirus type 3 has been isolated in people with VAPP who do not have immunodeficiencies while wild polio type 2 viruses has been isolated in individuals with immunodeficiencies (Burns *et al*., 2014). Individuals with B-cell deficiency, T-cell deficiency, major histocompatibility complex deficiency or combination of the above are at a higher risk of VDPV and VAPP (Macklin *et al.*, 2017).

Patients with primary immunodeficiency disorders either X-linked or sporadic agammaglobulinaemia (XLA), affecting the B-cell system appear to be at the highest risk for prolonged poliovirus (and other enterovirus) replication and excretion (Aghamohammadi *et al*., 2017). Patients with primary immunodeficiency (PID) are more likely to contract viral infections and shouldn't receive live vaccinations. Such individuals may have delayed excretion and viral divergence leads to their acting as reservoirs in the community and spreading the altered virus and endangering efforts to eradicate polio (Galal *et al.,* 2018).Immunocompromised individuals who take the oral polio vaccine may experience prolonged excretion of the poliovirus, which could result in the spread of highly diverse vaccine-derived polioviruses (VDPVs), raising concerns about the possibility of polio eradication worldwide (Mohanty *et al.,* 2017).

Persons with primary immune deficiency disorders (PID), especially those disorders affecting the B-cell system, are at substantially increased risk of paralytic poliomyelitis and can excrete poliovirus chronically (Li *et al.,* 2016).On average, the duration of excretion of poliovirus among iVDPV cases is relatively short (approximately 1 year) and has further declined in recent years. This declining length of poliovirus excretion for all serotypes, but especially type 2, is likely to be associated with the shift in cases from high to middle income countries. This may be caused by shorter survival of iVDPV cases in middle-income countries or a higher likelihood of spontaneous cessation of excretion (Macklin *et al.*, 2017).

2.5 Poliovirus Mucosal Replication

The main pillar of global polio end game is immunization strategies which target to prevent paralytic polio by limiting enteric replication of the virus and reducing the risk of transmission of neurovirulent strains. Given that OPV replicates in the gastrointestinal tract (GIT), it confers intestinal immunity and subsequently lowers the shedding of poliovirus once an individual is exposed to a live virus. On the contrary, IPV, induces resilient systemic immunity though has minimal effect on virus replication in the gastrointestinal tract on previously unexposed persons (Connor *et al.,* 2022). Infants vaccinated with tOPV have vigorous intestinal IgA and strain-specific neutralizing responses. Given that IPV alone cannot induce mucosal immunity; its best results are obtained when IPV is given to persons previously primed with OPV. Successful polio eradication strategies should therefore rely on mechanisms that limit the replication of poliovirus in the GIT and subsequent shedding in stool (Wright *et al*., 2016).

Infants given OPV can shed vaccine in their stools for up to 60 days. After administration of OPV alone, vaccine poliovirus serotypes were recovered in feces within 1 week and for as long as 31-60 days in 30%-80% of subjects after 1 or 2 doses and in 30%-50% after immunization with > or = 3 doses (Van Damme *et al.,* 2019).

2.6 Poliovirus Neuron Attachment and Replication

Poliovirus is transmitted through the oral-fecal route; it enters the body via the oral route. As compared to other pathogens, poliovirus is resistant to acid in the stomach cause initial infection in the throat and tonsils. The virus multiplies in the alimentary mucosa, and possibly in the tonsils and Peyer's patches. From the gut, the virus infects the lymph nodes (lymphatic system) and then move to the blood stream. From the blood stream, the virus can directly cross the Blood -Brain Barrier (BBB) into the Central Nervous System (CNS) and infect the neurons. It is not a common occurrence for viruses to cross the BBB. However, this virus does so by infecting the endothelial cells (trans endothelial transport) or injury (van den Pol, 2013).The poliovirus receptor (PVR) CD155 is a member of the Immunoglobulin G (IgG) super

family. All the three polio serotypes use the same receptor. CD 155 is required but alone cannot lead to an infection. Given that other non-neuronal cells express CD155 but are not so susceptible to poliovirus infection indicate infection requires interplay of many factors. Human motor neurons and muscle end plates express PVR which can either be membrane-bound or in releasable form. The membrane-bound receptor is the critical one for Poliovirus entry into a cell. A key feature in the global polio eradication is that poliovirus predominantly infect human cells and that there are no animal reservoirs (Koyuncu *et al.*, 2013).

The long-term paralysis that occurs in 1% of patients is the result of PV infection and irreversible killing of motor neurons in the anterior horn, resulting in flaccid paralysis of denervated muscles, or less commonly killing of motor neurons in the bulbar region of the brainstem, affecting muscles innervated by cranial nerves and thus interfering with breathing, speaking, or swallowing. Since little can be done to cure paralytic poliomyelitis, most efforts focus on prevention of infection, first by immunization and second by improving sanitation in regions at risk (van den Pol, *et al.*, 2013).

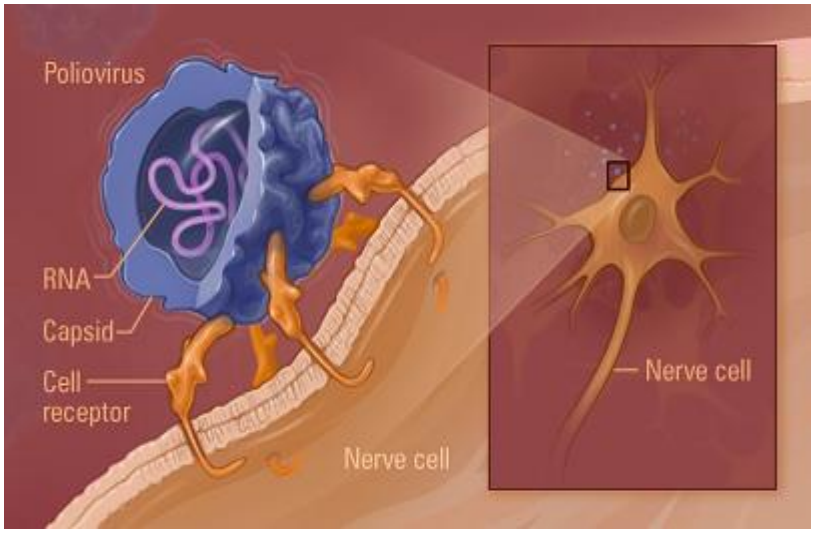

Figure 2.3: Poliovirus Bound to a Neuron Receptor (Illustration Courtesy of Americanhistory.si.edu)

2.7 Global Trends in Polio

Nearly three decades after the establishment of the Global Polio Eradication Initiative (GPEI), there has been a global decline from more than 350,000 paralysis in 1998 to 140 cases in 2020. At the moment, it's only the wild poliovirus type 1(WPV1) that still in circulation in Afghanistan and Pakistan. In the year 2020, Afghanistan reported 56 cases of WPV1 as compared to 29 cases in the year 2019 while Pakistan reported 84 cases in 2020 as compared to 147 cases in 2019. As GPEI puts more focus on final endemic WPV reservoirs, the current poliomyelitis outbreaks have been caused by circulating vaccine-derived poliovirus (cVDPV) that result from the attenuated oral poliovirus vaccine (OPV) virus regaining neurovirulence after prolonged circulation in under immunized populations. Globally, 32 countries reported cVDPV outbreaks in 2020; four countries reported type 1 (cVDPV1), 26 countries reported type 2 (cVDPV2) while two countries reported both type 1 and 3. As a result, the GPEI strategy (2022-2026) has incorporated the use of type 2 novel oral poliovirus vaccine (nOPV2) to avoid new emergences of cVDPV2 during outbreak responses (Bigouette *et al*., 2021).

A risk of further circulation of poliovirus in inadequately immunized populations arises when immunodeficient persons with persistent vaccine-related poliovirus infection may serve as a potential reservoir for reintroduction of polioviruses into the general population after wild poliovirus eradication. The oral polio vaccine which contains live attenuated poliovirus of serotype 1, 2 and 3 is the major tool that has been utilized in the global polio eradication strategies. As a result of the administration of OPV to a susceptible person, the polio vaccines establish an infection and subsequent replication in the pharynx and the intestines for a period of 4-6 weeks hence enabling the vaccinated individuals to acquire humoral and mucosal immunity. In some situations, the replication process may result in the mutation of Sabin strains towards more genetically stable variants that may revert to neurovirulence just like the wild polioviruses. These variants may cross the blood brain barrier and infect the central nervous system and leading to paralysis that cannot be distinguished from those caused by wild polioviruses. These are termed as vaccine-associated paralytic poliomyelitis (VAPP) (Platt *et al.*, 2014).

VAPP is a rare occurrence of paralytic polio among OPV vaccinated individuals or susceptible contacts of recipients, which occurs in an estimated rate of 1 case per every 2 million OPV doses administered. The risk of VAPP is determined by the general immunity of the population. Countries that have a large number of their population being susceptible are likely to have higher cases of VAPP. The susceptibility to VAPP can occur across all age groups and can be linked to history under immunization and lack of natural immunity from wild poliovirus in polio-free areas (Burns *et al*., 2014).

Polioviruses can be definitively categorized by the sequence properties of their genomes. Wild polioviruses have no genetic evidence of derivation from any vaccine strain and demonstrate capability of continuous person-to-person transmission. Sequences encoding their major capsid protein, VP1, are used during polio surveillance. Apart from the wild polioviruses, there are vaccine-related polioviruses which can be classified as OPV-like isolates meaning they are identical to the vaccine or VDPV isolates. OPV-like isolates have limited divergence from their parental OPV strains and are ubiquitous wherever OPV is used while VDPV isolate have higher level ofVP1 sequence divergence from their parental OPV strains; type 1 and 3 have more than 1% divergence while type 2 has more than 0.6% divergence. (Thompson *et al*., 2020: Burns *et al*., 2014: Jorba *et al*., 2017) This divergence points to prolonged replication or transmission of the vaccine virus. VDPVs can further be characterized as; circulating VDPVs (cVDPVs), when there is evidence of person-to-person transmission in the community; immunodeficiency-associated VDPVs(iVDPVs), which are isolated from persons with PIDs who have prolonged VDPV infections; or ambiguous VDPVs(aVDPVs), which are either clinical isolates from persons with no known immunodeficiency or sewage isolates whose ultimate source is unknown(Burns *et al*., 2014). VDPVs are defined primarily on their estimated period of replication and not their phenotypic characteristics. There is high probability that majority of OPV-like isolates have regained higher neurovirulence capabilities and increased transmissibility. Circulating VDPVs pose the same public health threat as wild polioviruses since they have recovered the biological properties of wild polioviruses and they have the potential to re-establish endemicity in settings of low polio vaccine coverage and they require the same control measures.

Immunodeficiency-associated VDPVs may be excreted by individuals with PIDs for many years with no apparent paralytic signs. However, individuals infected with iVDPVs are at risk of developing paralytic poliomyelitis and may infect others, presenting the potential risk of outbreaks (Hovi *et al*., 2013).

The first polio reported outbreak caused by cVDPVs was detected on the island of Hispaniola in the year 2000; this resulted in the paralysis of 21 children and clearly demonstrated for the first time the potential for vaccine strains to circulate and cause paralytic disease in the population Duintjer *et al.,* 2013).Absence of wild poliovirus of the same serotype and low vaccination coverage were associated with the outbreaks; however, it is not clear whether low population immunity alone is sufficient for the emergence of cVDPVs or if there are other factors contributing to the same (Cooper *et al.,* 2022).An explanation that is complementary is that only after essential mutations for virulence and transmissibility are acquired within an immunodeficient individual vaccine virus able to circulate and cause disease in the population. Shedding of iVDPVs for duration of at least 6 months has been identified in 19 patients with B cell immune deficiency disorders. In majority of patients who shed the virus for >10 years, some have developed paralytic polio, demonstrating that prolonged replication even within a single individual increases the virulence of these vaccine- related viruses (Macklin *et al.,* 2017).

No evidence of paralytic disease resulting from secondary exposure to iVDPVs has been shown; however, since most contacts of these individuals are immune to poliovirus, the significance of this observation is uncertain. Studies that have been designed to search for more patients with prolonged shedding of vaccine virus show that these cases are rare to get (DeVries *et al*., 2011).Currently there is no evidence to date indicating that immune deficiency caused by HIV is associated with VDPVs; however, few studies have been conducted to directly measure this association (Vidya *et al*., 2012).

A small increase in duration of viral shedding of several weeks to months may enhance the evolution and spread of vaccine viruses given the geographic foci of high HIV prevalence in several OPV-using countries. Therefore, a large study in a

high-risk setting in terms of HIV prevalence, OPV exposure, and conditions that facilitate vaccine virus spread which includes low vaccination coverage, high population density, poor hygiene, and tropical climate was needed to evaluate the risk of persistence of vaccine virus shedding among persons with HIV infection (Holubar *et al.*, 2017).

Oral poliovirus vaccine has been a vaccine of choice in developing countries for both persons infected with HIV and the uninfected. Previous studies in Democratic Republic of Congo showed high protective antibody titres in HIV infected children who had received the three OPV doses (Manirakiza *et al.*, 2010). Shedding of poliovirus in immunized persons is usually between 2-3 months (Troy *et al.*, 2013). OPV associated disease incidence is estimated to be 1 case for every 750 000 doses among first-dose recipients and 1 case for every 6.4 million doses (all doses) among contacts (Kalkowska *et al.*, 2019).

The immune response to the poliovirus vaccine in HIV-exposed children can be different compared to HIV-negative children. HIV-exposed children may have a weaker antibody response to the poliovirus vaccine, which can result in lower levels of protection against the disease.

This is because HIV can impair the immune system, particularly the function of CD4+ T cells, which play a critical role in the body's ability to mount an effective response to vaccines. Additionally, HIV-exposed children may have a higher risk of developing vaccine-associated paralytic polio (VAPP) due to their weakened immune system.

To address these concerns, the World Health Organization (WHO) recommends that HIV-exposed children receive the inactivated poliovirus vaccine (IPV) instead of the oral poliovirus vaccine (OPV), which has been associated with a higher risk of VAPP in immunocompromised individuals. The IPV is a safe and effective vaccine that is administered as an injection and has been shown to provide good protection against polio in HIV-exposed children.

It is important to note that despite the potential for a weaker immune response to the poliovirus vaccine in HIV-exposed children, vaccination remains a critical tool in the global effort to eradicate polio. Through continued vaccination efforts and public health interventions, we can work towards a future where polio is no longer a threat to global health.

Further, in all cases (infected and uninfected), the poliovirus infections were cleared in the course of the observation period and the overall patterns of excretion did not differ by HIV status. The pointed phenomenon of non-variation in excretion by HIV or non-HIV status have also been noted in a prospective, longitudinal, observational study in Zimbabwe (Troy *et al*,. 2013). Effect of Vaccination with OPV leads to decreased Poliovirus shedding which is considered a marker for the development of mucosal intestinal immunity (Hird, 2012) in the present study there was a decrease in poliovirus shedding in weeks 6 and 8 suggesting that both HIV infected and HIV uninfected children developed intestinal mucosal immunity after the OPV booster dose. Age was also not a factor that affected the pattern or duration of OPV shedding. Findings from South Africa (among three HIV infected children) have observed that HIV-infected children shed vaccine-related polioviruses in specimens obtained between 15 and 42 months after the last known dose of OPV, suggesting possible long-term persistence (Halpern *et al*., 2018). However, further molecular studies showed that for all three cases, there was a divergence of nucleotide sequences in the VP1 region of 0.3% to 0.6% showing close genetic relatedness to prototype Sabin strains. Thus, it was interesting to estimate the frequency of such individuals in countries with different socioeconomic contexts and different health policies for poliomyelitis control. Findings from a study done after national immunization week in a Mexican community primarily found that Serotype 2 circulated longer and was transmitted more readily than serotypes 1 or 3 vaccinated with IPV. This may be part of the reason why most isolated cVDPV has been serotype 2 (Troy *et al.*, 2013). In our presented findings though in a shorter time period but more participants, indicate that the ability to clear OPV is preserved in both HIV-infected and HIV-uninfected children.

A number of factors could potentially influence the course of poliovirus infection in an HIV-infected host, including the degree of immunosuppression, previous OPV vaccination history, and patient age. The CD4 lymphocyte count of HIV-infected individuals is an important predictor of HIV disease progression and acquired immune deficiency syndrome (AIDS)-associated mortality (Crum-Cianflone & Sullivan, 2017).

2.8 The Polio Eradication Initiative

The Global Polio Eradication Initiative (GPEI) was launched in 1988 with the goal of completely eradicating wild polioviruses by year 2000. More than thirty years later, the goal has not been achieved, although substantial progress has been made, with wild poliovirus reported in only 2 countries in 2019. GPEI utilized four strategies to interrupt the transmission of WPV; enhancing routine childhood immunization with the oral OPV, conducting supplementary immunization activities (SIAs), conducting surveillance for WPV through investigation of acute flaccid paralysis (AFP) cases among children under 15 years of age, and laboratory characterization of polioviruses isolated from stool specimens and conducting mop-up campaigns in focal areas surrounding recently identified polio cases (Wassilak, 2010).

The milestones achieved by the GPEI are mainly attributed to extensive OPV and IPV coverage. Though the goals of GPEI have not been achieved, the number of WPV paralysis cases have diminished significantly from nearly 350,000 children paralyzed annually in 125 endemic countries in 1988, to 140 wild-type polio cases reported in 2020 only in Pakistan and Afghanistan.

Pakistan and Afghanistan face challenging epidemiological conditions such as; high population densities, high birth rates, poor sanitation, insecurity, nomadic practices and competing health priorities making polio eradication much more challenging. Additionally, the polio programme has resorted to repeated mass vaccination campaigns due to lack of markers for prior vaccinations and the fact that acute flaccid paralysis (AFP) can be caused by other viruses and conditions, which makes laboratory confirmation compulsory and leads to a lag between symptoms and confirmation of polio (González, 2021).

Additionally, the rise in WPV cases from 33 in 2018 to 140 in 2020 is attributed to and the impact of the COVID-19 pandemic in the response capacity of health systems and prohibition of house-to house vaccination campaigns in regions with civil strife. Furthermore, there has been a recent rise in cVDPV; with 318 cases reported in 17 countries in 2019 and majority being in Africa. Such outbreaks are linked to the neuro-conversion of OPV used in routine vaccination to virulent strains. This has led to an action by many of these countries to introduce inactivated poliovirus vaccine (IPV) – a safe and effective alternative for routine immunization – using one of two approaches: replacement of OPV by IPV and introduction of a sequential IPV/OPV schedule. Trivalent OPV (tOPV) which contains live attenuated strains of serotypes 1, 2 and 3 of poliovirus has been replaced with bivalent OPV (bOPV) (Bandyopadhyay, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted at Cottolengo children's home an orphanage situated in the Karen area of Nairobi along the Langata road. This home is private managed by the Catholic Church. The home hosts orphaned children whose parents have died of HIV related complication and have no other member of family with ability to take care of them. The home has children with ages ranging from 4months to 18years of age. Most children are HIV positive and a few children who are HIV negative are five years and below. This is due to the fact that children who test negative and are above two years of age are transferred to Thomas Banados children home for adaption. The population of children in this home is about 500 children in total with target population for this study being 23 children as per February 2012.

3.2 Study Design

The study adopted a longitudinal study design and aassessed the rate of poliovirus shedding among HIV-1-infected children who received tOPV at Cottolengo Children's Home based on practicality, feasibility, ethical considerations, and the potential public health impact.

The primary aim of our study was to determine the rate of poliovirus shedding among children aged under 5 years who were vaccinated with tOPV. A cross-sectional design was suitable for obtaining a snapshot assessment of poliovirus shedding at a single point in time, providing valuable insights into the status of the population

Understanding the rate of poliovirus shedding among HIV-1-infected children who received tOPV is important for vaccine policy and infection control measures. The study sort to characterize the health outcome of the study population was determined after uptake of tOPV

3.3 Study Variables

3.3.1 Dependent Variable: Rate of Vaccine Shedding among Vaccinated Children

This was the proportion of vaccinated children who excrete (shed) the vaccine virus in their stool after receiving a vaccination. The measurement was expressed as a percentage or proportion of children who test positive for the vaccine virus among the total number of vaccinated children in the study. The samples were collected at multiple time points to provide insights into variations in shedding rates over time.

3.3.2 Independent Variables

3.3.2.1 Socio-Demographic Factors: Age and Sex

Age can be a significant independent variable affecting the rate of vaccine shedding. Younger children may have different immune responses or shedding patterns compared to older individuals. For example, infants and toddlers might shed the vaccine virus differently than adolescents or adults. Sex can also play a role in the rate of vaccine shedding. Biological differences between males and females may lead to variations in immune responses, which can impact shedding.

3.3.2.2Vaccination History and Vaccine Type Used

A child's vaccination history, including the number of previous vaccinations and the timing of those vaccinations, can influence the rate of vaccine shedding. For instance, a child who has received multiple doses of a vaccine may have a different shedding pattern than one who has only received a single dose.

The specific type of vaccine administered can significantly affect shedding rates. Different vaccines may use varying strains of the virus or have different vaccine components, which can impact shedding. Researchers should categorize participants based on the type of vaccine they received (e.g., trivalent poliovirus vaccine, monovalent vaccine) and investigate whether shedding rates vary between vaccine types. (Troy *et al.,* 2013).

Independent variables represent potential factors that may influence the rate of vaccine shedding. To assess their actual impact, researchers would typically conduct statistical analyses to determine whether there are significant associations or correlations between these variables and the rate of shedding.

3.4 Study Population

This was a longitudinal study of duration of poliovirus vaccine shedding among children who lived in a small orphanage for HIV-infected children in Nairobi, Kenya. Cottolengo (capacity, 500 children) admits mainly orphaned children between the age of 4months and 18 years whose parents have died of HIV related complications. At the orphanage, children receive adequate nutrition and medical care, including immunizations. Anti-retroviral treatment was not routinely available at the time the study was conducted.

Medical records at the orphanage were reviewed to identify each child's HIV status and obtained Age, Sex, and immunization data. Final HIV infection status of the participants was assigned at the time of sample collection in September 2013, when all the study participants were aged ≤ 59 months. Children who had reverted to seronegative were classified as HIV-uninfected and served as the control group. The dates of the last OPV exposure were defined as the date of the national immunization days (NIDs) for all children ≤ 59 months both who received and for those who did not receive routine OPV doses after study enrolment.

3.5 Sample Size Determination

The sampling universe;

The sampling universe was finite and well defined making it easy to collect samples therefore all the children of the target group were included in the study. The sampling of all the children in the home made the data optimum for the said universe because it fulfilled the requirements of efficiency, representativeness, reliability and flexibility. This was to ensure that study gave a true representativeness of the under 5s and was not biased.

The target population of children below 5 year of age in cottolengo childrens' home for the study was 26 children. The test group had 10 HIV +ve children while the control had 16 HIV-ve children. The use of children from the same children's home decreased the rate of drop out among study participants. Only HIV positive children were required in the study, HIV negative children were recruited to the study to act as controls, thereby reducing sampling bias. This group were added to determine/establish if there are any differences in viral shedding rate between the two groups.

3.5.1 Inclusion Criteria

Children of under 5 year of age.

All children under five of whom the legal guardian has assented to participate in this study

3.5.2 Exclusion Criteria

All children above five of whom the ministry of health don't target for routine or supplementary immunization

3.6 Laboratory Methods

All virus isolation and real time PCR procedures and primers were done according to WHO polio manual v4 (World Health Organization 2004)

3.6.1 Collection and Preparation of Fecal Samples for Virus Isolation

Stool specimens were collected at site during week 2, week 4, week 6 and week 8 after vaccination with tOPV from children residing at the orphanage who were ≤ 59 months of age at the time of enrollment. The intervals between specimen collections were 2 weeks. Data collection tool attached in Appendix I

3.6.2 Procedure for Preparation of Fecal Samples for Virus Isolation

Labelled Centrifuge tubes were filled with 10 ml PBS (sigma), 1 g of glass beads and 1 ml chloroform. Under a Bio Safety Cabinet II (BSC-II), approximately 2 g of each faecal sample were transferred to a labelled centrifuge tube. Centrifuge tubes was closed securely and shaken vigorously for 20 minutes using a mechanical shaker.

Samples were centrifuged for 20 minutes at 1500xg in a refrigerated centrifuge ensuring that centrifuge caps were securely in place and centrifuge buckets were sealed. Working in a BSC, supernatant was transferred from each sample tube into two labelled externally threaded screw-capped storage vials

3.6.3 Virus Inoculation

Virus isolation was done on RD and L20B cell line to determine presence of the Poliovirus in the samples.

Procedure:

Two tubes of RD and two of L20B were labelled with the specimen number for each specimen that was inoculated at the same time with 0.2ml of specimen extract. One tube of each cell type was labelled as a negative control. This is in accordance to WHO polio manual

Specimens were incubated in the stationary-sloped (5°) position at 36°C and examined daily, using a standard or inverted microscope, for the appearance of cytopathic effect (CPE) for at least one week, then record of CPE (1+ to 4+) to indicate the percentage of cells affected (1+ to up to 25%; 2+ to 25 to 50%; 3+ to 50 to 75% and 4+ to 75 to 100%), toxicity [denoted as 1], degeneration or contamination [denoted as 2].

3.6.4 Result Interpretation

Characteristic enterovirus CPE of rounded, retractile cells detaching from the surface of the tube, were recorded and allowed to develop until at least 75% of the cells are

affected (3+ CPE). This was then stored at -20°C for a second passage in a tube containing 2 ml of medium. Second passage material was pooled for typing and Inter Typing Differentiation (ITD). A blind passage was performed for samples with no CPE after seven days, and examined for a further seven days. Negative cultures were examined for a total of at least 14 days before being discarded.

Any culture positive in RD cells but negative after 14 days in L20B cells were re-passaged in L20B cells and examined for seven days to exclude the possibility that they are polioviruses.

3.7 Characterization of Shed Poliovirus Vaccine Types by Real Time PCR

Isolates positive on L20B cell lines (a mouse cell line) were amplified using real time Poliovirus diagnostic PCR kit. Primers used have specificity for the enterovirus group and Sabin type-specific for each of the three serotypes.

3.7.1 Primers for Intra-Typing Differentiation (ITD) and Vaccine Derived Poliovirus (VDPV)

Intratypic differentiation of isolates was done using real time PCR targeting the VP1 gene (~900 nucleotides) were used to determine the type of poliovirus and the degree of its relatedness to the prototype Sabin strains. The primer sequence that was used for the real time PCR was as shown in table 3.1 and 3.2 below;

Table 3.1: Primer or Probe Sequence for Intratypic Differentiation (ITD) as per CDC Protocol (Gerloff et al., 2018)

Specificity	Primer or Probe (polarity)	Primer or Probe Sequence (5'→3')
Pan-Enterovirus	PCR-1(A)	GCGATTGTCACCATWAGCAGYCA
	PCR-2 (S)	GGCCCCTGAATGCGGCTAATCC
(Gerloff N *et al., 2018)*	PanEV Probe (S)	FAM-CCGACTACTTTGGGWGTCCGTGT-BHQ1
***Pan-Poliovirus**	panPV/PCR-1(A)	AYRTACATIATYTGRTAIAC
***Gerloff N et al.*, 2018)**	panPV/PCR-2(S)	CITAITCIMGITTYGAYATG
	panPV Probe21A(A)	FAM-TGRTTNARIGCRTGICCRTTRTT-BHQ1
***Serotype 1**	seroPV1A(A)	ATCATIYTPTCIARPATYTG
(Gerloff N *et al., 2018)*	seroPV1,2S(S)	TGCGIGAYACIACICAYAT
	seroPV1 Probe 16A(A)	FAM-TGCCYAVICCYGIGMIADYGC-BHQ1
Serotype 2	seroPV2A (A)	AYICCYTCIACIRCICCYTC
(Gerloff N *et al., 2018)*	seroPV1,2S (S)	TGCGIGAYACIACICAYAT
	seroPV2,Probe 5S (S)	FAM-CARGARGCIATGCCCICARGGIATNGG-BHQ1
***Serotype 3**	seroPV3A (A)	CCCIAIPTGRTCRTTIKPRTC
(Gerloff N *et al., 2018)*	sero PV 3S (S)	AAYCCITCIRTITTYTAYAC
	seroPV3,Probe 11S (S)	FAM-CCRTAYGTNGGITTRGCVAAYGC-BHQ1
Sabin 1	Sab1/PCR-1(A)	CCACTGGCTTCAGTGTTT
(*Gerloff N et al., 2018)*	Sab1/PCR-2(S)	AGGTCAGATGCTTGAAAGC
	Sab1/Probe (A)	CY5-TTGCCGCCCCCACCGTTTCACGGA-BHQ3
Sabin 2	Sab2/PCR-1(A)	CGGCTTTGTGTCAGGCA
(*Gerloff N et al., 2018)*	Sab2/PCR-2(S)	CCGTTGAAGGGATTACTAAA
	Sab2/Probe (S)	FAM-ATTGGTTCCCCCGACTTCCACCAAT-BHQ1
Sabin 3	Sab3/PCR-1(A)	TTAGTATCAGGTAAGCTATC
(*Gerloff N et al., 2018)*	Sab3/PCR-2(S)	AGGGCGCCCTAACTTT
	Sab3/Probe (S)	ROX-TCACTCCCGAAGCAACAG-BHQ2

Degenerate primers: K=G and T; M=A and C; R=A and G; Y=C and T; I=Degenerate base analog, Inosine; P=Degenerate base for (TC).
*the study used degenerate PCR conditions with these primer sets.

Table 3.2: Primer or Probe Sequence for Sabin Vaccine Derived Poliovirus (VDPV)

Primer specificity	Primer and Probe sequences 5'→3'
S1 VDPV VP1	Sense CATGCGTGGCCATTATA
(Target aa# 99)	Anti-Sense CAAATTCCATATCAAATCTA
(*Gerloff N et al., 2018)*	VP1 Probe FAM-CACCAAGAATAAGGATAAGC-BHQ1
S2 VDPV VP1	Sense GACATGGAGTTCACTTTTG
(Target aa# 143)	Anti-Sense CTCCGGGTGGTATATAC
(*Gerloff N et al., 2018)*	VP1 Probe FAM-CATTGATGCAAATAAC-BHQ1
S3 VDPV VP1	Sense CATTTACATGAAACCCAAAC
(Target aa# 285-290)	Anti-Sense TGGTCAAACCTTTCTCAGA
(*Gerloff N et al., 2018)*	VP1 Probe FAM-TAGGAACAACTTGGAC-BHQ1

3.7.2 Materials and Equipment for Real Time PCR

The details of these are in appendix 5

3.7.3 Sample Preparation (WHO, 2004; Gerloff *et al.*, 2018)

Isolates that were L20B positive were aliquoted first in 1ml cryovials and transferred to the molecular laboratory for further analysis. In the molecular laboratory 50 µl amounts of the isolates were placed into a tube and spun using a bench top micro centrifuge at 6,500 rpm at room temperature for 2 minutes to get a clean RNA from the supernatant.

Spun isolates were stored at 4°C awaiting inoculation into master mix.

3.7.4 Real-Time RT-PCR Reactions (Gerloff *et al.,* 2018)

To determine the serotypes being shed by study participants , all six ITD assays were tested against L20B positive isolates (*n* = 53) representing all PV serotypes that were isolated in cell cultures of L20B cells (recombinant murine cells that express human poliovirus receptor [PVR]) and human rhabdomyosarcoma cells (RD; ATCC CCL-136) inoculated.

The six rRT-PCR assay used in the study identified between one and four different targets and followed a specific algorithm (see table 3.1 and 3.2 for targets). The quadruplex EV-Sabin assay includes primers and probes for the detection of any enterovirus (EV) and the oral vaccine strains (Sabin 1, 2, and 3) The other five assays detect any poliovirus (PanPV), WPV1, PV2, and WPV3

The master mix for the ITD PCR consisted of 1 ml of Buffer B, 2.8 µl of 1 M DTT, 27.6 µl of 40 U/µl RNase inhibitor, 18.0 µl of 20 U/µl Transcriptor RT and 54.8 µl of 5 U/µl Taq polymerase (Quanta Biosciences, Beverly, MA), 1 µl of primer-probe mixture (contained in the ITD 5.0 kit; CDC, Atlanta, GA) and 1 µl of template (cell culture supernatant or extracted viral RNA). Reaction strips were placed in real-time thermocycler and run cycle as follows:

a) RT reaction, 42°C, 45 min.

b) Inactivate RT, 95°C, 3 min.

c) PCR cycles (all primers sets): Using an ABI 7500 (or equivalent): 95°C for 24 sec, 44°C for 30 sec, then a 25% ramp speed to 60°C for 24 sec, for 40 cycles The endpoint fluorescent data was collected at the end of the anneal step.

d) The appropriate dye filter was selected to correspond with the assay being used. *(*Gerloff *et al.,* 2018). A reduced ramp rate of 25% between annealing and elongation was applied on the Applied Biosystems 7500 real-time PCR system [Thermo Fisher Scientific].

Table 33: Master Mix Guide

Sample number	Primer amount (microliters)	Buffer B + enzyme Mix (microliters)
1	19	5
2	38	10
3	57	15
4	76	20
5	95	25
6	114	30
7	133	35
8	152	40

3.7.5 Result Interpretation

The negative and positive control data was used to validate assay before reading data from samples. The cycle threshold value (Ct) of between 10-28 (a cycle number where a PCR product is seen via fluorescence) was used to determine the positivity or negativity of each curve. These Ct values were calculated automatically by the ABI 7500 software.

The Ct value cutoff was 30, with values less than 30 as positive and values more than 30 as negative.

3.8 HIV Status Data and CD4 Count

Secondary HIV status and CD4 results data was abstracted from the cottolengo children home database. The use of this data from the home was time-saving, reduced harm on study participants and cost-efficient. The type of data is routinely performed at the home for admission, management and handling of the children admitted at the home.

3.9 Data Management

3.9.1 Primary Data Collection

All samples were coded at site and entered into a bio data collection tool. The Data was then entered into a Microsoft Office spreadsheet referencing the unique laboratory number, CD4 count, and the type(s) of vaccine poliovirus shed and period of shedding. The data was backed up weekly using CD-ROM's. The Bio data collection tool for this study was kept in a box file under lock and key at KEMRI's EPI laboratories in the Center for Virus Research.

Samples were collected and used according to ethical regulations. Data got from the sample processing was entered into excel spread sheets and hard copies kept under lock and key. The data was imported into SPSS for windows computer software which was used for processing and analysis. Data was subjected to descriptive statistics to summarize results into percentages and frequencies. Univariate data was expressed inform of tables and graphs. Bivariate data was used to determine the relationships between the dependent and independent variables.

Data were managed using excel spread sheet and Statistical Package for Social Sciences (SPSS) version 21. Quantitative data was analyzed by means of descriptive statistics. Variables considered while estimating the proportions shedding vaccine poliovirus serotypes included time from the last OPV dose administered, HIV status, poliovirus seroconversion, and age. Immuno competence was defined per the WHO/CDC age specific clinical categories and immunologic status: 1) no evidence of immunosuppression – age <12 months: CD4+ count ≥ 1500, and/or CD4% ≥ 25%; age 1–5 years: CD4+ count ≥ 1000 and/or CD4+% ≥ 25%; 2) moderate immunosuppression – age <12 months: CD4+ count 750–1499, and/or CD4% 15–24%; age 1–5 years: CD4+ count 500–999 and/or CD4+% 15–24%; 3) severe immunosuppression – age <12 months: CD4+ count <750, and/or CD4% <15%; age 1–5 years: CD4+ count <500 and/or CD4+% 15% [36, 37]. In the rRT-PCR for ITD and VDPV, primers that specifically differentiate between wild and vaccine derived polio were utilized. Pearson's chi-squared test was applied to establish whether there was a significant difference in the distribution of poliovirus serotypes between the

HIV-infected and HIV-uninfected groups. P values of equal or less than 0.05 ($p \leq 0.05$) were considered to be statistically significant.

3.10 Ethical Consideration

This research study was presented to the center Steering committee and Scientific Steering Committee (SSC 2352) and ethical committee at Kenya Medical Research Institute (KEMRI), and Jomo Kenyatta University of Agriculture and Technology (JKUAT) for approval. Informed consent was sort to collect the blood and stool samples from the cottolengo children's home administration since the children are minors and are orphans. This study was laboratory based, in which the selected blood and stool samples were subjected to molecular analysis.

3.11 Biosafety

Biosafety level 2 (BSL-2) practices and facilities were applied while handling clinical material from the cases. Disposable gloves were worn when working with the known HIV and poliovirus infectious materials. Work areas were decontaminated with 0.5% sodium hypochlorite prepared fresh each month. Gloves, micro pipette tips, tubes, micro tubes and any other disposable materials and equipment used in the laboratory when handling the samples were autoclaved before being discarded. All the information obtained was handled as strictly confidential, data password protected and accessible only to the principal investigator. The participants in the study were kept anonymous using assigned laboratory numbers. All the findings of the study were communicated to the cottolengo children's home.

CHAPTER FOUR

RESULTS

4.1 Study Enrollment

The study recruited 26 children ≤ 59 months from the home. On profiling the children HIV status as at September 2013 (the time when national immunization activities was done), 10 of these were confirmed to be HIV-infected from the records filed in the Home, while 16 children had sero-negative status and were therefore reclassified as HIV-uninfected.

The mean of CD4 count among the HIV infected children as captured from the data collected from the home was 1450/mm^3 confirming no child was considered immunodepressed among the enrolled despite having the virus and factoring the age range of those enrolled [Malhotra, 2006; Giorgi, 1992]. The mean age of the study population was 28.8 months, while the median age was 30 months (25-48 months range). Of the children recruited, majority were in the 25-48 months (n=12; 46.2%) age category followed by 13-24 months age category (n=8; 30.8%). There were slightly more females (n= 14; 53.8%) recruited than males (n=12; 46.2%).

4.2 The Duration of Vaccine Shedding in Children Vaccinated with Trivalent Poliovirus

In the genetic characterization of PV isolates, the VP1 gene was the gene of interest for the isolates. Poliovirus isolates appeared “Sabin-like” in rRT PCR intratypic differentiation tests, indicating the lack of any major antigenic changes. All the poliovirus isolates that tested positive during the intratypic differentiation (ITD) for any Sabin-like polioviruses (n = 53) from the 26 study subjects on week 2–6 were tested for possible recombination by real-time PCR (table 4.1); all did amplify with the VP1 genomic region using the homotypic Sabin-specific primers, ruling out the evidence of recombination with other circulating vaccine-related or wild polioviruses. One HIV-infected participant shed a combination of poliovirus type 2

sabin like and non-polio enterovirus (NPEV) up to week 6, but continued shedding NPEV up to week 8.

Table 4.1: Duration of Vaccine Shedding in Study Subjects from Week 2 to Week 8

week	Total, N = 26, (%)	percentage (%) shedding poliovirus	percentage (%) shedding non-polio enterovirus	percentage (%) shedding no virus
Week 2	26 (100%)	26(100%)	0	0
Week 4	26 (100%)	26(100%)	0	0
Week 6	26 (100%)	1 (3.8)	0	25 (96.2)
Week 8	26 (100%)	0	1 (3.8)	25 (96.2)

4.3 The Types of Vaccine Derived Polioviruses Being Isolated from Stool Samples of the Vaccinated Children

Type 1 poliovirus was detected in 16 specimens (including 3 specimens from HIV-infected children), type 2 in 3 specimens (2 from HIV-infected children), and type 3 in 20 specimens (6 from HIV-infected). More than one poliovirus type was present in 14 specimens (6 from HIV-infected). The twenty six children (10 HIV-infected and 16 HIV-uninfected) had more than one specimen positive for poliovirus. There were no significant differences in the distribution of poliovirus serotypes between the HIV-infected and HIV-uninfected groups with the virus shedding pattern between the HIV positive and the HIV negative groups showing no significant difference ($P > 0.05$) by sex or age as well. Among the study participants (Table 4.2), Poliovirus serotype 3 was shed by majority of the children (38.5%) in week 2 and week 4 respectively with the highest shedding among ages 13-24 months (15.4% and 19.2%) in week 2 and 4 in the order. However, Poliovirus serotype 1 was also shed most by

ages 25-48 months (15.4%). There was no evidence from our data that age of the study participants was a contributing factor to prolonged (≥6 months) poliovirus shedding (table 4.2).

Table 4.2: Duration of Shedding Vaccine after Immunization Verses the Proportional Study Subjects at the Time Points and HIV Status

Parameters (time and poliovirus)	**Total, N = 26, (%)**	**HIV(+), n = 10, (%)**	**HIV(−), n = 16, (%)**
2 weeks			
PV 1	9 (34.6)	3 (30)	6 (37.5)
PV 2	1 (3.8)	1 (10)	0 (0)
PV 3	10 (38.5)	4 (40)	6 (37.5)
PV 1 + 2	1 (3.8)	1 (10)	0 (0)
PV 1 + 3	3 (11.5)	1 (10)	2 (12.5)
PV 1 + 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 2 + 3 + NPEV	1 (3.8)	0 (0)	1 (6.3)
4 weeks			
PV 1	7 (26.9)	2 (20)	5 (31.3)
PV 2	1 (3.8)	1 (10)	0 (0)
PV 3	10 (38.5)	3 (30)	7 (43.8)
PV 1 + 2	1 (3.8)	1 (10)	0 (0)
PV 1 + 3	5 (19.2)	3 (30)	2 (12.5)
PV 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 3 + NPEV	1 (3.8)	0 (0)	1 (6.3)
6 weeks			
Negative	24 (92.3)	9 (90)	15 (93.8)
PV 1	0 (0)	0 (0)	0 (0)
PV 2	1 (3.8)	1 (10)	0 (0)
NPEV	1 (3.8)	0 (0)	1 (6.3)
8 weeks			
Negative	25 (96.2)	10 (100)	15 (93.8)
NPEV	1 (3.8)	0 (0)	1 (6.3)

4.4 Comparison of Poliovirus Shedding Against Study Subject Age

The subjects in both HIV infected and HIV un-infected were 26. Of these participants 12(46%) were male while 14(54%) were female. The age range distribution was as follows; <12 months were 4, 13 to 24 months were8, 25 to 48 months were 12 and 49 to 60 months were 2.

Poliovirus serotype 3 was shed in large numbers in both week 2 and week 4 among age group 13 to 24 months. Serotype 2 was the least shed in the same period as seen in table 4.3. There was a higher shedding of serotype 1+3 combination as compared to the rest.

Most of the study subjects were in the age group 25 to 48 months giving it the highest shedding rate.

Table 4.3: Duration and Proportions of Participants Shedding Vaccine after Immunization Verses the Age of the Study Subjects

Parameters	**Total, n = 26 (%)**	**< 12 months**	**13–24 months**	**25–48 months**	**49–60 months**
2 weeks					
PV 1	9 (34.6)	1 (3.8)	2 (7.7)	4 (15.4)	2 (7.7)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 3	10 (38.5)	3 (11.5)	4 (15.4)	3 (11.5)	0 (0)
PV 1 + 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 3	3 (11.5)	0 (0)	2 (7.7)	1 (3.8)	0 (0)
PV 1 + 2+3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
1 + 2 + 3 + NPEV					
4 weeks					
PV 1	7 (26.9)	2 (7.7)	1 (3.8)	3 (11.5)	1 (3.8)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 3	10 (38.5)	2 (7.7)	5 (19.2)	3 (11.5)	0 (0)
PV 1 + 2	1 (3.8)	0 (0)	0 (0)	0 (0)	1 (3.8)
PV 1 + 3	5 (19.2)	0 (0)	2 (7.7)	3 (11.5)	0 (0)
PV 2 + 3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 2 + 3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
1 + 3 + NPEV					
6 weeks					
Negative	24 (92.3)	4 (15.4)	8 (30.8)	10 (38.5)	2 (7.7)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
8 weeks					
Negative	25 (96.2)	4 (15.4)	8 (30.8)	11 (42.3)	2 (7.7)
NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)

4.5 Study Subject Demographics

The mean age of the HIV positive study population was 32.4 months, while the median age was 36 months(21-40 ranges); the mean age of the male study participants was 31.1 months with a median of 33 months (21-40 range) whereas the mean age of corresponding females was 40 months and with a median of 36 months (31-60 range).

For the HIV negative study population the mean age was 26.2 months with a median of 27 months; the mean age of the male study participants was 28.6 months while the females was 25.3 months with a median age of 30 and 24 months for male and female in the order (table 4.4.).

The mean of CD4 count among the HIV infected children was 1450/mm^3 confirming no child was considered immunodepressed among the enrolled despite having the virus and factoring the age range of those enrolled (Malhotra, 2017).

Table 4.4: Study Subject Demographics and CD4 Mean

Characteristics of the HIV Positive study subjects (n = 10)		
	Frequency of n	**Percentage (%)**
Age (months)		
< 12	1	10
13–24	2	20
25–48	5	50
49–60	2	20
Sex		
Female	3	30
Male	7	70

Sex	Mean CD4 count (mm^3)	CD4 range (mm^3)
Male	1302	1000–1900
Female	1795	1600–2000

Characteristics of the HIV negative (control) study subjects (n = 16)		
	Frequency of n	Percentage (%)
Age (months)		
< 12	3	18.7
13–24	6	37.5
25–48	7	43.8
Sex		
Female	11	68.8
Male	5	31.2

Table 4.5: Comparing Poliovirus Shedding Against Age and Sex

Table 4.5A: Association between sex and polio shedding

	Column and Row Totals		
	Shedding (a)	**Not shedding (b)**	***Marginal Row Totals***
Males (c)	27	11	38
Females (d)	28	14	42
Marginal Column Totals	55	25	80 (Grand Total)

The Fisher exact test statistic value is 0.8099. The result is not significant at $p < .05$.

Fisher Exact Test Statistic Value: 0.8082

Null Hypothesis (H0): There is no association between HIV status and poliovirus vaccine shedding.

Alternative Hypothesis (H1): There is an association between HIV status and poliovirus vaccine shedding.

Since the p-value is much greater than 0.05, we fail to reject the null hypothesis.

This indicates that there is no statistically significant evidence to suggest an association between HIV status and polio vaccine shedding in the sample studied.

Table 4.5B: Association between HIV status and polio shedding

	Column and Row Totals		
	Shedding (a)	**Not shedding (b)**	***Marginal Row Totals***
HIV Infected	23	9	32
HIV uninfected	33	15	48
Marginal Column Totals	56	24	80 (Grand Total)

The Fisher exact test statistic value is 0.8082. The result is not significant at $p < .05$.

The p-value of HIV status Vs Polio vaccine shedding of 0.8082 is significantly higher than common significance levels that ranges 0.05, 0.01, or 0.001. This p-value of 0.8082 suggests a high probability that any observed difference in polio vaccine shedding between HIV-infected and HIV-uninfected children is due to random chance rather than a true associate

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This study provides valuable insights into the interaction between HIV status and poliovirus shedding in children under 59 months during a national immunization campaign. The findings have implications for understanding the dynamics of vaccine-derived poliovirus shedding in HIV-infected versus HIV-uninfected populations, which is critical for optimizing immunization strategies.

The study evaluated vaccine poliovirus shedding duration in stool of HIV infected and HIV uninfected Kenyan children who received OPV according to the National immunization activities schedule. The study recruited 26 children, with a nearly balanced gender distribution (14 females and 12 males). The children were stratified into HIV-infected (n = 10) and HIV-uninfected (n = 16) groups. The mean age of the participants was 28.6 months, with the majority (46.2%) falling within the 25–48 month age category. Interestingly, while the HIV-infected group had a slightly higher mean age (32.4 months) compared to the HIV-uninfected group (26.2 months), no child in the study was considered immunodepressed despite their HIV-positive status. This may indicate effective management of HIV in these children, potentially influencing the study's findings on poliovirus shedding.

Conversely, in the HIV-infected group, males had a slightly higher mean age (28.6 months) compared to females (25.3 months), with median ages also showing a similar trend (30 months for males and 24 months for females). There was no difference in shedding among males and females due to the p velue>0.05

Poliovirus shedding of single serotype or shedding of multiple serotypes was noted in all study subjects in weeks 2 and 4 respectively regardless of the HIV status. One of the key findings was the lack of prolonged poliovirus shedding beyond 8 weeks post-immunization, which was consistent across both HIV-infected and HIV-uninfected children. This suggests that HIV infection, at least in the context of well-

managed cases with higher CD4 counts, does not necessarily lead to prolonged shedding of vaccine-derived poliovirus (VDPV). This is significant because prolonged shedding in immunocompromised individuals can contribute to the emergence of vaccine-derived polioviruses that may revert to neurovirulence, posing a public health risk.

The study identified that all poliovirus isolates were vaccine-derived, with no significant difference in shedding patterns between HIV-positive and HIV-negative children. Poliovirus serotype 3 was the most frequently shed serotype, particularly during weeks 2 and 4 post-immunization. There was no evidence suggesting that the age of participants influenced the duration of poliovirus shedding. Moreover, while type 1 and type 2 polioviruses were also detected, they were less prevalent, and type 2 was only found in a few specimens. The absence of significant differences in poliovirus shedding between the two groups (HIV-infected and uninfected) reinforces the notion that HIV infection, when well-controlled, may not exacerbate the risk of prolonged poliovirus shedding.

Genetic analysis of the poliovirus isolates revealed no major antigenic changes, as all isolates were "Sabin-like" in rRT-PCR intratypic differentiation tests. This indicates the lack of recombination with other circulating vaccine-related or wild polioviruses. The fact that no recombination events were detected is crucial, as such recombination could potentially lead to the emergence of virulent strains, complicating eradication efforts. The detection of both poliovirus type 2 Sabin-like and non-polio enterovirus (NPEV) in one HIV-infected participant up to week 6, and continued NPEV shedding up to week 8, suggests that co-infections may occur without necessarily extending the shedding duration of poliovirus itself.

However, this study had some limitations, other than the small sample size, the present study population possibly present a segment of vulnerable population well catered for within the orphanage in terms of any arising medical and nutritional attention needs hence enhanced immune competence. Thus, the study participants segment may not be completely representative of the typical general population of HIV-infected children in Kenya and in other developing countries where nutrition

support and cultural barriers would be a challenge to effective HIV status management and hence possible response to OPV.

Cognizant of the fact that approximately 90% of the 2 million children with HIV live in Africa (Troy *et al*,. 2013), the presented finding shed insights into context specific setting results that can be the basis for comparison with other pockets of findings in the country and sub-Saharan region to information and/or formulation of a basis for a possible population based and more rigorous representative national or regional evidence for policy relevant findings in the pan African context. This is in appreciation and consideration of the fact that progress towards eradication of poliovirus circulation in most countries of the world is more tenable than ever before and discussions are underway on workable and context resilient vaccination policies in the post-eradication era ((Hird, 2012). Thus, potential or possible large scale research evidence anchored on and/or informed by pockets of preliminary small scale observations, will be core in streamlining evidence based policy and operational guidelines for success of any vaccination programs post eradication of polio and in the overall continuum of healthcare.

5.2 Conclusion

This study provides important insights into the dynamics of poliovirus shedding in children under 59 months, particularly in the context of HIV infection. The findings suggest that well-managed HIV-infected children do not exhibit prolonged shedding of vaccine-derived poliovirus (VDPV), aligning their response to immunization with that of HIV-uninfected children. This is significant for public health, as it supports the continued use of oral polio vaccines (OPV) in HIV-infected populations without increased risk of VDPV persistence or transmission.

The study also highlights that while all detected polioviruses were vaccine-derived and no major antigenic changes were found, continuous surveillance is crucial to detect any potential shifts that could lead to the emergence of virulent strains. The absence of significant differences in poliovirus shedding patterns between HIV-infected and uninfected groups further reinforces the safety and efficacy of OPV in both populations.

5.3 Recommendations

The study supports the continued use of OPV in both HIV-infected and uninfected children. It is recommended that national immunization programs maintain their efforts to reach all children, regardless of HIV status, to ensure high coverage and prevent poliovirus transmission.

Monitoring poliovirus shedding in both HIV-infected and uninfected children, particularly in regions with varying levels of HIV management should continue with focus on detecting any prolonged shedding or recombination events that could signal the emergence of vaccine-derived or wild-type poliovirus strains.

Ensuring effective management of HIV in infected children is crucial. Higher CD4 counts were associated with the absence of prolonged poliovirus shedding in this study, indicating the importance of maintaining robust antiretroviral therapy (ART) programs alongside immunization efforts.

while the study did not find significant gender-related differences in the poliovirus shedding patterns, the age distribution by gender within the HIV-infected and uninfected groups suggests underlying factors that could influence health outcomes. Further studies are recommended to delve deeper into these gender dynamics, particularly in the context of HIV and other infectious diseases, to better inform public health strategies and ensure that both male and female children receive optimal care and protection

Additional studies should explore larger populations and diverse settings to confirm these findings and assess other factors that might influence poliovirus shedding, such as nutritional status, co-infections, and different vaccine schedules. This study had some limitations and may not be completely representative of the general population of HIV infected children in Kenya. The sampling universe was finite; the present study population that are well catered for in terms of medical and nutritional attention needs hence enhanced immune competence.

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APPENDICES

Appendix I: Data Collection Tool

Introduction to the study

Routine and supplementary oral administration of OPV strains produce a local immune response in the lining of the intestines which is the primary site for poliovirus replication. Mucosal immunity decreases the replication and excretion (shedding) of the virus, and thus provides a potential barrier to its transmission. Although OPV is a safe vaccine, on rare occasions adverse events may occur. Vaccine-associated paralytic poliomyelitis (VAPP) is the most important of these rare adverse events. Cases of VAPP are clinically indistinguishable from poliomyelitis caused by WPV, but can be distinguished by laboratory analysis. Sabin viruses can spread in populations where the coverage of OPV is low and they can acquire the neurovirulence and transmissibility characteristics of WPV. This may result in polio cases and outbreaks as circulating vaccine-derived poliovirus (cVDPV). These serious events become increasingly unacceptable as the number of paralytic cases due to circulating wild poliovirus declines, and when transmission of wild virus ends, the complete cessation of paralytic disease due to poliovirus will lead to the end of OPV usage.

Study title

Poliovirus shedding among HIV infected Kenyan Children Vaccinated with trivalent polio vaccine

Study aim

To asses any prolonged viral shedding among HIV positive subjects living in cottolengo children's home

Investigators	Role on Project	Institution
Joanne Hassan	Principal Investigator	KEMRI

Prof. Michael Kiptoo.	Co-Investigator.	SEKU
Prof. Laura Wangai	Co – Investigator	KyU
Dr. Peter Borus	Co – Investigator	KEMRI
Dr. Sepha Mabeya	Co – Investigator	JKUAT

PART A. *To be completed at the home*

Date of collection……..Study No…….

Age……..Sex…….……

Facility identity……

Date of last vaccination…….No. of doses received……

Stool sample laboratory identification:

1…….

Other Secondary information:

1. Date of last CD 4 Count:……Results:…….
2. Date of last viral load:……..Results:……
3. Date of last known OPV vaccination…….

PART B *To be filled after completion of test*

Week 2

1. Poliovirus shedding results……

   CPE level…….ITD type…….

   Kit lot number…….Exp……..

Week 4

2. Poliovirus shedding results……

   CPE level…….ITD type…….

   Kit lot number…….Exp……..

Week 6

3. Poliovirus shedding results……

   CPE level…….ITD type…….

   Kit lot number…….Exp……..

Week 8

4. Poliovirus shedding results……

   CPE level…….ITD type…….

   Kit lot number…….Exp……..

Appendix II: Conscent Form

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI)

Cottolengo children's Home

Poliovirus shedding among HIV infected Kenyan Children Vaccinated with trivalent polio vaccine

Investigators	Role on Project	Institution
Joanne Hassan	**Principal Investigator**	**KEMRI**
Dr. Michael Kiptoo.	**Co-Investigator.**	**SEKU**
Dr. Laura Wangai	**Co – Investigator**	**KyU**
Dr. Sepha Mabeya	**Co – Investigator**	**JKUAT**
Dr. Peter Borus	**Co – Investigator**	**KEMRI**

Investigators Statement

This is a research study which will be carried out by researchers from Kenya Medical Research Institute (KEMRI), institute of tropical medicine and infectious diseases (ITROMID), Jomo Kenyatta University of Agriculture and Technology (JKUAT) and world health organization (WHO). This consent form should provide for you information that you will need to help you decide whether you/your child will participate in the study or not. Please read it carefully. You may ask any question concerning the purpose of the research, procedures that will be followed, your rights as a participant in the study, risks and benefits of the study. Your children's participation or lack of participation in this study will not affect the treatment you will receive at the site.

Introduction and Purpose of the Study

Polio is a viral disease transmitted primarily through the ingestion of material contaminated with the virus found in stool. Not washing hands after using the bathroom and drinking contaminated water were common culprits in the transmission of the disease. Symptoms of this may include; mild flu-like symptoms such as mild upper respiratory infection, diarrhea, fever, sore throat, and a general feeling of being ill.in 1%-5% show neurological symptoms such as sensitivity to light and neck stiffness may occur and in 0.1%-2% of cases paralytic polio may occur.

Poliovirus prevention in Kenya is routinely done using oral poliovirus vaccine(OPV).The problem with OPV was that, in very rare cases, paralytic polio could develop either in immunized children or in those who came in contact with them.

The main purpose of the study is to determine the rate of poliovirus shedding among HIV positive children aged 5years and below to determine prolonged virus replication in the gut and shedding that will eventually serve as a reservoir for poliovirus after the eradication of paralytic poliovirus.

Procedures

If you agree to participate or for your child to take part in the study, the age, gender, your HIV status, clinical signs and symptoms will be recorded in a questionnaire. A stool sample and 2mls of blood sample will be collected after filling the questionnaire from the patient. The sample will be taken to the laboratory for further analysis. The sample will be stored in the freezer and may be used in future for further analysis. This further analysis may include isolation of the Poliovirus and further characterization of these pathogens.

Risks

The blood sample that will be taken from the patient that don't have records of HIV status and CD4 count may make them feel uncomfortable unlike the stool specimen which may not feel uncomfortable.

Benefits

The outcome of this study will guide policy makers on the correct vaccine to be used for our population, especially those children of vaccination age who are HIV-infected and to reduce paralysis caused by poliovirus to children less than 15 years of age.

Other Information

Participation in the study may involve loss of privacy. Any information given to the study will be kept private. Your name/child's name will not be used in any report coming from this study. The questionnaires and the consent form will be safely kept where only the study staff may have access to the information.

Signature of investigator **Date**

Subject Statement and Signature

The study has been explained to me. I volunteer/ I have volunteered for my child to participate in this study. I have had a chance to ask questions. If I have more questions I can ask one of the investigators listed. If I have questions about my rights as a research participant I can ask the chairperson of the KEMRI Review Committee.

Signature or fingerprint of participant or participants' parent/guardian

Appendix III: Ethical Clearence

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **June 12, 2013**

TO: **JOANNE HASSAN,**
PRINCIPAL INVESTIGATOR

FOR DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

THROUGH : **DR. FRED OKOTH;**
DIRECTOR, CVR
NAIROBI

Forwarded June 20th 2013

Dear Madam,

RE: **SSC PROTOCOL NO. 2352 – REVISED (*RE-SUBMISSION*): POLIOVIRUS SHEDDING AMONG HIV INFECTED KENYAN CHILDREN VACCINATED WITH TRIVALENT POLIO VACCINE AT COTTOLENGO CHILDREN'S HOME**

Reference is made to your letter dated May 30, 2013. The ERC Secretariat acknowledges receipt of the revised proposal on May 31, 2013.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the 211th meeting held on February 6, 2013 have been adequately addressed.

The study is granted approval for implementation effective this **12th day of June 2013**. Please note that authorization to conduct this study will automatically expire on **June 11, 2014**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **April 30, 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

CENTRE FOR VIRUS RESEARCH
20 JUN 2013
P.O. Box 54628 - NAIROBI

Appendix IV: Publication

Hassan et al. BMC Res Notes (2017) 10:555
DOI 10.1186/s13104-017-2843-y

BMC Research Notes

RESEARCH ARTICLE Open Access

Vaccine-related poliovirus shedding in trivalent polio vaccine and human immunodeficiency virus status: analysis from under five children

Joanne Hassan[1,2*], Laura Wangai[3], Peter Borus[4], Christopher Khayeka-Wandabwa[5,6*], Lucy Wanja Karani[1], Mercy Kithinji[7] and Michael Kiptoo[1,8]

Abstract

Background: Poliomyelitis is an acute viral infection caused by poliovirus and transmitted via the fecal–oral route. The causative agent is one of the three serotypes of poliovirus (serotypes 1, 2, 3) that differ slightly in capsid protein. Prolonged vaccine-related poliovirus shedding in human immunodeficiency virus (HIV) positive individuals has been linked to possible reservoir for reintroduction of polioviruses after eradication. The study therefore aimed at estimating the duration for vaccine-related poliovirus shedding among potentially and HIV-infected persons.

Methods: Poliovirus excretion was studied following vaccination of children aged ≤ 59 month per human immunodeficiency virus status after national immunization days. Their medical records were reviewed to identify the child's HIV status, demographic and immunization data. Sequential stool samples were collected at site 2nd, 4th and 8th week after trivalent oral poliovirus vaccine (tOPV) was administered. To isolate suspected polioviruses and non-polio enteroviruses, characterize poliovirus subtypes by intratypic differentiation and Sabin vaccine derived poliovirus, real time polymerase chain reaction was applied. Shedding for ≥ 24 weeks was defined as long-term persistence.

Results: The mean age of the study population was 28.6 months, while the median age was 24 months. Of the children recruited, majority were in the 25–48 months (n = 12; 46.2%) age category. All the HIV-positive children (n = 10) had mild symptomatic HIV status and did shed vaccine-related polioviruses between weeks 2 and 4 respectively. No participant shed polioviruses for ≥ 6 weeks.

Conclusions: It was evident mildly symptomatic HIV+ children sustain the capacity to clear vaccine-related poliovirus. The oral poliovirus vaccine-2 (Sabin like) that was detected in one HIV-infected child's stool 6 weeks after the national immunization days was predominantly non revertant. There was no evident prolonged poliovirus shedding among the participants enlisted in the present study. High powered studies are desired to further corroborate these findings.

Keywords: Poliomyelitis, Poliovirus, Poliovirus shedding, HIV, Immunization, Vaccination, Real time polymerase chain reaction

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Background

After polio eradication, it is broadly advanced that paralytic poliomyelitis will mainly occur as a consequence of sustained use of live oral poliovirus vaccine (OPV) [1–6]. Vaccination policies on the post-eradication era discussions are underway with progress toward eradication of poliovirus (PV) circulation in most regions and countries globally [1, 7]. After the global eradication of polioviruses the potential of persistence and circulation vaccine-derived polioviruses (VDPV) is a risks that cannot be underestimated when advancing strategies towards stopping polio vaccination [7].

According to World Health Organization (WHO) OPV is considered safe and immunogenic in HIV-infected persons [8, 9] and is widely used as a primary approach for global polio eradication initiative for instance in Africa where about 90% of the two million children with HIV live [10]. In this population segment of HIV positive children few cases of vaccine-associated paralytic poliomyelitis (VAPP) have earlier been reported [11, 12]. In consideration of the high proportions of potentially and HIV positive persons in developing countries eligible for and therefore exposed to OPV [9, 13], and the public health implications of possible prolonged VDPV persistence, the need to evaluate and appraise the risk progressively should be emphasized. Genetically, VDPVs have been shown to have > 1% sequence difference from the Sabin vaccine virus [14]. Mutations of OPV can lead to a form that can achieve neurological infection and consequently cause paralysis [15] this neurological infection include paralysis caused by VDPV and is indistinct from that caused by wild polioviruses by means of sequencing [16]. However, this is a rare occurrence with the rate of vaccine-associated paralytic poliomyelitis (VAPP) varying by region and at about 1 case per 750,000 vaccine recipients [17]. Reported outbreaks of VAPP tend to happen in areas of low OPV coverage and more likely to occur in adults than in children with pre-assumption being OPV is itself protective against the related outbreak strain [4, 18, 19].

Further circulation risk of poliovirus in ineffectively immunized persons arises when immunocompromised individuals with persistent VDPV may serve as a latent reservoir for possible reintroduction of PV into the overall population following wild poliovirus elimination. HIV infection during perinatal phase is correlated with humoral and cellular immunodeficiencies. It has been advanced that perinatally HIV infected children have been identified to have a polyclonal hypergammaglobulinemia paradoxically linked with reduced antibody responses to specific antigens [20–22]. Furthermore, individuals with certain defects of antibody production are known to be affected by chronic VDPV persistence [1, 23], with limited insights about PV persistence among HIV+ persons. In associated studies conducted, a case of enterovirus excretion was noted in an HIV+ child for up to 6 months [24, 25] and related findings pointed to the concern that, HIV-positive children are more prone to enteroviruses as compared to their healthy counterparts [24, 25]. Further findings have pointed to varied observations of non-severe or severe enterovirus infection among HIV-infected individuals [26–28]. Available findings do not support the increased risk and severity of wild or vaccine-associated poliomyelitis in HIV-infected individuals. Immunization using OPV of HIV-infected children results in protective, although rather lower, antibody titres as compared with uninfected children, with a comparable low rate of adverse events across the groups [29, 30]. In this regard, whether children perinatally infected with HIV may be source of VDPV is a query yet to be fully explored. In order to provide more insights, the study aimed to examine vaccine derived poliovirus shedding among potentially and HIV positive children who are under 5 years of age to asses if there is prolonged virus replication and shedding that would eventually serve as a reservoir for paralytic poliovirus. We conducted a prospective cross sectional study evaluating stool shedding of vaccine strains and polio seroconversion in Kenyan children who received OPV per the national immunization schedule. The highly desired incremental evidence [31–33] could additionally benefit the global and regional policy environment on shaping the discourse around public health implications of potential VDPV, inform the potential need for large scale rigorous studies and the contention on the risk of the VDPV persistence among HIV-infected persons [4, 7, 19, 31].

Methods

Study population and specimen collection

This was a prospective cross sectional study conducted at Cottolengo children's orphanage home in Nairobi, Kenya from August 2013 until June 2014. The orphanage has a capacity of 500 children and admits mainly orphaned children between birth and 18 years whose parents have died of HIV related complications. The institution accords the children with adequate nutritional and medical care inclusive of comprehensive immunization. The reliable operational scope of the children's home alongside its progressively appraised medical records and tracking systems with demographic, clinical and immunization database, provided a rigorous and dependable platform for the prospective study. In addition, prior experience with similar target population reliability in closely related settings has been proven [10, 34]. For inclusion into the study, stepwise considerations and parameters were followed as elaborated. All children

enrolled in the study were below the age of 5 years. Medical records at the orphanage were reviewed to identify each child's HIV status and obtain age, sex, and immunization data. Final HIV infection status of the participants was assigned at the time of sample collection in September 2013. One blood vial sample for confirmatory HIV status and CD4+ count per national screening protocol [National AIDS and STI Control Programme (NASCOP)] was collected in August 2013 just before NIDs (effective September), Children whose status had reverted to seronegative were categorized as HIV-uninfected and assigned to the control group. All enrolled infants received trivalent oral poliovirus vaccine (tOPV) during the September NIDs. Serial stool specimens collection at site was done during the 2nd, 4th, 6th and 8th week after vaccination with tOPV. The last OPV exposure dates were defined as the date of the national immunization days (NIDs) for all children $\leq$ 59 months. Severely ill children were excluded. Informed consent of legal foster parents/tutors was obtained before inclusion in the study. Thus, infants were included in the study if (1) their age was $\leq$ 59 months at the time of enrollment (2) had their HIV status confirmed (3) their OPV status history could be verified, and (4) they had available stool. The entire stool samples from HIV-infected and uninfected children were processed in stool-shedding analysis. The data collection processes were performed by trained clinical nurse and laboratory technologist on the methodology and objectives in coordination by investigators JH and MK1 at the facility. All samples were processed at Kenya Medical Research Institute Nairobi (KEMRI).

Laboratory analysis determination of HIV-1 infection status and T cell subset analysis

The whole blood samples were collected in an ethylenediaminetetraacetic acid-containing Vacutainer (purple-top), transported on ice to the Centre for virus research HIV laboratory at Kenya Medical Research Institute (KEMRI) for analysis. HIV status was determined by HIV PCR using an Amplicor HIV DNA Test Version 1.5 kit and a GeneAmp 9700 thermal cycler. HIV enzyme-linked immunosorbent assay (ELISA) was performed to confirm HIV status at 18 months of age. Confirmation was defined as $\geq$ 2 positive HIV tests with $\geq$ 1 done as part of the study, or $\geq$ 1 positive HIV test done as part of the study and clinical depiction strongly suggestive of HIV infection. In line with their HIV status, the children were classified into two groups: (i) HIV-infected (n = 10), and (ii) HIV-uninfected (n = 16). CD4 T-cell count was carried out on the HIV positive using a FACS Calibur Flow Cytometer (Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA), as per the manufacturer's instructions [35, 36].

Virus isolation and identification

Stool specimens were transported on frozen ice packs to the Centre for virus research Poliovirus laboratory at Kenya Medical Research Institute (which is also a WHO National lab in the AFRO region). Virologic isolation and identification was done according to WHO Polio Laboratory Network Standard Protocols (Polio laboratory manual 4th edition, 2004). Briefly, stool extracts were inoculated in RD (human rhabdomyosarcoma derived cells) and murine L20B (a transgenic mouse L cells) cell lines. The L20B cell line was used to distinguish polioviruses from non-polio enteroviruses because the mouse cell lines is genetically modified to express human cellular receptor for polioviruses allowing selective poliovirus culture. Positive RD cell cultures were passaged in L20B to distinct poliovirus and non-polio enteroviruses. Positive L20B isolates were harvested for PCR.

Real time reverse transcriptase-polymerase chain reaction (rRT-PCR) for intratypic differentiation (ITD) and Sabin VDPV

The poliovirus diagnostic real -time RT PCR was performed on L20B positive cell cultures that were suspected to contain poliovirus. Briefly, the viral RNA (vRNA) was converted to complementary DNA (cDNA) using reverse transcriptase. The cDNA was amplified in a PCR reaction using Taq polymerase. The PCR products were detected and identified by hybridization with specific Taqman® probes. Both the cDNA synthesis and the PCR reaction used multiple sets of oligonucleotide primers that were tagged with probes with different specificities. This combination of primers and probes resulted in the serotype identification and intratypic differentiation of poliovirus isolates (as described in World Health Organization, Polio Laboratory Manual 4 ed. 2004, Geneva.). Using an ABI 7500 system the run conditions used were as follows: 95 °C for 24 s, 44 °C for 30 s, then a 25% ramp speed to 60 °C for 24 s, for 40 cycles. The primers (manufactured by Center for Disease Control and Prevention, USA) targeting the VP1 gene ~ 900 nucleotides were utilized to determine if the sample contained polioviruses or non-polio enterovirus. The type of poliovirus was determined as wild type or vaccine derived (sabin) using primer specific for wild and VDPV. This was done using the detailed primer sequence in Table 1.

The vaccine derived poliovirus samples were further tested for the degree of relatedness to prototype Sabin strains per standard procedure from the kit manufacturer (Center for Disease Control and Prevention, USA). The primer sequence used for the real time PCR was as shown in Table 2.

Table 1 Primer or probe sequence for intratypic differentiation (ITD)

Specificity	Primer or probe (polarity)	Primer or probe sequence (5′ → 3′)
Pan-enterovirus	PCR-1(A)	GCGATTGTCACCATWAGCAGYCA
	PCR-2 (S)	GGCCCCTGAATGCGGCTAATCC
	PanEV probe (S)	**FAM**-CCGACTACTTTGGGWGTCCGTGT-**BHQ1**
Pan-poliovirus[a]	panPV/PCR-1(A)	AYRTACATIATYTGRTAIAC
	panPV/PCR-2(S)	CITAITCIMGITTYGAYATG
	panPV Probe21A(A)	**FAM**-TGRTTNARIGCRTGICCRTTRTT-**BHQ1**
Serotype 1[a]	seroPV1A(A)	ATCATIYTPTCIARPATYTG
	seroPV1,2S(S)	TGCGIGAYACIACICAYAT
	seroPV1 probe 16A(A)	**FAM**-TGCCYAVICCYGIGMIADYGC-**BHQ1**
Serotype 2	seroPV2A (A)	AYICCYTCIACIRCICCYTC
	seroPV1,2S (S)	TGCGIGAYACIACICAYAT
	seroPV2,probe 5S (S)	**FAM**-CARGARGCIATGCCCICARGGIATNGG-**BHQ1**
Serotype 3[a]	seroPV3A (A)	CCCIAIPTGRTCRTTIKPRTC
	sero PV 3S (S)	AAYCCITCIRTITTYTAYAC
	seroPV3,probe 11S (S)	**FAM**-CCRTAYGTNGGITTRGCVAAYGC-**BHQ1**
Sabin 1	Sab1/PCR-1(A)	CCACTGGCTTCAGTGTTT
	Sab1/PCR-2(S)	AGGTCAGATGCTTGAAAGC
	Sab1/probe (A)	**CY5**-TTGCCGCCCCCACCGTTTCACGGA-**BHQ3**
Sabin 2	Sab2/PCR-1(A)	CGGCTTTGTGTCAGGCA
	Sab2/PCR-2(S)	CCGTTGAAGGGATTACTAAA
	Sab2/probe (S)	**FAM**-ATTGGTTCCCCCGACTTCCACCAAT-**BHQ1**
Sabin 3	Sab3/PCR-1(A)	TTAGTATCAGGTAAGCTATC
	Sab3/PCR-2(S)	AGGGCGCCCTAACTTT
	Sab3/probe (S)	**ROX**-TCACTCCCGAAGCAACAG-**BHQ2**

Degenerate primers: K=G and T; M=A and C; R=A and G; Y=C and T; I = degenerate base analog, Inosine; P = degenerate base for (TC)

[a] We used degenerate PCR conditions with these primer sets

Table 2 Primer or probe sequence for Sabin vaccine derived poliovirus (VDPV)

Primer specificity	Primer and probe sequences 5′ → 3′
S1 VDPV VP1 (Target aa# 99)	Sense CATGCGTGGCCATTATA
	Anti-sense CAAATTCCATATCAAATCTA
	VP1 probe **FAM**-CACCAAGAATAAGGATAAGC-**BHQ1**
S2 VDPV VP1 (Target aa# 143)	Sense GACATGGAGTTCACTTTTG
	Anti-sense CTCCGGGTGGTATATAC
	VP1 probe **FAM**-CATTGATGCAAATAAC-**BHQ1**
S3 VDPV VP1 (Target aa# 285–290)	Sense CATTTACATGAAACCCAAAC
	Anti-sense TGGTCAAACCTTTCTCAGA
	VP1 probe **FAM**-TAGGAACAACTTGGAC-**BHQ1**

Statistical analysis

Data were managed using excel spread sheet and Statistical Package for Social Sciences (SPSS) version 21. Quantitative data was analyzed by means of descriptive statistics. Parameters considered in estimating the proportions shedding vaccine poliovirus serotypes entailed time from the last OPV dose administered, HIV status, PV seroconversion, and age. Immuno competence was defined per the WHO/CDC age specific clinical categories and immunologic status: (1) no evidence of immunosuppression—age < 12 months: CD4+ count ≥ 1500, and/or CD4% ≥ 25%; age 1–5 years: CD4+ count ≥ 1000 and/or CD4+ % ≥ 25%; (2) moderate immunosuppression—age < 12 months: CD4+ count 750–1499, and/or CD4% 15–24%; age 1–5 years: CD4+ count 500–999 and/or CD4+ % 15–24%; (3) severe immunosuppression—age < 12 months: CD4+ count < 750, and/or CD4% < 15%; age 1–5 years: CD4+ count < 500 and/or CD4+ % 15% [36, 37]. In the rRT-PCR for ITD and VDPV, primers that specifically differentiate between wild and vaccine derived polio were utilized. Pearson's Chi squared test was applied to determine whether there was a significant difference in the distribution of PV serotypes between the HIV+ and HIV− groups. P values of equal or less than 0.05 ($P \leq 0.05$) were considered to be statistically significant.

Results

The study recruited 26 children ≤ 59 months from the home. On profiling the children HIV status as at September 2013 (the time when national immunization activities was done), 10 were confirmed to be HIV-infected, whereas the rest (16 children) had sero-negative status and were therefore categorized as HIV-uninfected. The mean of CD4 count among the HIV infected children was 1450/mm^3 confirming no child was considered immunodepressed among the enrolled despite having the virus and factoring the age range of those enrolled [37, 38]. The mean age of the study population was 28.6 months, while

the median age was 24 months. Of the children recruited, majority were in the 25–48 months (n = 12; 46.2%) age category followed by 13–24 months age category (n = 8; 30.8%). There were slightly more females (n = 14; 53.8%) recruited than males (n = 12; 46.2%). The demographic characteristics of the study subjects are as elaborated in Table 3. The mean age of the HIV positive study population was 32.4 months, while the median age was 30 months; the mean age of the male study participants was 29.1 months with a median of 24 months whereas the mean age of corresponding females was 40 months with a median of 36 months. For the HIV negative study population the mean age was 26.2 months with a median of 27 months; the mean age of the male study participants was 28.6 months while the females was 25.3 months with a median age of 30 and 24 months for male and female in that order. Medical records review showed that only one child had been ill during the immunization rounds period which could have been a contraindication to supplemental doses of OPV during NIDs.

Table 3 The demographic characteristics of the study subjects

Characteristics of the HIV positive and negative (control) study subjects (N = 26)		
	Frequency of N	**Percentage (%)**
Age (months)		
< 12	4	15.4
13–24	8	30.8
25–48	12	46.2
49–60	2	7.7
Sex		
Female	14	53.8
Male	12	46.2
HIV status		
Positive	10	38.5
Negative	16	61.5
Characteristics of the HIV positive study subjects, CD4 count mean and ranges (n = 10)		
	Frequency of n	**Percentage (%)**
Age (months)		
< 12	1	10
13–24	2	20
25–48	5	50
49–60	2	20
Sex		
Female	3	30
Male	7	70
Sex	**Mean CD4 count (mm^3)**	**CD4 range (mm^3)**
Male	1302	1000–1900
Female	1795	1600–2000
Characteristics of the HIV negative (control) study subjects (n = 16)		
	Frequency of n	**Percentage (%)**
Age (months)		
< 12	3	18.7
13–24	6	37.5
25–48	7	43.8
Sex		
Female	11	68.8
Male	5	31.2

Findings of poliovirus isolation in stool specimens by HIV serostatus are as presented in Table 4. A total of 80 stool specimens were obtained during the 8 week study period, 31 from HIV-infected children and 49 from HIV-uninfected children. Of the collected specimens 53 were positive for suspected poliovirus, 23 from HIV-infected children and 30 from HIV-uninfected children. There was a positive viral culture results at some point for each child during the study period.

On poliovirus shedding duration, there was no observable evidence of prolonged PV or non-polio enteroviruses (NPEV) shedding with no PV detected in any of the specimens at 8 weeks after the last OPV dose. It was evident; all PV isolates from the enrolled study participants were vaccine-related. At some point of the study each study subject shed one form or another of the vaccine derived poliovirus as seen on Table 5. Type 1 poliovirus was detected in 16 specimens (three specimens from HIV-infected children), type 2 in three specimens 2 of which were from HIV-infected children and type 3 in 20 specimens (six from HIV-infected). More than one poliovirus type was identified in 14 specimens of which six were from HIV-infected children. More than one specimen among the 26 children (10 HIV-infected and 16 HIV-uninfected) was positive for poliovirus. There was no observable significant difference in the distribution of PV serotypes between the HIV positive and HIV negative groups with the virus shedding pattern between the groups in the order showing no significant difference as well ($P > 0.05$) by sex or age. Among the study participants (Table 6), poliovirus serotype 3 was shed by majority of the children (38.5%) in week 2 and week 4 respectively with the highest shedding among ages 13–24 months (15.4 and 19.2%) in week 2 and 4 in the order. However, poliovirus serotype 1 was also shed most by ages 25–48 months (15.4%). There was no evidence from our data that age of the study participants was a contributing factor to prolonged (≥ 6 months) poliovirus shedding.

In the genetic characterization of PV isolates, the VP1 gene was the gene of interest for the isolates. Poliovirus isolates appeared "Sabin-like" in rRT PCR intratypic

Table 4 Virus isolation verses HIV status of study subjects

Sample size	Total, n	HIV(+), n	HIV(−), n
Children in the study	26	10	16
Isolation rate for suspected polioviruses and NPEV			
Specimens with suspected polioviruses or NPEV isolated in week 2	26	10	16
Specimens with suspected polioviruses or NPEV isolated in week 4	26	10	16
Specimens with suspected polioviruses or NPEV isolated in week 6	1	1	0
Specimens with suspected polioviruses or NPEV isolated in week 8[a]	1	0	1

[a] One study subject who was HIV-uninfected shed NPEV up to week 8 of sample analysis

differentiation tests, indicating the lack of any major antigenic changes. All the poliovirus isolates that tested positive during the intratypic differentiation (ITD) for any Sabin-like polioviruses ($n = 53$) from the 26 study subjects on week 2–6 were tested for possible recombination by real-time PCR; all did amplify with the VP1 genomic region using the homotypic Sabin-specific primers, ruling out the evidence of recombination with other circulating vaccine-related or wild polioviruses. One HIV-infected participant shed a combination of poliovirus type 2 sabin like and non polio enterovirus (NPEV) up to week 6, but continued shedding NPEV up to week 8.

Table 5 Duration of shedding vaccine after immunization verses the proportional study subjects at the time points and HIV status

Parameters (time and poliovirus)	Total, N = 26, (%)	HIV(+), n = 10, (%)	HIV(−), n = 16, (%)
2 weeks			
PV 1	9 (34.6)	3 (30)	6 (37.5)
PV 2	1 (3.8)	1 (10)	0 (0)
PV 3	10 (38.5)	4 (40)	6 (37.5)
PV 1 + 2	1 (3.8)	1 (10)	0 (0)
PV 1 + 3	3 (11.5)	1 (10)	2 (12.5)
PV 1 + 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 2 + 3 + NPEV	1 (3.8)	0 (0)	1 (6.3)
4 weeks			
PV 1	7 (26.9)	2 (20)	5 (31.3)
PV 2	1 (3.8)	1 (10)	0 (0)
PV 3	10 (38.5)	3 (30)	7 (43.8)
PV 1 + 2	1 (3.8)	1 (10)	0 (0)
PV 1 + 3	5 (19.2)	3 (30)	2 (12.5)
PV 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 2 + 3	1 (3.8)	0 (0)	1 (6.3)
PV 1 + 3 + NPEV	1 (3.8)	0 (0)	1 (6.3)
6 weeks			
Negative	24 (92.3)	9 (90)	15 (93.8)
PV 1	0 (0)	0 (0)	0 (0)
PV 2	1 (3.8)	1 (10)	0 (0)
NPEV	1 (3.8)	0 (0)	1 (6.3)
8 weeks			
Negative	25 (96.2)	10 (100)	15 (93.8)
NPEV	1 (3.8)	0 (0)	1 (6.3)

Discussion

Oral poliovirus vaccine has been a vaccine of choice in developing countries for both persons infected with HIV and the uninfected. Previous studies in Democratic Republic of Congo showed high protective antibody titres in HIV infected children who had received the three OPV doses [29, 30]. Shedding of poliovirus in immunized persons is usually between 2 and 3 months [29, 30, 39]. We evaluated vaccine poliovirus shedding duration in stool of HIV positive and HIV negative Kenyan children who received OPV per the National immunization activities schedule. We observed that although prolonged poliovirus shedding was not associated with the children's HIV status, multiple serotypes shedding was noted in all study subjects in 2nd and 4th weeks respectively. In resonating Kenyan findings, to evaluate the potential for VDPV persistence among HIV-infected persons, a proportion of HIV seropositive children enrolled was confirmed to be HIV-infected. There was no clinical indication of prolonged persistence of vaccine-related polioviruses or NPEV. Further, in all cases (infected and uninfected), the poliovirus infections were cleared with the overall trends of excretion not differing by HIV status. The pointed phenomenon of non-variation in excretion by HIV or non-HIV status have also been noted in a longitudinal outcomes [10]. Subsequent to vaccination with OPV there is decreased Poliovirus shedding which is broadly considered a marker for the development of mucosal intestinal immunity [40]. In the present study, there was a decrease in poliovirus shedding in weeks 6 and 8 suggesting that both HIV infected and uninfected children developed intestinal mucosal immunity after OPV dose. Age was also not a factor that affected the pattern or duration of OPV shedding. Prior reporting has observed that HIV-infected children shed vaccine-related polioviruses in between 15 and 42 months based on specimens obtained after the last known OPV dose consequently

suggesting possible long-term persistence [41]. However, further molecular findings pointed to the fact that for all three cases, there was a variance of nucleotide sequences in the VP1 region of 0.3–0.6% showing close genetic relatedness to prototype Sabin strains. Thus, it was worth estimating the frequency of such individuals in countries with varying socioeconomic and health policies context for poliomyelitis control. In our presented findings though in a shorter time period but more participants, affirms the ability to clear OPV is preserved across HIV-infected and uninfected children.

A range of factors could possibly influence the course of poliovirus infection among the HIV-infected and these include the degree of immunosuppression, previous OPV vaccination history, and patient age. The CD4 lymphocyte count of HIV-infected individuals is a significant predictor of HIV progression as well as acquired immune deficiency syndrome (AIDS)-associated mortality [30]. In the present study, following HIV status evaluation, we considered CD4 count to assess immune competence and there after adopted the "gold standard" for assessing mucosal immunity after vaccination with PV vaccines which consists of measuring virus excretion in stool after challenge with OPV. The absence of or reduced shedding is an indicator of mucosal intestinal protection. Further, immune protection to poliomyelitis is known to prevail in two forms—humoral and mucosal where humoral immunity protects from paralytic poliomyelitis and protection against disease correlates with induction of serum poliovirus-neutralizing antibody [42, 43]. On the contrary, mucosal immunity is presumed to protect against poliovirus entry into and transmission from the intestinal and nasopharyngeal mucosae which is the primary sites of poliovirus replication. The clinical categorization and immunologic profiles of HIV positive children was assigned according to the 1994 Centers for Disease Control and Prevention (CDC) revised classification which is age specific defined immunologic status [36]. Considering HIV infection is mainly a mucosal disease and the gastrointestinal tract (GIT) is the foremost site for the virus replication given that it houses most of the body's lymphocytes (including activated memory CD4+ T cells that are preferential targets for HIV), infection and progression of HIV is mainly characterized by incessant activation, rapid turnover, and activation-induced cell death of CD4+ and CD8+ T cell populations [44, 45]. The

Table 6 Duration and proportions of participants shedding vaccine after immunization verses the age of the study subjects

Parameters	Total, n = 26 (%)	< 12 months	13–24 months	25–48 months	49–60 months
2 weeks					
PV 1	9 (34.6)	1 (3.8)	2 (7.7)	4 (15.4)	2 (7.7)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 3	10 (38.5)	3 (11.5)	4 (15.4)	3 (11.5)	0 (0)
PV 1 + 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 3	3 (11.5)	0 (0)	2 (7.7)	1 (3.8)	0 (0)
PV 1 + 2+3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 2 + 3 + NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
4 weeks					
PV 1	7 (26.9)	2 (7.7)	1 (3.8)	3 (11.5)	1 (3.8)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 3	10 (38.5)	2 (7.7)	5 (19.2)	3 (11.5)	0 (0)
PV 1 + 2	1 (3.8)	0 (0)	0 (0)	0 (0)	1 (3.8)
PV 1 + 3	5 (19.2)	0 (0)	2 (7.7)	3 (11.5)	0 (0)
PV 2 + 3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 2 + 3	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
PV 1 + 3 + NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
6 weeks					
Negative	24 (92.3)	4 (15.4)	8 (30.8)	10 (38.5)	2 (7.7)
PV 2	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)
8 weeks					
Negative	25 (96.2)	4 (15.4)	8 (30.8)	11 (42.3)	2 (7.7)
NPEV	1 (3.8)	0 (0)	0 (0)	1 (3.8)	0 (0)

degree of immune activation represents an independent and more powerful predictor of CD4+ T cell homeostasis, immune and disease progression [46, 47]. Thus we did not directly target mucosal immunity parameters due to associated determinants [48] nonetheless, progressive validation of mucosal immunity markers is desired depended on how beneficial they will be in relation to transition from using OPV to IPV [40, 48]. Overall mean CD4 counts established in the HIV infected children was not characteristic to progression of disease and the PCR analysis of viral protein 1 (VP1) region of the Sabin like isolates showed no difference with the one of the vaccine administered therefore there was no reversion to neurovirulence.

However, this study had some limitations, other than the small sample size, the present study population possibly present a segment of vulnerable population well catered for within the orphanage in terms of any arising medical and nutritional attention needs hence enhanced immune competence. Thus, the participants segment may not be completely representative of the typical general population of HIV-infected children in Kenya and in other developing countries where nutrition support and cultural barriers would be a challenge to effective HIV status management and hence possible response to OPV.

Cognizant of the fact that about 90% of the two million HIV positive children live in Africa [10], the presented finding shed insights into context specific setting results that can be the basis for comparison with other pockets of findings in the country and sub-Saharan region to information and/or formulation of a basis for a possible population based and more rigorous representative national or regional evidence for policy relevant findings in the pan African context. This is in appreciation and consideration of the fact that progress towards eradication of poliovirus globally is more tenable than ever before and discussions are underway on workable and context resilient vaccination policies in the post-eradication era [1, 40, 49]. Thus, potential or possible large scale research evidence anchored on and/or informed by pockets of preliminary small scale observations, will be core in streamlining evidence based policy and operational guidelines for success of any vaccination programs post eradication of polio and in the overall continuum of healthcare.

Conclusion

In consideration of the scope of the presented findings, it is indicative that HIV+ children retain the capacity to clear vaccine-related PV. The OPV-2 (Sabin Like) that was detected in one HIV-infected child's stool 6 weeks after the NID was predominantly non-revertant. Elaborate longitudinal and high powered studies covering full range of HIV disease and robust follow up period are needed to fully shed more insights on the patterns of VDPV excretion that can potentially lead to revertant poliovirus among HIV+ persons and further quantify the risk magnitude of long-term VDPV replication in this group.

Abbreviations

PV: poliovirus; tOPV: trivalent oral poliovirus vaccine; NPEV: non-polio enteroviruses; ITD: intratypic differentiation; VDPV: vaccine derived poliovirus; PCR: polymerase chain reaction; VAPP: vaccine-associated paralytic poliomyelitis; VDPV: vaccine-derived polioviruses; VP1: viral protein 1; HIV: human immunodeficiency virus; NIDs: national immunization days; NASCOP: National AIDS and STI Control Programme; WHO: World Health Organization; rRT-PCR: real time reverse transcriptase- polymerase chain reaction; cDNA: complementary deoxyribonucleic acid; ITD: intratypic differentiation.

Authors' contributions

This work was carried out in collaboration between all authors. JH, LW, PB, LWK, MK1 and MK2 conceived/designed the study and were involved in data collection. JH, LW, PB, CKW, LWK, MK1 and MK2 gave substantial inputs in the draft manuscript. JH and CKW gave substantial inputs to the plan for analysis and data analysis. All the authors participated in drafting and revising the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data and study tools/materials that support the findings of this study are available from the Centre for virus research Poliovirus laboratory at Kenya Medical Research Institute (which is also a WHO National lab in the AFRO region) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Centre for virus research Poliovirus laboratory at Kenya Medical Research Institute repository.

Consent for publication

Not applicable.

Ethical approval and consent to participate

The study protocol was approved by the Kenya Medical Research Institute (KEMRI) Ethics Review Committee (SSC Protocol No. 2352). Written informed consent of legal foster parents/legal tutors was obtained before inclusion in the study. The ethically approved guidelines were strictly adhered to during the research.

Appendix V: Materials and Equipment for Real Time PCR

The following materials and consumables were required for PCR;

- Autoclave Balance Biological Safety Cabinet (one for reagents and another for sample preparation)
- Refrigerator, 4°C
- Vortex mixer
- Real-time PCR machine AB7500
- Microcentrifuge tubes, 1.5 ml, low adsorption
- Micropipette tips, aerosol-resistant, 20 µl and 200 µl capacity
- Paraffin film or flexible 96-well microtiter plates
- 8-well PCR strips, 0.2 ml per well, and optical caps to specific to your real-time machine
- Powder free gloves
- Ice bucket and ice
- Laboratory coat with cuffs
- Microcentrifuge tube racks
- Microcentrifuge with 8-well strip
- Adaptor Micropipettes 20 µl and 200 µl capacities
- 8-well strip PCR racks

Reagents:

From Roche Applied Science

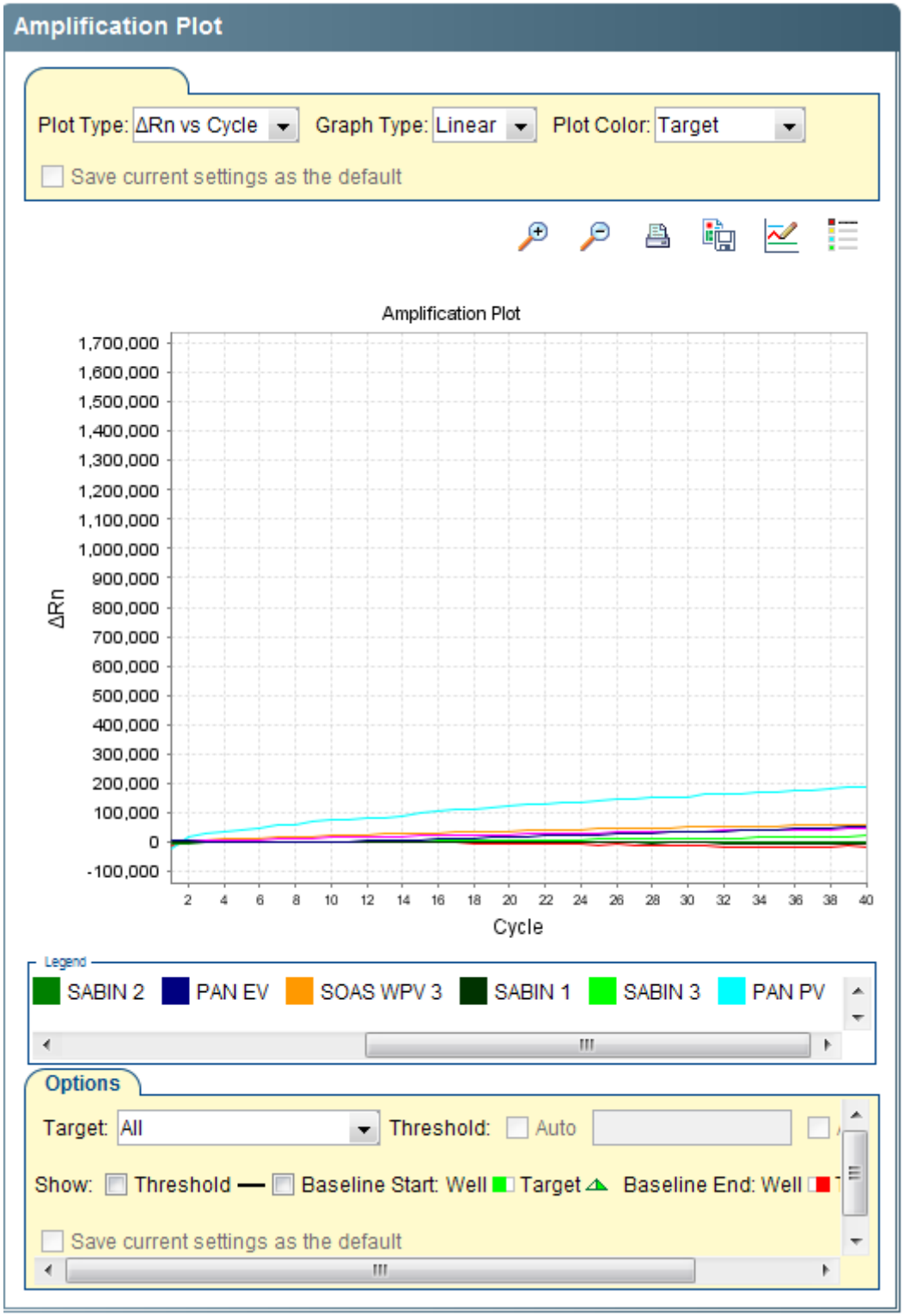
- Protector RNase Inhibitor 40 U/µL
- Taq DNA Polymerase 5 U/µL
- Transcriptor RT 20 U/µL (or AMV RT 25 U/µL)

From CDC

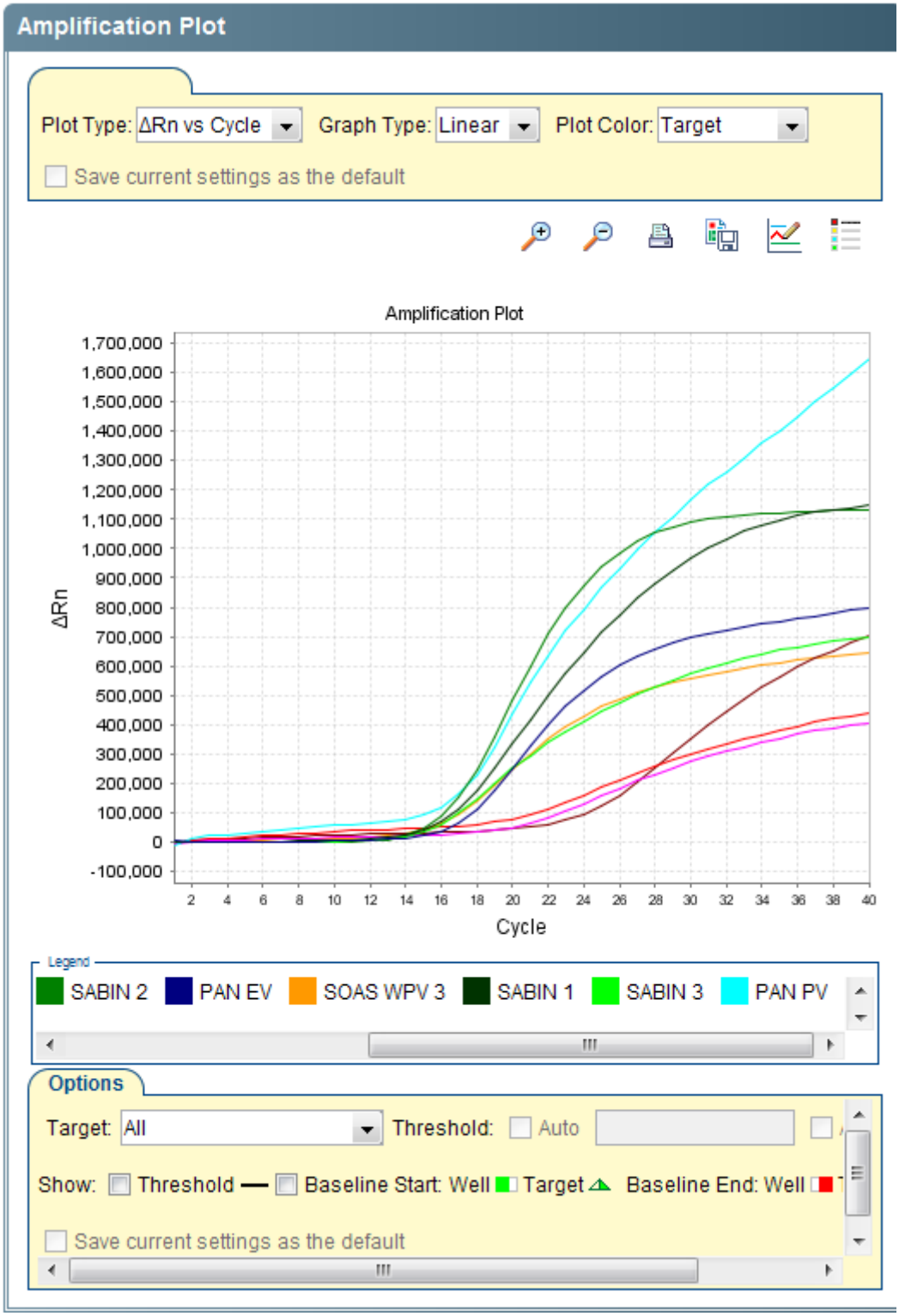
- CDC Poliovirus Diagnostic rRT-PCR kit
- CDC VDPV rRT-PCR Screening kit

Annex VI: Real Time PCR Amplification Plots

Amplification plot Negative control plots



Amplification plot Positive Controls



Amplification plot samples