Prevention of Mother-to-Child Transmission of HIV-1 in Lesotho

Appolinaire Tiam

Thesis for the degree of Philosophiae Doctor (PhD)
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Name: Appolinaire Tiam

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To all women living with HIV and children who are HIV-exposed

Scientific environment

This thesis work is the result of a close collaboration between the Center for International Health, Faculty of Medicine, University of Bergen and the Elizabeth Glaser Pediatric AIDS Foundation, particularly their activities in Lesotho.

The Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) is an international, non-governmental organization headquartered in Washington, DC. EGPAF's mission is to end HIV/AIDS globally in children, youth, and families. EGPAF has been supporting the Lesotho Ministry of Health (MoH) and the Christian Health Association of Lesotho (CHAL) since 2003, with an initial focus on establishing programs for the prevention of mother-to-child transmission of HIV. However, since 2010, EGPAF's program expanded to include HIV care and treatment for pregnant and breastfeeding women, their children and their families.

EGPAF also has an extensive research portfolio in Lesotho. During my coursework with the University of Bergen's Center for International Health, I served as Principal Investigator on multiple EGPAF studies, which I have used to build the work of this thesis with significant support from my supervisors. Below are the logos of the collaborating institutions: Elizabeth Glaser Pediatric AIDS Foundation (EGPAF), Ministry of Health of Lesotho, and University of Bergen.

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Lastly, I am eternally indebted to my wife, Dr. (Mrs) Mayowa Tiam, for accepting my repeated absence from home especially during our transition to settle in the USA. Thank you for all your support my love and my friend. To my son Fotie Tiam and my daughter Peace Megne Tiam for encouraging me with kind words and speaking research language to me. You are both Gems.

My joy will be full if only one more HIV-exposed child can be alive and free of HIV.

PREFACE

The Lesotho Programme for Prevention of Mother-to-Child Transmission of HIV-1 (PMTCT) was launched in Lesotho in 2005. I joined the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) in 2008, first as the Senior HIV Care and Treatment Advisor and subsequently, as Technical Director in the Lesotho program. I oversaw the PMTCT program implemented by EGPAF in six of the ten districts of Lesotho. In 2010, with additional funding from the United States Agency for International Development (USAID), EGPAF scaled up PMTCT services across the whole Kingdom of Lesotho. As part of my work, I was co-chair of the national PMTCT and pediatric HIV care and treatment technical working group (TWG). During this period, there were several changes to the PMTCT guidelines at both the global and national levels. As part of the TWG, we carried out site supportive supervision, during which we explored site-level data to understand if changes to the guidelines had the necessary impact on outcomes. However, we soon realized that, the real issue with PMTCT was not only the drug regimen, but the effectiveness of the program itself. For example, deoxyribonucleic acid polymerase chain reaction (DNA PCR) testing is recommended for early HIV diagnosis of infants at six to eight weeks of age to assess intrauterine and intrapartum HIV transmission. While the DNA PCR sample may be collected on-time in the facility, the laboratory system defines if the child's caregiver receives the result on time to inform the course of further care for the child. In addition, traditionally, monitoring the results of infant HIV tests at six to eight weeks of age has continued to be the public health approach to measure PMTCT program effectiveness in many countries. However, in populations with high rates of breastfeeding among HIV-exposed infants (HEI), as recommended by World Health Organization (WHO), HEI continue to be at risk of HIV infection throughout breastfeeding period. This motivated me to conduct the studies upon which this thesis was written.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, referred to in the text by their respective Roman numerals.

Paper I	Tiam A, Gill MM, Hoffman HJ, Isavwa A, Mokone M, Foso M, Safrit JT, Mofenson LM, Tylleskär T, Guay L. Conventional early infant diagnosis in Lesotho from specimen collection to results usage to manage patients: Where are the bottlenecks? PLoS ONE 2017 12(10): e0184769. https://doi.org/10.1371/journal. pone.0184769
Paper II	Tiam A, Kassaye SG, Machekano R, Tukei V, Gill MM, Mokone M, Letsie M, Tsietso M, Seipati I, Barasa J, Isavwa A, Tylleskär T, Guay L. Comparison of 6-week PMTCT outcomes for HIV-exposed and HIV-unexposed infants in the era of lifelong ART: Results from an observational prospective cohort study. PLoS ONE 2019 14(12): e0226339. https://doi. org/10.1371/journal.pone.0226339
Paper III	Appolinaire Tiam, Michelle M. Gill, Rhoderick Machekano, Vincent Tukei, Majoalane Mokone, Shannon Viana, Mosilinyane Letsie, Mots'oane Tsietso, Irene Seipati, Cecilia Khachane, Marethabile Nei, Florence Mohai, Thorkild Tylleskär, Laura Guay. 18-24-month HIV-free survival as measurement of the effectiveness of prevention of mother-to-child transmission in the context of lifelong antiretroviral therapy: results of a community-based survey. PLoS ONE 2020, accepted for publication

All papers are open access and freely available.

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ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
aOR	adjusted Odd Ratio
ART	Antiretroviral therapy
AZT	Zidovudine
CCR5	chemokine receptor type 5
CD4	Cluster of Differentiation 4
CDC	Centers for Disease Control and Prevention
CHAL	Christian Health Association of Lesotho
CI	Confidence interval
CSW	Commercial sex workers
CTX	Cotrimoxazole
CXCR4	chemokine receptor type 4
DNA	Deoxyribonucleic Acid
DTG	Dolutegravir
EGPAF	Elizabeth Glaser Pediatric AIDS Foundation
EID	Early infant diagnosis
ELISA	Enzyme Immunosorbent Assay
EPI	Expanded Program for Immunization
FANC	Focused Antenatal Care
GDP	Gross Domestic Product
HEI	HIV-exposed infant
HIV	Human Immunodeficiency Virus
HTS	HIV testing services
HUI	HIV-unexposed infant
IRB	Institutional Review Board
LTFU	Loss to follow-up
MBP	Mother-baby pack
MCH	Maternal and child health care
MOH	Ministry of Health
MSM	Men who have sex with men
MTCT	Mother-to-child transmission of HIV-1
NAT	Nucleic Acid Testing
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PHDP	Positive Health Dignity and Prevention
PMTCT	Prevention of mother-to-child transmission of HIV-1
POC	Point-of-care (testing)
PrEP	Pre-Exposure Prophylaxis
REC	Research Ethics Committee
RR	Relative Risk
sdNVP	Single dose Nevirapine
TAT	Turnaround time

TDF	Tenofovir Disoproxil Fumarate
TNA	Total Nucleic Acid
USAID	United States Agency for International Development
USD	United States Dollars
VHW	Village Health Workers
VL	Viral load
WHO	World Health Organization
3TC	Lamivudine

DEFINITIONS

This thesis uses the following operational definitions:

Caregiver: a person who provide essential care to a young child who has not reached the age of maturity. S/he can be biological parents, grandparents, other family relatives, or legal guardians.

Conventional EID testing: Provision of early infant HIV testing for children in a centralized laboratory.

HIV-exposed infant: A child who is born from an HIV-positive mother or who is being breastfed by an HIV-positive woman.

Option B+: Provision of lifelong antiretroviral therapy to HIV-positive pregnant and breastfeeding women irrespective of their CD4 or WHO clinical stage.

Point-of-Care Diagnosis: A test that can be provided in lower health facility or at the point where care is being provided to the patients

ABSTRACT

The number of new pediatric HIV-1 infections is reducing globally as services for the prevention of mother-to-child transmission of HIV-1 (PMTCT) are being scaled up. Lesotho has one of the highest HIV burdens globally with an estimated HIV prevalence of 25.7% in antenatal care (ANC). In 2013, the Ministry of Health of Lesotho adopted lifelong antiretroviral therapy (ART) for all HIV-positive pregnant and breastfeeding women, regardless of clinical or immunologic status (Option B+). Although this is expected to decrease mother-to-child transmission (MTCT) in general, program effectiveness data are only starting to emerge.

In this thesis, I present results of three studies: conventional early infant diagnosis (EID) turnaround time (TAT); six-week PMTCT outcomes for HIV-exposed and HIV-unexposed infants in the era of lifelong ART, and 18-24-month HIV-free survival measured through community survey.

The overall aim of the study was to assess the effectiveness of the PMTCT program by determining birth outcomes and HIV-free survival of a prospective cohort of HIV-exposed infants compared to HIV-unexposed infants. In addition, we assess HIV-free survival among HIV-exposed children identified in the community who were born 18-24 months prior to study initiation. This was built on a baseline evaluation where we described turnaround time for EID when conventional EID was used.

Methods: The baseline study was a retrospective cohort where data were abstracted from routine clinical records in health facility. The prospective observational cohort study included HIV-positive and HIV-negative women attending ANC and their infants up to 24 months postpartum; enrolled in the study June 2014 – February 2016. Study visits for HIV-positive mothers were three-monthly until 24 months after delivery, while HIV-negative mothers had study visits every three to six months after delivery. Demographic, social, and medical data were collected from participants during clinic visits through interviews and extraction of medical record information. For the community cross-sectional study, we captured the mortality and HIV infection outcomes of HIV-exposed children who were born after the introduction of Option B+.

For all studies, quantitative data analysis was performed using Stata. Categorical variables were summarized using frequencies and percentages of participants, while continuous variables were summarized using means and standard deviations or medians and interquartile ranges, as appropriate. Maternal baseline characteristics were stratified by HIV status. For the baseline study, turnaround time geometric means (with 95% CI) were calculated and compared using linear mixed models. For the prospective cohort study, we compared birth outcomes between HEIs and HIV-unexposed infants (HUIs). Categorical variables were compared using Chi-square tests and continuous variables using t-tests or Wilcoxon rank-sum tests, as appropriate. HIV-free survival was estimated as the proportion of children alive and HIV-negative among HEI. For the community cross-sectional survey, the difference in survival between subgroups was determined using the log-rank test.

Results: Concerning the baseline study, of the total of 1,187 infants reviewed, the turnaround time was 61.7 days (95% CI: 55.3-68.7). The longest turnaround time was time of results from central laboratory to district hospital, 23.3 days (95% CI: 18.7-29.0). Mean times from specimen transfer to the central laboratory and for result transfer from central laboratory to district hospital were significantly shorter in the Lowlands Region (0.9 and 16.2 days, respectively), compared to Highlands Region (6.0 [p = 0.030] and 34.3 days [p = 0.0099].

Results of the cohort study showed that prematurity was more frequent among HEI, 7.8% vs. 3.6%, although there was no difference in rates of congenital anomalies between HEI (1.0%) and HUI (0.6%). For HEI, cumulative HIV-1 transmission was 0.9% (N = 4/431) (95% CI: 0.25–2.36) at birth

and 1.0% (N = 6/583) (95% CI: 0.38–2.23) at six weeks. Among liveborn infants, six-week HIV-free survival for HEI was 95.6% (95% CI: 93.7–97.1).

For the cross-sectional community survey, the MTCT rate was 5.7% [95% CI: 4.0-8.0] and the reported mortality rate was 2.6% [95% CI: 1.6-4.2] among HIV-exposed children compared to 1.4% (95% CI: 0.9 – 2.3) among HIV-unexposed children. The estimated HIV-free survival was 91.8% [95% CI: 89.2-93.8] among HEI. Disclosure of mother's HIV status (aOR = 4.9; 95% CI: 1.3-18.2) and initiation of cotrimoxazole prophylaxis in the child (aOR = 3.9; 95% CI: 1.2-12.6) were independently associated with increased HIV-free survival while child growth problems (aOR = 0.2; 95% CI: 0.09 – 0.5) were independently associated with reduced HIV-free survival.

Conclusion: The turnaround study showed that average EID turnaround time was two months; the longest period of delay was transfer of results from central laboratory to district hospital. Meanwhile the prospective cohort study showed that implementation of universal maternal ART lowers MTCT at six weeks of age with no differences in congenital anomalies or early mortality between HEIs and HUIs. Of note, HEIs were reported to have high rates of prematurity. From the community cross-sectional survey, despite scale up of lifelong ART among pregnant and breastfeeding women, HIV has a significant effect on survival among HIV-exposed children compared to unexposed children.

1.0 INTRODUCTION

1.1 The HIV life cycle

HIV-1 replication starts as soon as the virus enters the human body (Figure 1). The host human cells must express CD4, a glycoprotein acting as a receptor found mainly on the surface of CD4+ T-lymphocytes (often called 'CD4 cells' for short) but also on monocytes/macrophages and dendritic cells [1,2]. The replication of HIV-1 starts when the virus particle comes into contact with CD4 glycoprotein. The viral surface protein gp120 sticks to CD4 and a co-receptor (chemokine receptor type 5 (CCR5) or the chemokine receptor type 4 (CXCR4) causing the viral envelop to fuse with the host cell membrane followed by release of the viral content into the host cell cytoplasm [3,4]. The reverse transcriptase enzyme immediately and reversely transcribes the viral ribonucleic acid (RNA) into deoxyribonucleic acid (DNA) which is transported into host cell nucleus to be integrated into human DNA by the viral enzyme integrase to form a provirus. One unique characteristic of HIV is its capacity to go dormant in the provirus state for a long time until when there is cellular activation. When activated, the provirus is transcribed into viral messenger RNA using human enzymes, then transported to the cytoplasm where the messenger RNA acts as blueprint for the production of new viral proteins, enzymes and viral particles. Viral particles, proteins and enzymes assemble into immature virions as the viral protease enzyme chops up long strands of protein to mature viral particles. The latter are capable to infect new host cells and the cycle starts all over again [5-8].

Once HIV infection is established, there is rapid multiplication of HIV in the body causing a large number of CD4 T-lymphocytes to be infected and destroyed (Figure 2). The destruction of CD4 cells is caused by several mechanisms induced by HIV cytopathic effects and include syncytial formation, cellular dysfunction, and apoptosis [9-11]. CD4 T-lymphocytes play a central role in the immune system. As CD4 T-lymphocytes are destroyed, there is rapid rise of the viral load with spread and seeding of the virus in many organs especially in the lymphoid system such as the spleen, lymph nodes, the thymus and gastrointestinal associated lymphoid system [10]. During this period, called 'the acute phase', due to high circulating viral load in the peripheral blood and body secretion especially in the genital and anal areas, HIV infected individuals are very infectious [12-14]. The concentration of virus particles in the blood, called 'viral load' (VL) remains high for 10-12 weeks after the initial infection, during which there is irreversible destruction of the reservoir of CD4 T-lymphocytes. The HIV virions also integrate in the resting T-lymphocytes DNA and remain dormant

for years. At the same time during this period, several individuals present with flu-like symptoms, the severity of which is a predictor of disease progression [15,16].

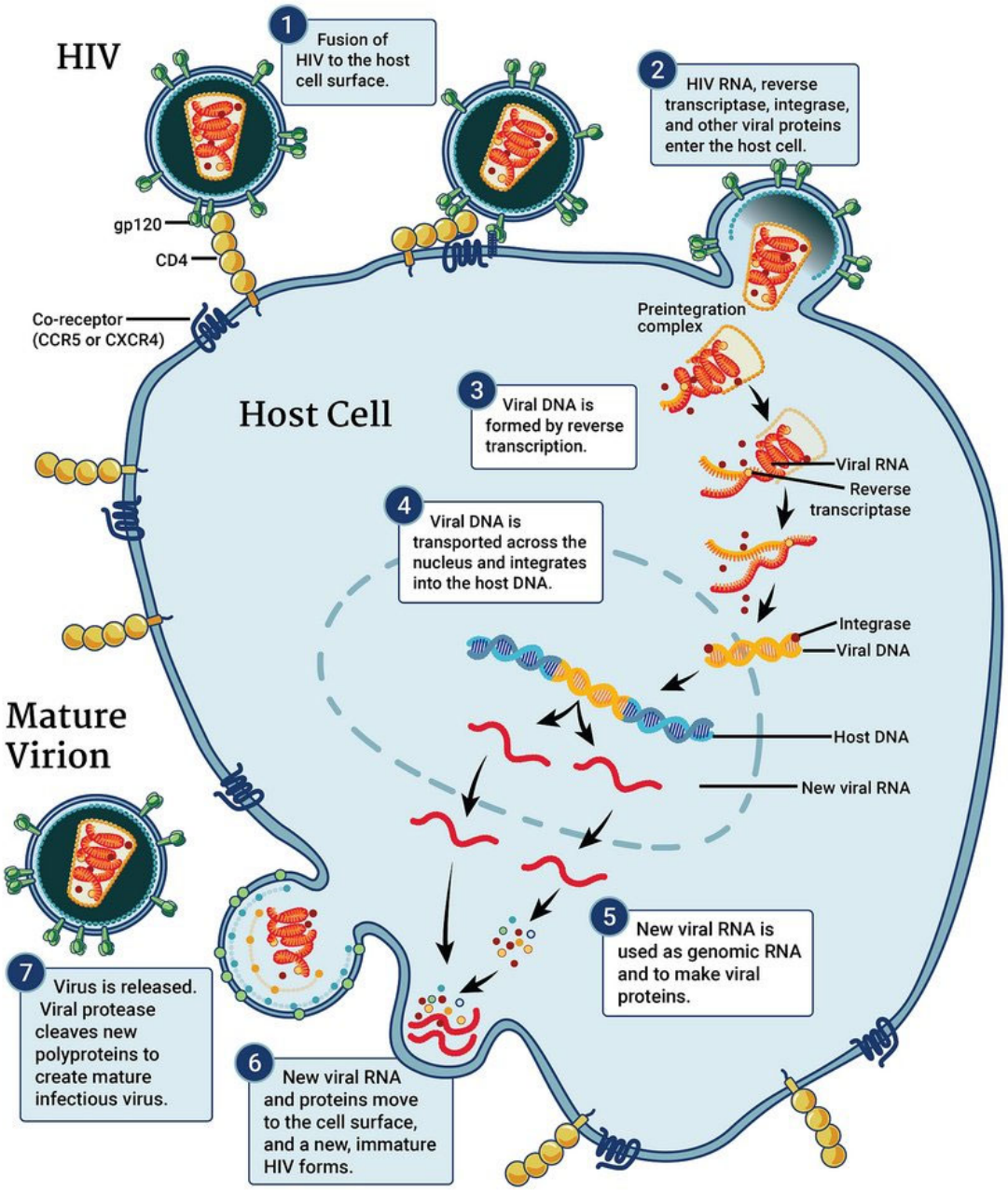


Figure 1. The HIV replication inside a human cell. [8]

HIV blood VL gradually reduces to a low set point while the CD4 cells count bounds back but never to the initial level [16]. If the patient is not treated, the CD4 cell count slowly declines over time leading to increasing degree of immunosuppression. The patient will eventually develop opportunistic infections. These were initially used by World Health Organization (WHO) to make a clinical staging of the disease [17-19]. This staging is still in use. As the disease progresses, the patient is prone to life threatening conditions such as pneumocystis pneumonia, disseminated tuberculosis, cryptococcal disease and opportunistic cancers especially when the CD4 drops below 200 cells/mm^3 [20,21].

With the introduction of antiretroviral therapy (ART) and the scale up of treatment across the globe, the HIV disease progression has changed significantly. Once initiated on effective treatment, there is suppression of viral replication to an undetectable level (<40 copies/mm^3) and CD4 recovery to almost initial pre-infection level [22-24]. The laboratory monitoring of HIV-positive patients is summarized in table 1.

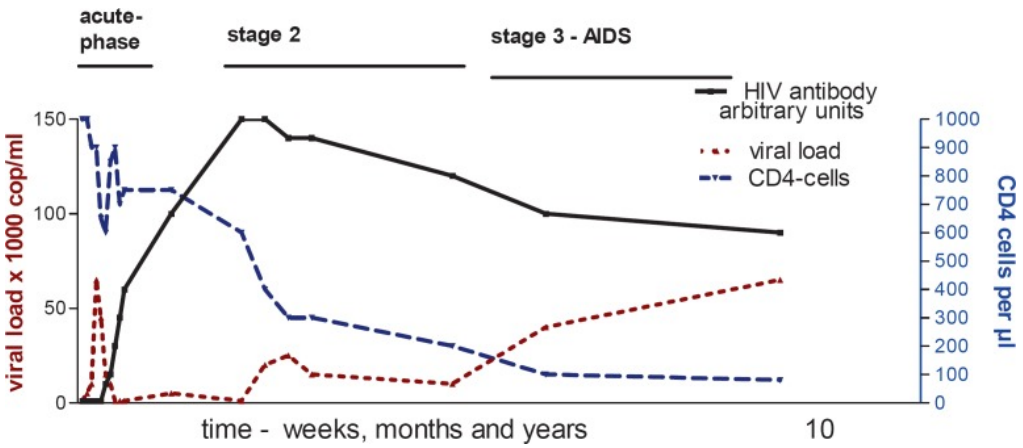


Figure 2. Temporal course of an untreated HIV infection, viral load in red and CD4 cell count in blue. [16]

1.2 Burden of HIV disease in the world

Since the first case of HIV was reported in 1981, more than 70 million people have been infected with the HIV virus [25]. In 2017, 36.9 million [31.1–43.9 million] people were living with HIV compared to 27.4 million [23.1–32.6 million] in 2000 (figure 3). This represents an estimated 0.8% [0.6-0.9%] of adults aged 15–49 years worldwide. The burden of the HIV epidemic continues to vary considerably between countries and regions; however, Africa continues to be home to 70% of those living with HIV [26, 27]. Women of reproductive age in Africa have higher HIV prevalence than men of similar age. Among adults aged 15-49 years it is estimated 18.2 million (15.6-21.4 million) women

and 16.4 million (13.9-20.4 million) men are living with the virus, respectively [26, 27]. An estimated 1.8 million (1.3-2.4 million) children aged 0-14 years are also living with the virus. Although HIV incidence is either stable or actually dropping in all age groups, among adolescents it has continued to rise with 1 million (0.54-1.5 million) female and 0.77 million (0.55-1.1 million) male adolescents living with HIV worldwide [26, 27].

Table 1. Important laboratory tests for management of HIV infection.

	Use	Normal values	Values in an HIV-infected individual
HIV antibody test	To see if a person is HIV-infected. Cannot be used for children below 18 months (maternal antibodies are still present)	Negative	Positive
CD4+ cell count	To see how suppressed the person's immune system is	Above 1500 cells/ml	Can go down to 0 cells/ml Very poor immunity if CD4 count <200 cells/ml The count usually increases if the person is on ART
Qualitative detection of HIV DNA or RNA by nucleic acid testing (NAT)	To see if an infant is infected	Negative (HIV-uninfected child)	Positive (HIV-infected)
VL = Quantitative detection of HIV DNA or RNA by NAT	To assess how intense the viral replication is in an HIV positive patient.	0	VL <40 copies/mm^3 means 'undetectable' / 'suppressed', the patient is well treated and adherent VL <1000 copies/mm^3 means not so well suppressed VL >1000 copies/mm^3 means virally 'unsuppressed'. The patient is a) not on treatment, b) not adherent or c) has viral resistance to the treatment
Resistance testing	To assess whether an HIV-infected child or HIV-positive adult failing ART treatment has resistance	HIV-uninfected persons do not have the virus and hence no resistance	Can go from 'susceptible' (no resistance detected) to any amount of mutations (the virus has developed resistance, often due to prolonged non-adherence)

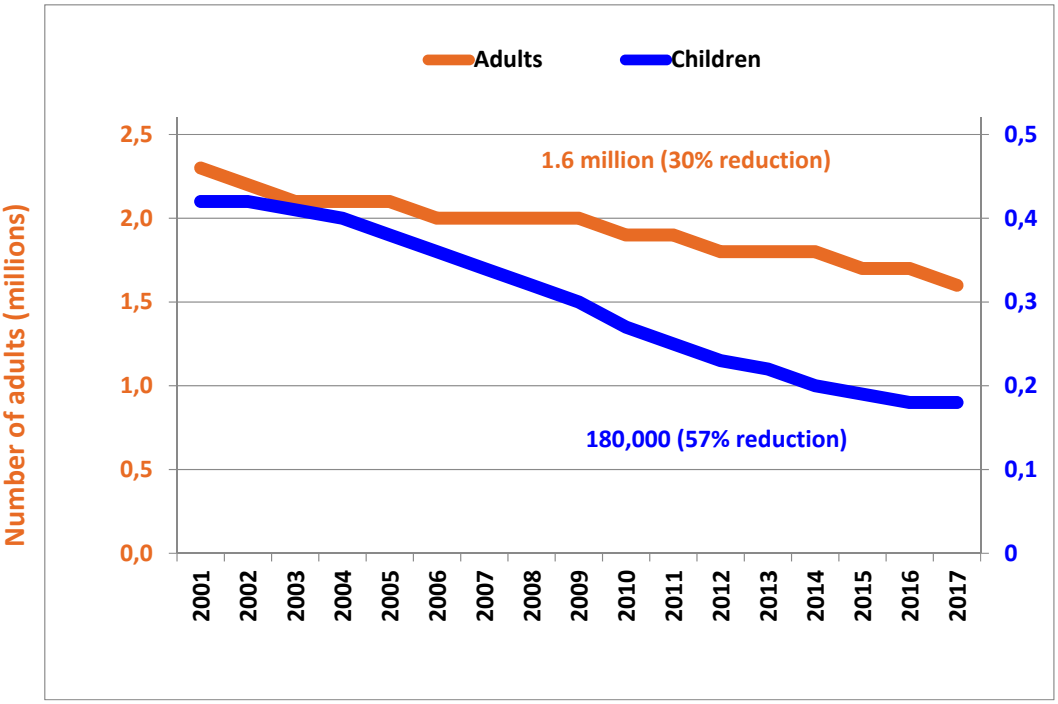


Figure 3. Global new HIV infections among adults and children (blue), 2001-2017 (Adapted [26, 27]).

1.3 Mother-to-child transmission of HIV-1

HIV can be transmitted from an HIV-positive mother to her infant during pregnancy, delivery and postnatally through breastfeeding. In the era before treatment for HIV-positive women and prophylaxis for HEI, the estimated risk of MTCT was a) 5-10% during pregnancy; b) 10-20% during labor; c) 5-20% during breastfeeding [28]. Therefore, in the absence of any intervention, MTCT rates range from 15-30% in a non-breastfeeding population and 25-45% in a breastfeeding population. Factors associated with MTCT are shown in Table 2.

A lot of progress has been made to reduce the risk of MTCT and in high-income countries, MTCT rates are below 2% [26]. In 2017, 80% of HIV-positive pregnant women received an intervention for PMTCT representing an excellent progress of more than 51% in comparison with PMTCT coverage in 2000, Figure 4 [26,27]. However, it is important to note that, close to 740,000 HIV-positive women of reproductive age became infected during 2016; 73% of whom are in 23 priority countries [26, 27]. In 2017, despite the roll out of lifelong combination ART in many countries, 180,000 children became HIV-infected; with over 90% being a result of MTCT [30]. MTCT rates in Africa vary from country to country.

Table 2. Summary of factors that increase risk of MTCT of HIV (Adapted from [29]).

Factors which may increase the risk of MTCT		
Pregnancy • High maternal VL (new infection or advanced AIDS) • Sexually Transmitted Infections (STIs) • Viral, bacterial, or parasitic placental infection • Poor maternal nutritional status • Chorioamnionitis (from an untreated STI or other infection)	**Labour and Delivery** • High maternal VL (new infection or advanced AIDS) • Rupture of membranes for more than 4 hours • Invasive delivery procedures that increase contact with mother's infected blood or body fluids (e.g. episiotomy, foetal scalp monitoring) • First infant in multiple birth • Preterm delivery • Low birth weight	**Breastfeeding** • High maternal VL (new infection or advanced AIDS) • Prolonged breastfeeding • Mixed feeding, particularly during the first 6 months of life (e.g. food or fluids in addition to breast milk) • Breast abscesses, nipple fissures, mastitis • Poor maternal nutritional status • Oral disease in the baby (e.g. oral thrush or sores)

Figure 4, with data drawn from the Spectrum model, also provides estimates that suggest fewer pregnant women were living with HIV over the past decade (about 1.4 million vs. prior estimates of about 1.5 million). ARV coverage rates for PMTCT appear to have plateaued over the last three to four years [26, 27].

1.4 **The context of lifelong antiretroviral treatment**

The World Health Organization (WHO) has led the coordination of building guidelines for PMTCT based on the most up-to-date evidence. These guidelines have evolved since the publication of the first WHO PMTCT guidelines in 2001. The guidelines are built on public health principles to reach the highest number of patients with the most efficient treatment available, Table 3. In 2001, the guidelines recommended the provision of prophylaxis mainly to HEI with zidovudine (AZT) or zidovudine in combination with lamivudine (AZT/3TC) for four weeks or single-dose nevirapine (sdNVP) during labor [31].

In 2004, WHO reviewed and published guidelines for PMTCT with prophylaxis called combination or more efficacious regimen given to HIV-positive pregnant women with CD4 $\geq$200cells/mm^3 from gestational age of 28 weeks as zidovudine monotherapy and during labor they are given sdNVP. All pregnant women with CD4<200 cells/mm^3 were initiated on lifelong therapy as per the care and treatment guidelines for adults [32]. This was the first-time pregnant women were being given ART for their own health.

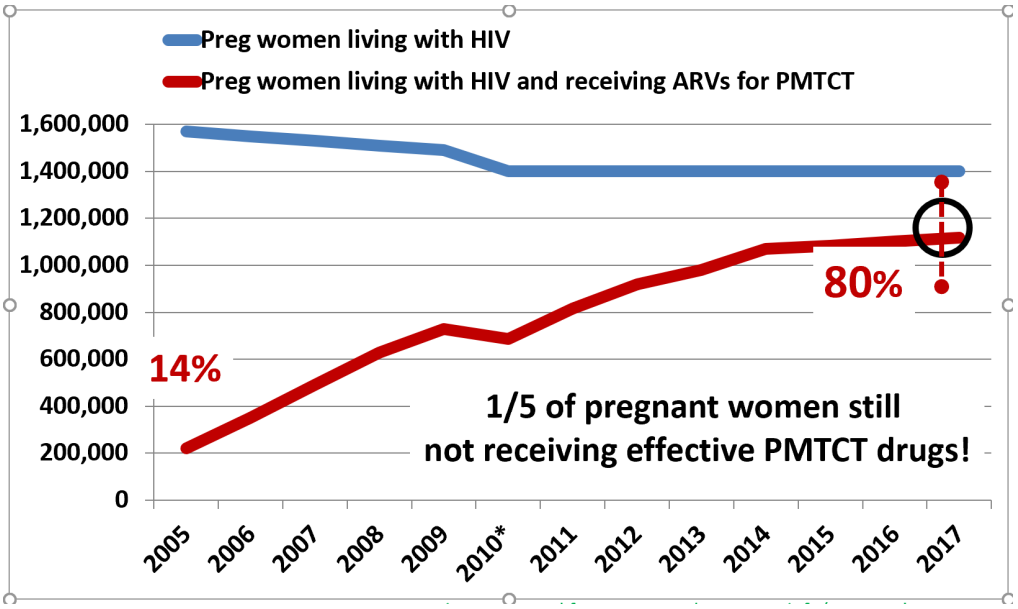


Figure 4. Global Access to Antiretroviral Drugs (ARVs) for PMTCT 2005-2017 (Adapted [26,] and [27]).

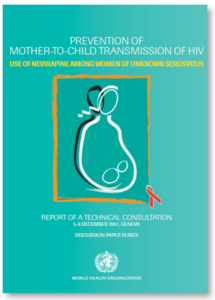
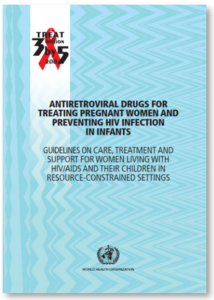
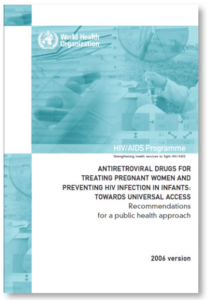
In 2006, based on the reported findings of high resistance to NVP among women after who received sdNVP, WHO reviewed the PMTCT guidelines to introduce coverage for "the non-reverse transcriptase inhibitors (NNRTI) tail". So, women with CD>200cells/mm received AZT from 28-week gestation, and sdNVP during labor and AZT/3TC for seven days after delivery [33].

In 2010, WHO decided to optimize PMTCT interventions for HIV-positive pregnant women. The new guidelines were based on an amalgamation of studies that demonstrated the efficacy of maternal ART during pregnancy and breastfeeding, or infant prophylaxis during breastfeeding to prevent MTCT in resource-limited settings [34-36]. The cut-off point for CD4 was increased to 350 cells/mm^3. For women with CD4>350; however, there were two streams of recommendation [37]:

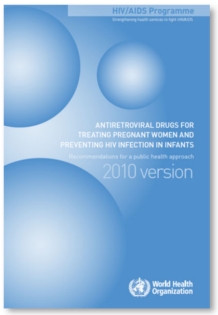
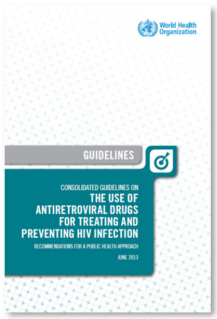
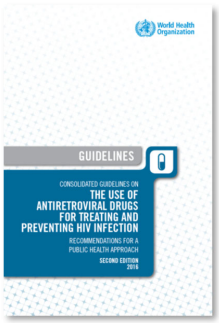
- Under Option A, HIV-positive women with CD4>350 cells/mm were initiated on AZT and 3TC from14-week gestation, then sdNVP during labor and the infant given daily NVP until six weeks of life.
- Under Option B, all HIV-positive women with CD4>350cells/mm^3 were initiated on ART during pregnancy and throughout breastfeeding period.

In 2013, WHO decided to make a single comprehensive document of "consolidated guidelines" for both treating and preventing HIV, including PMTCT. In this review of the guidelines, WHO kept

Table 3. World Health Organization Historical overview of the guidelines for prevention of mother-to-child transmission of HIV-1 (Adapted from [31-33] and 37-39]).

Year	2001	2004	2006
WHO guidelines			
Testing and counselling approach	Voluntary counselling and testing	Voluntary counselling and testing	Voluntary counselling and testing
General antiretroviral treatment for all HIV-infected individuals	No recommendations	ART if CD4 <200	ART if CD4 <200
Prevention of mother-to-child transmission of HIV-1	4 weeks AZT/AZT+ 3TC or sdNVP	AZT from 28 weeks + sdNVP	AZT from 28 weeks + sdNVP + AZT/3TC for 7 days
Monitoring	Clinical monitoring for mothers and their infants	CD4 monitoring of the mothers	CD4 monitoring for mothers
HIV diagnosis in HIV exposed infants	Western blot or ELISA	Western blot or ELISA	ELISA, DNA PCR, Rapid HIV testing

Option B and increased the cut-off point for CD4 count to 500 cells/mm^3. A major drawback of the Option B was that as soon as a mother stopped breastfeeding, she was supposed to stop ART only to start again as soon as she fell pregnant. In high-fertility areas, this created a lot of confusion. In addition, some mothers who were not eligible for ART for their own health, experienced immunological worsening of their disease and therefore became eligible for ART soon after stopping ART during pregnancy and breastfeeding. Therefore, WHO introduced Option B+ which initiated HIV-positive pregnant and breastfeeding women on lifelong ART irrespective of their CD4 cells count. HEI would be given daily NVP or AZT/3TC until six weeks of age [38].

2010	2013	2015-2016
		
Provider initiated testing and counseling with opt-out	Provider initiated testing and counselling with opt-out	Provider initiated testing and counselling with opt-out Self-testing (in some context)
ART if CD4 <u>≤</u>350	ART if CD4 <u>≤</u>500	ART regardless of CD4
<u>Option A</u> AZT from 14 weeks + sdNVP+ AZT/3TC for 7 days + infant NVP if breastfeeding <u>Option B</u> ART during pregnancy and breastfeeding	<u>Option B</u> ART during pregnancy and breastfeeding <u>Option B+</u> Lifelong ART	Lifelong ART for all pregnant and breastfeeding women
<u>CD4 monitoring of HIV positive mothers</u>	<u>CD4 monitoring of HIV positive mothers</u>	VL monitoring
<u>DNA PCR, Rapid HIV test</u>	<u>DNA PCR</u>	DNA PCR Total Nucleic Acid (TNA) using point of care machines

These guidelines were then aligned with the WHO 2016 HIV treatment guidelines for HIV-positive patients which recommends lifelong treatment irrespective of CD4 cells count [39]. All children are given daily nevirapine or AZT/3TC for six weeks. One important point in the 2016 guidelines, is the use of VL to monitor treatment success. Therefore, HIV-positive pregnant or breastfeeding women on ART were expected to have VL done every six months. Ideally, all HIV-positive patients on ART were expected to have an undetectable VL (VL<40 copies/mm^3) by six months after initiation of treatment and subsequently thereafter. In the public health approach, the term VL suppression was introduced when VL was less than 1,000 copies/mm^3 as infectivity is considered low [39].

1.5 General overview of the Kingdom of Lesotho

The Kingdom of Lesotho – Lesotho, for short – is a mountainous, landlocked country located completely within South Africa in the Southern African region. Lesotho is a sovereign, democratic and independent nation.

Figure 5. Administrative map of Lesotho. The capital Maseru is on the north-western border [40].

Lesotho, with the capital Maseru, was formerly known as the Basutoland and was renamed the Kingdom of Lesotho when she gained its independence from the United Kingdom on October 4th,

1966. Lesotho is a constitutional monarchy and the current king (King Letsie III) has been head of state since 1996 [41, 42]. The latitudinal and longitudinal extent of the country is 29°30' South and 28°30' East, respectively. The total length of its border is 909 kilometers and Lesotho has a surface area of 30,000 square kilometers, about 10% of the size of Norway or as large as the county of Vestland surrounding Bergen.

Lesotho has four ecological zones (Lowlands, Foothills, Highlands, and the Senqu River Valley) and 10 districts, namely Berea, Botha-Bothe, Leribe, Mafeteng, Maseru, Mohale's Hoek, Mokhotlong, Qacha's Nek, Quthing and Thaba Tseka [43]. Lesotho has four seasons: Spring (September 21st to December 20th); Summer (December 21st to March20th); Autumn (March 21st to June20th); and Winter (June 21st to September20th). Throughout the year, temperatures range from negative six degrees Celsius in winter to 35 degrees Celsius in summer. During winter, there is heavy snowfall, especially in the mountains. Lesotho has a population of 2.2 million with a population density of 64 persons per square kilometer, Table 4. The fertility rate in Lesotho is 3.09 births per women with a population growth of 1.3%.

Table 4. Key selected socio-demographic and health indicators in Lesotho (Adapted from [98] and [42].

Indicators	2016/2017
Total population	2.2 million inhabitants
GDP per capita	1,181.81 USD
Adult literacy	97%
Life expectancy	54 years
Total fertility rate	3.1 children per women
Maternal mortality ratio	420 per 100 000 live births
Under-five mortality rate	94 per 1000 live births
Neonatal mortality rate	38 per 1000 live births
Population growth	1.3% per year
HIV prevalence among general population (ages 15-49 years)	23.8%
HIV incidence among general population (ages15-49 years)	1880 per 100 000 population
Number living with HIV	320 000
Number of HIV related deaths	4900
Antiretroviral therapy coverage	53%
BCG Immunization coverage among 1-year-olds	98%
Polio immunization coverage among 1-year-olds	90%
Penta 3 immunization coverage among 1-year-olds	93%
Measles immunization coverage among 1-year-olds	90%

Lesotho is a middle-income country with a Gross Domestic Product (GDP) per capita of USD $1,181.81 as of fiscal year 2017 [41]. The government of Lesotho, in the current budget, spends 12.7%

of the total budget on healthcare which still falls short of the Abuja Declaration recommendation of 15%, but is higher than many other countries in the region [42].

1.6 The health system in Lesotho

Located in Maseru, the capital, the Lesotho Ministry of Health (MOH) provides overall leadership for the health sector. The mission of the MOH is to "provide an efficient and compassionate health care and social welfare system, with particular emphasis on the prevention and eradication of priority health and social welfare problems that are amenable to cost-effective intervention." [43, 44]. the MOH plans to reach its mission through an effective and effective approach whereby affordable interventions with high health impacts are implemented in the country. Among others, the MOH coordinates formulation of policy, and stakeholder engagement during the consultation process for policy development. It sets standards and quality assurance, ensures capacity development for health care professionals to keep them abreast of international standards, provides supportive supervision and technical support to health facilities, and monitors and evaluates implementation of various national guidelines. The MOH also develops the budget for the health sector and ensures its appropriate implementation. However, in Lesotho, the MOH provides care to only 60% of the population while the remaining 40% receive their care from the Christian Health Association of Lesotho (CHAL), Figure 6 [45]. CHAL brings all churches together under one board to have concerted effort to provide health services. In recent times, the MOH signed a memorandum of understanding (MOU) with CHAL to provide funding to cover the cost of medicines and salaries for health care workers. In addition, there has been an increased presence of international non-governmental organizations to support the MOH in fighting the HIV scourge.

The national health structure consists of primary, secondary and tertiary levels of care. Currently, Lesotho has 372 health facilities including: 1 referral hospital, 2 specialized hospitals, 18 district hospitals, 3 filter clinics, 188 health centers, 48 private surgeries, 66 nurse clinics and 46 pharmacies [43, 45]. The first point of entry into the health system is the health center, which is the primary health care facility. The MOH owns 42% of the health centers, and 58% of the hospitals while CHAL owns 38% of the health centers and 38% of the hospitals (Figure 5). The rest of facilities are owned by private practitioners who are located mainly in the districts of Maseru, Berea, Leribe, and Mafeteng [41, 45].

Figure 6 shows the distribution of public, not-for-profit health facilities in Lesotho. Private or for-profit health facilities are not shown here because they are more unstable in their existence.

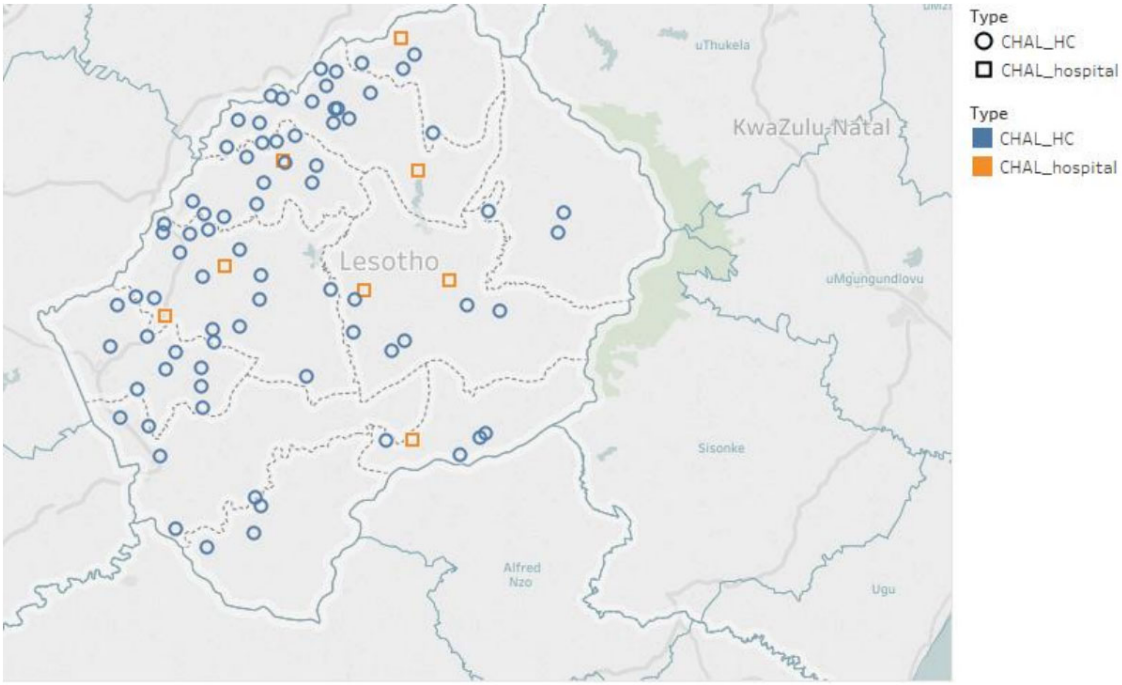


Figure 6. Distribution of health centers and hospitals run by the non-governmental organization CHAL (Adapted from [41]).

The primary health care level consists of health centers, health posts, and all community-level initiatives including all staff working at this level. There are village health workers (VHWs) who serve as the first point of contact for health with 40 household per VHW giving a total estimate of 6,000 VHWs in Lesotho. At the district level, there are district hospitals and district management teams which are the secondary level of care and supervise health centers. The National Referral Hospital and the two specialized hospitals namely Mohlomi Mental Hospital and Bots'abello Leprosy Hospital constitute the tertiary level of care in Lesotho. Patients requiring more advanced care including cancer patients, patients requiring a transplant, etc. are referred to South Africa [45].

As demonstrated in the most recent survey data, individuals living in the urban/peri-urban settings have the highest access to and likelihood of using available health services [41, 46, 47]. Infant feeding practices also vary across the four ecological zones.

1.7 Antenatal care (ANC)

The WHO now talks about positive pregnancy experience which is defined as: (a) maintaining physical and sociocultural normality; (b) maintaining a healthy pregnancy for mother and baby

(including preventing and treating risks, illness and death); (c) having an effective transition to positive labor and birth, and (d) achieving positive motherhood (including maternal self-esteem, competence, and autonomy) [48-50].

In Lesotho, there are various ANC interventions at different levels of care, Figure 7. In the figure, the maternal worker is a VHW who in this model accompanies the pregnant woman to facilitate her movement through the health system [51].

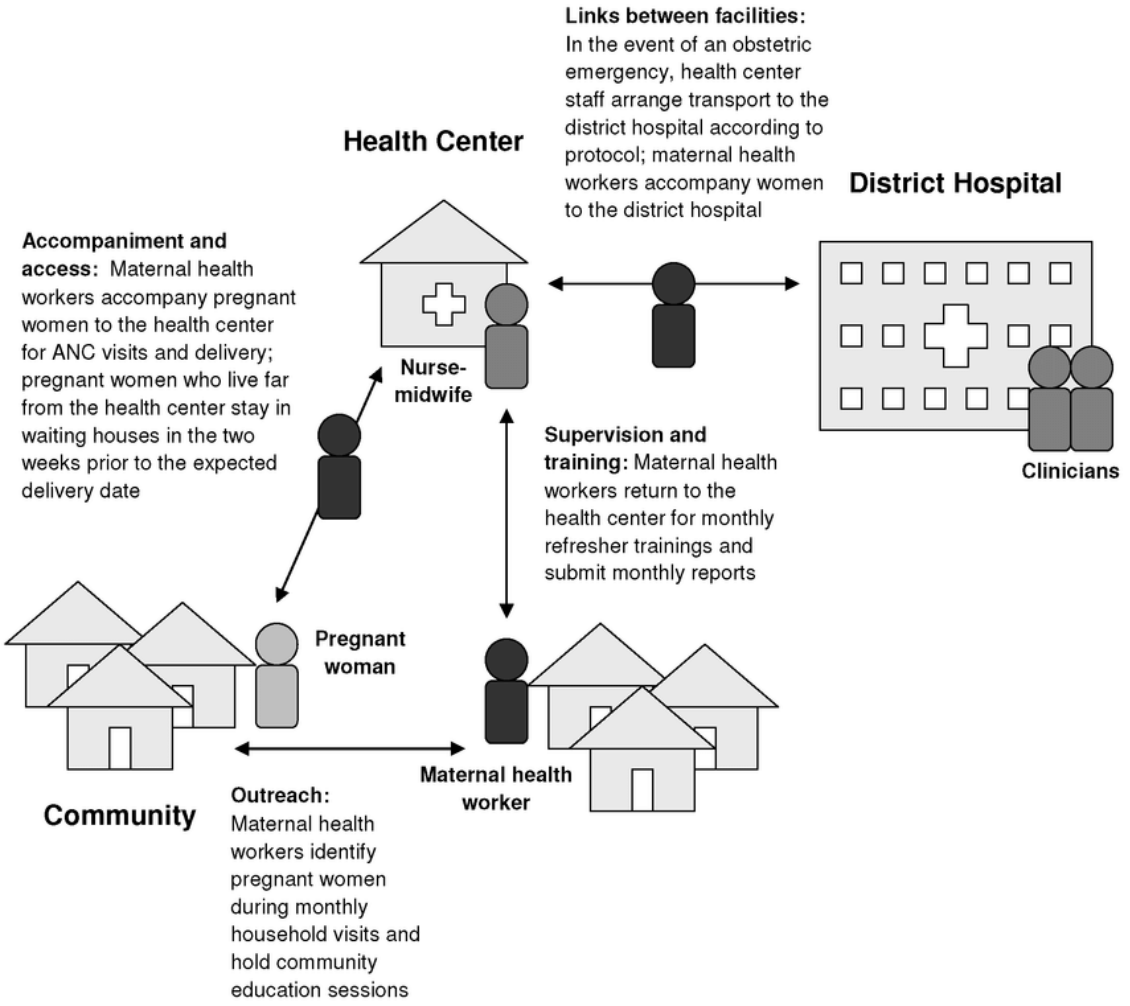


Figure 7. Schematic of the continuum of care for maternal health in Lesotho [51].

In Lesotho, the focused antenatal care (FANC) approach, which requires four ANC visits during pregnancy, is the norm with more than 90% of women attending four ANC visits [52, 53]. Most women are attended to by qualified health care professionals especially nurses/midwives. Facility-

based delivery is also high and most deliveries (70-85%) are attended to by skilled health workers [41, 53, 54]. Maternal and child health indicators have continued to improve after a deep dive during the initial phase of the HIV epidemic, before effective treatment was rolled out. In Lesotho hospitals and health clinics, once enrolled for care in ANC, pregnant women are assigned an ANC identification number that is recorded both in the register book and in the woman's handheld card. The latter is brought by the pregnant woman to every visit. The ANC facility register contains the woman's ANC identification number and demographic and pregnancy-related information. Table 5 shows the newborn and child mortality in Lesotho.

Table 5. Newborn and child mortality in Lesotho in view of the current targets [55].

Indicator	Baseline value (2015)	Current value (2016)	Projected (1) value (2030)	Target value (2030)	Comment	Colour code (2)
Under-five mortality rate (per 1000 live births)	97.9	93.5	80.9	25.0	The pace is not fast enough to meet the SDG target. An annual reduction of about 9.1% is required to achieve the 2030 target.	
Neonatal mortality rate (per 1000 live births)	39.1	38.5	37.4	12.0	The pace is not fast enough to meet the SDG target. An annual reduction of about 7.9% is required to achieve the 2030 target.	
Infants receiving three doses of hepatitis B vaccine (%)	93.0		100.0	100.0	Progress in reducing under-five mortality rate is good. If the annual reduction of 05% is maintained, the country is on track to achieve the 2030 target.	

1.8 The HIV situation in Lesotho

The HIV epidemic in Lesotho is a generalized and mature epidemic, with heterosexual contact as the main transmission method. In addition, mobility of the population into South Africa in search of employment has been cited as one of the major factors [56, 57]. Since 2005, the government of Lesotho has considered HIV as national emergency because of the magnitude of the epidemic.

According to UNAIDS, in 2018, HIV prevalence in Lesotho was 23.6% among the population aged 15-49 years. Currently there are an estimated 340,000 people living with HIV in Lesotho [85]. The

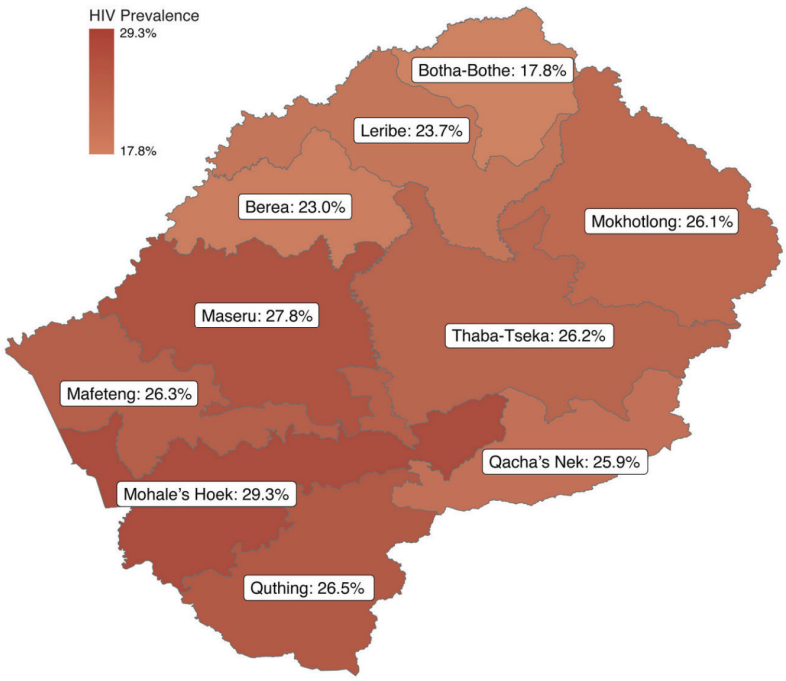


Figure 8. Lesotho HIV prevalence distribution by district [47].

top five population groups most affected by HIV are commercial sex workers, factory workers, men who have sex with men, prison inmates, and pregnant women with HIV prevalence of 71.9%, 42.7%, 32.9%, 31.0%, and 25.9%, respectively [52, 58]. In 2016, the HIV prevalence among children aged 0-14 years in Lesotho was 2.1% with 2.6% of girls infected compared to 1.5% of boys [47, 52]. In 2018, there were 13,000 new HIV infections and 6,100 deaths among people living with HIV [52]. There are about 13,000 children living with HIV in Lesotho, over 95% of whom were infected vertically from their mothers. PMTCT has had a significant impact over the years and the number of newly infected children has reduced from 4,400 in 2009 to 1,300 in 2015 [41, 47, 52].

The HIV prevalence also varies from district to district with the highest prevalence of 29.3% found in Mohale's Hoek District and the lowest prevalence of 17.8% in Butha-Buthe District (Figure 8). Maseru District has the third highest HIV prevalence in pregnant women attending ANC and is the largest of the Lesotho's 10 health districts. In the national ANC HIV Surveillance Report of 2018, HIV prevalence in ANC in Maseru District was 29.5% (CI 25.9%-33.3%) [56, 58].

The prevalence in various population categories varies by age and sex is shown in Figure 9. Women are infected at an earlier age than men and have the highest HIV prevalence (45.5%) between 35 and 39 years of age. Men have their highest prevalence (43.5%) in the age group 40-44 years [47].

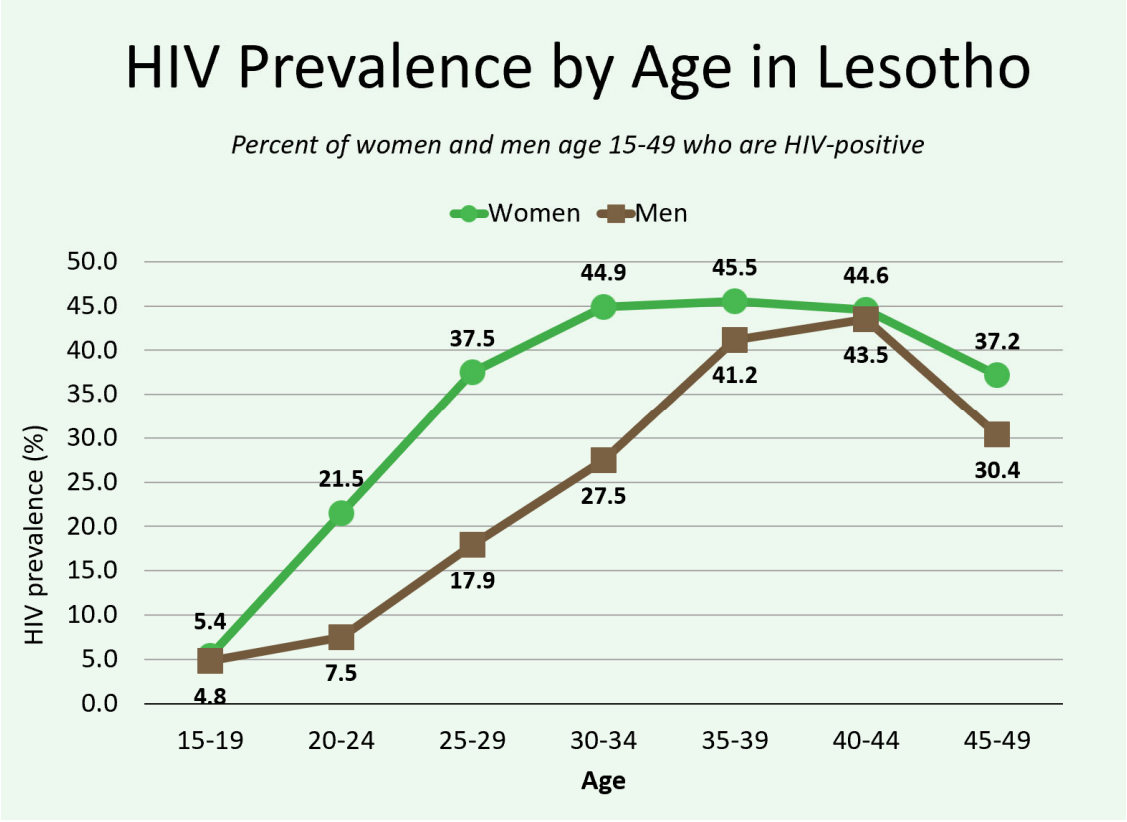


Figure 9. HIV prevalence Lesotho distributed by age and sex [47].

The Lesotho HIV epidemic has remained stable since 2004 with HIV prevalence of 23.4%, 23.0% and 24.6% in 2004, 2009 and 2014, respectively, Figure 10.

Furthermore, the prevalence is much higher in urban areas compared to rural areas, 30.0% vs. 21.8%, respectively (Figure 11). This disparity is noted among women and men where women continue to have higher prevalence in both urban (35.6%) and rural (26.4%) areas compared to men who have a prevalence of 23.1% in urban area and 16.2% in rural area [47].

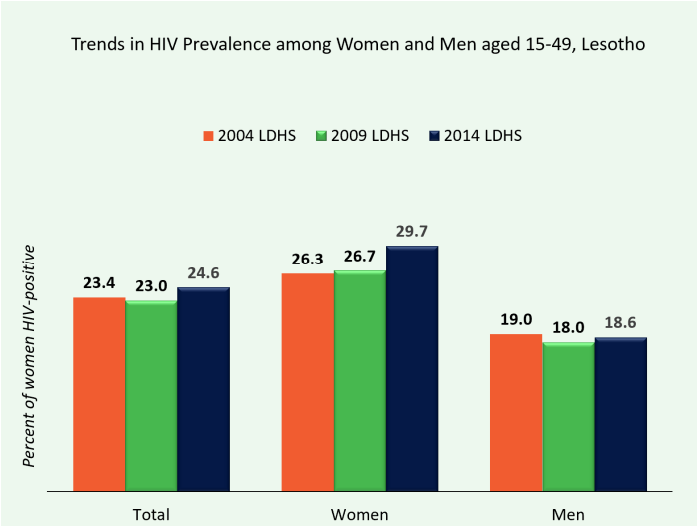


Figure 10. HIV prevalence among Basotho adults aged 15-49 years. The prevalence continues to be much higher among women compared to men [47].

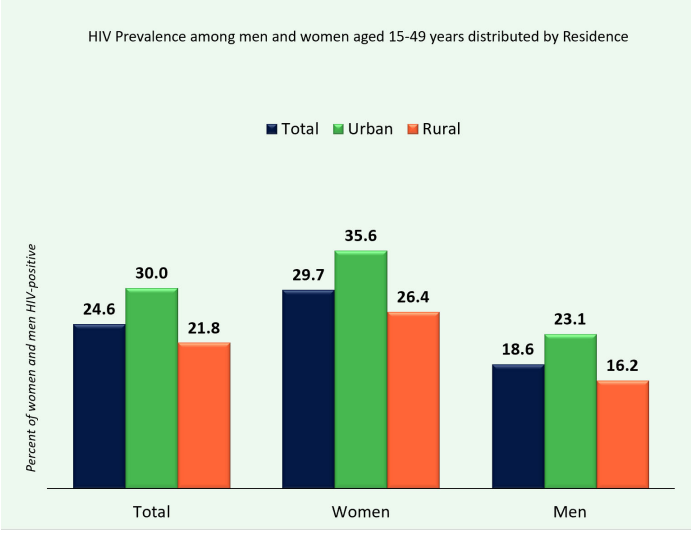


Figure 11. HIV Prevalence among Basotho aged 15-49 years by urban and rural areas [47].

Factors contributing to HIV transmission in Lesotho include gender norms, multiple concurrent sexual partners, low use of female and male condoms, stigma and discrimination, internal migration from the mountainous rural areas to urban lowland areas, and external migration to South Africa. Among commercial sex workers (CSW) particularly, there are reports of high rates of sexual violence and rape. Among men who have sex with men (MSM), poor access to health services due to stigma

and abuse continue to fuel the epidemic [59-61]. The drivers of HIV epidemic in Lesotho have been summarized in Table 6.

Table 6. Factors driving HIV epidemic in Lesotho (Adapted from [47], [62] and [63]).

Risk factors for HIV transmission	Contextual factors contributing to HIV epidemic
Multiple concurrent sexual partners	Poverty
Lack of condom use	Socio-cultural factors
Lack of circumcision or traditionally circumcised	Low educational status of young girls and women
Married or widowhood	Human rights, stigma and discrimination against certain groups such as MSM and CSW
Men who sex with men	Lack of access to prevention, care and treatment services
Transactional sex	Urban location
Migrant workers (internal or external)	
Alcohol and illicit drug use	

HIV has had a devastating effect on Lesotho, making its population to drop from 2.2 million in 1986 to 1.8 million in 2006 [64]. The country has continued to make great gains in care of people living with HIV. After the launching of the "Know your Status" campaign in 2000 by the King of Lesotho, testing rates increased rapidly. Among women, the coverage of HIV testing rose from 12% in 2004 to 66% in 2009 and 84% in 2014 while for men, it has risen more slowly 9%, 37% and 67% in 2004, 2009 and 2014, respectively [47]. This was coupled with the introduction of free ART in 2005 [59, 65]. Progress has been made and the number of new cases has continued to drop (Figure 12).

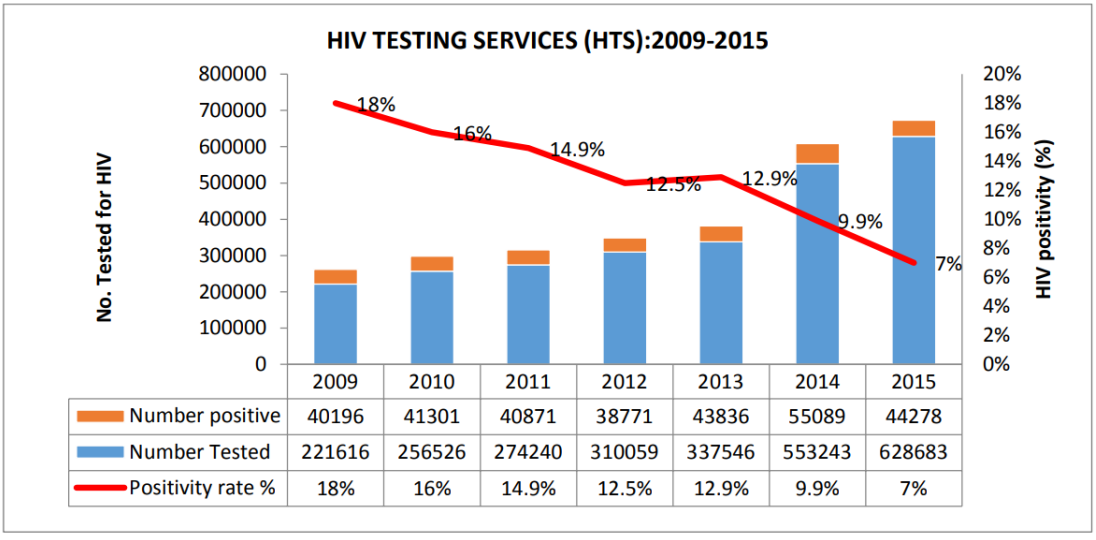


Figure 12. Trends in HIV Testing Services, 2009 – 2015 [45]

1.9 Prevention of mother to child transmission of HIV (PMTCT) in Lesotho

The PMTCT program started in Lesotho in 2003 with the goal of universal access to PMTCT. Indeed, Lesotho has rapidly expanded its national PMTCT program to reach 100% facility coverage and over 80% of women in need of services [55]. Over the years, Lesotho has aligned its national guidelines with the WHO recommendations, Table 7.

Table 7. Overview of the changes in the PMTCT program in Lesotho

WHO Guidelines by year (see table 3)	Implemented in Lesotho	Main differences
2001	In 2003	Voluntary HIV testing, single dose nevirapine
2004	In 2005	ART for CD4<200cells/mm^3 If CD4≥200cells/mm^3: AZT from 28 weeks gestation, single dose nevirapine during labor
2006	In 2007	ART for CD4<200cells/mm^3 If CD4≥200cells/mm^3: AZT from 28 weeks gestation, single dose nevirapine during labor and AZT/3TC for 7 days postpartum
2010	In 2011	Option A: ART for CD4<350cells/mm^3 If CD4≥350cells/mm^3: AZT from 14 weeks gestation plus, single dose nevirapine during labor and AZT/3TC for 7 days postpartum; Nevirapine for infants. Monitor with CD4 testing.
2013	In 2013	Option B+: Start all pregnant women on ART irrespective of their CD4. Infant nevirapine
2016	In 2016	Treatment for all: Start all pregnant women on ART irrespective of their CD4, infant nevirapine. monitor treatment with VL testing six-monthly

The comprehensive approach to PMTCT has four prongs, Table 8.

Lesotho began implementation of the adapted WHO 2010 PMTCT guidelines using “Option A” in 2011 and transitioned from “Option A” of the 2010 WHO PMTCT guidelines to “Option B+,” meaning universal ART to all HIV-positive pregnant and breastfeeding women in April 2013 [54]. This is still the situation today. The guidelines provide a comprehensive package to pregnant women, Figure 13.

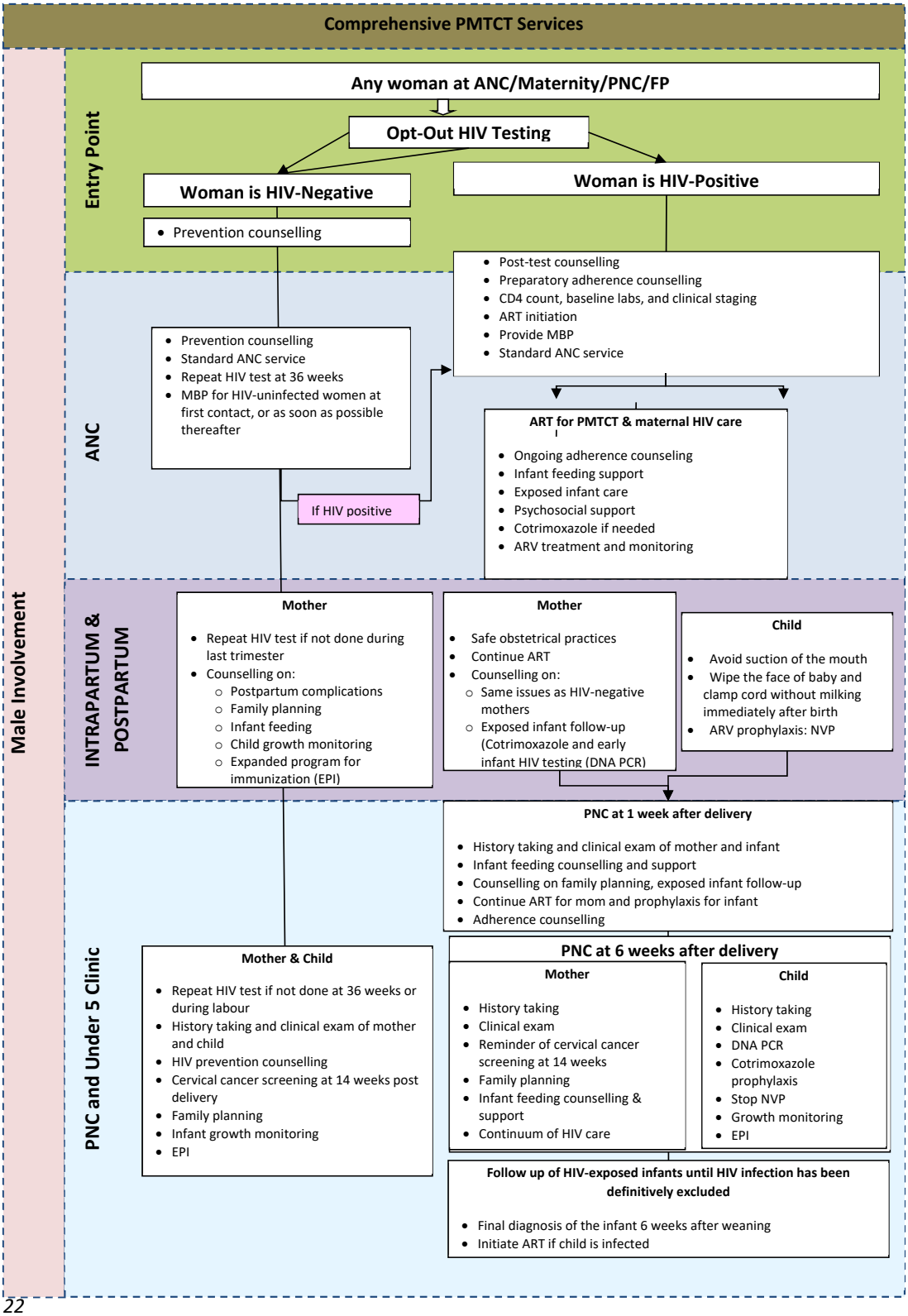
The first-line ART regimen included in the 2013 Lesotho PMTCT guidelines consists of one combination pill taken once per day containing tenofovir 300 mg, lamivudine 300 mg, efavirenz 600 mg [54].

Table 8: Prongs of PMTCT as implemented in Lesotho [54].

Component	Target population	Additional information
1: Primary prevention of HIV infection	Women and men who are sexually active	This aims to prevent men and women from ever contracting HIV. If new HIV infections are prevented, fewer women will have HIV and fewer infants will be exposed to HIV.
2: Prevention of unintended pregnancies among women infected with HIV	HIV-infected women	This addresses the long-term family planning and contraceptive needs of women with HIV. If women who are infected with HIV do not have unintended pregnancies there will be fewer infants exposed to HIV.
3: Prevention of HIV transmission from women infected with HIV to their infants	HIV-infected women	This focuses on: Access to HIV testing and counselling during ANC, labour and delivery, and the postpartum period Provision of ART to mother and infant Safer delivery practices to decrease the risk of infant exposure to HIV Infant feeding information, counselling and support for safer practices
4: Provision of treatment, care and support to women infected with HIV, their infants and their families	HIV-infected women, their children and families	This addresses the treatment, care and support needs of HIV-infected women, their children and families.

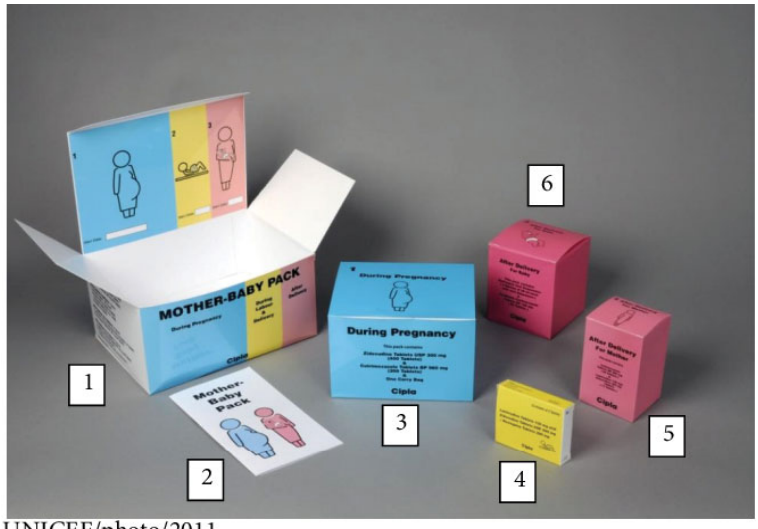
Pregnant women receiving ART should be monitored for possible medication side effects, efficacy of treatment, treatment failure, adherence and drug-drug interactions. Pregnant women receiving ART should be assessed clinically at 2 weeks, 1 month, 2 months, 3 months, 6 months and then at least every 6 months. VL should be checked every 6 months. Pregnant women receiving tenofovir should also have creatinine checked every 6 months [54]. Pregnant women on zidovudine-based regimens should have hemoglobin checked monthly.

A Mother-Baby Pack (MBP) is offered to all pregnant women at their first visit to the ANC (Figure 14). The MBP was designed to help address high rates of loss to follow-up (LTFU) during pregnancy due to weather, transportation and financial challenges. The pack is intended to simplify supply chain management and minimize treatment interruption due to stock-outs. Since the majority of women attend ANC at least once, the pack is provided at the first visit to maximize uptake of essential medications during pregnancy. Initially, three types of MBPs were developed: MBP1 for HIV-negative women, MBP2 for HIV-positive women eligible for PMTCT prophylaxis, and MBP3 for


Comprehensive PMTCT Services
Male Involvement
Entry Point
Any woman at ANC/Maternity/PNC/FP
Opt-Out HIV Testing
Woman is HIV-Negative
Prevention counselling
Woman is HIV-Positive
Post-test counselling
Preparatory adherence counselling
CD4 count, baseline labs, and clinical staging
ART initiation
Provide MBP
Standard ANC service
ANC
Prevention counselling
Standard ANC service
Repeat HIV test at 36 weeks
MBP for HIV-uninfected women at first contact, or as soon as possible thereafter
If HIV positive
ART for PMTCT & maternal HIV care
Ongoing adherence counseling
Infant feeding support
Exposed infant care
Psychosocial support
Cotrimoxazole if needed
ARV treatment and monitoring
INTRAPARTUM & POSTPARTUM
Mother
Repeat HIV test if not done during last trimester
Counselling on:
Postpartum complications
Family planning
Infant feeding
Child growth monitoring
Expanded program for immunization (EPI)
Mother
Safe obstetrical practices
Continue ART
Counselling on:
Same issues as HIV-negative mothers
Exposed infant follow-up (Cotrimoxazole and early infant HIV testing (DNA PCR)
Child
Avoid suction of the mouth
Wipe the face of baby and clamp cord without milking immediately after birth
ARV prophylaxis: NVP
PNC and Under 5 Clinic
PNC at 1 week after delivery
History taking and clinical exam of mother and infant
Infant feeding counselling and support
Counselling on family planning, exposed infant follow-up
Continue ART for mom and prophylaxis for infant
Adherence counselling
Mother & Child
Repeat HIV test if not done at 36 weeks or during labour
History taking and clinical exam of mother and child
HIV prevention counselling
Cervical cancer screening at 14 weeks post delivery
Family planning
Infant growth monitoring
EPI
PNC at 6 weeks after delivery
Mother
History taking
Clinical exam
Reminder of cervical cancer screening at 14 weeks
Family planning
Infant feeding counselling & support
Continuum of HIV care
Child
History taking
Clinical exam
DNA PCR
Cotrimoxazole prophylaxis
Stop NVP
Growth monitoring
EPI
Follow up of HIV-exposed infants until HIV infection has been definitively excluded
Final diagnosis of the infant 6 weeks after weaning
Initiate ART if child is infected

← Opposite page: Figure 13. Flow chart for PMTCT Services in Lesotho [54].

HIV-positive women on ART. However, in 2013 guidelines, the MOH maintained only MBP1 and MBP3 because there was no longer prophylaxis.



UNICEF/photo/2011

Figure 14. Mother-Baby pack, (MBP). Counterclockwise from the left. (1) Outer pack identical for all women. (2) Instruction in local language (Sesotho). (3) Blue inner box contains medicine to be taken during pregnancy: (i) ferrous sulphate, folic acid, and Vitamin B-complex for all women; (ii) ART for HIV-positive women. (4) Yellow inner box containing ART medicine to be taken during delivery. (5) Pink inner box containing medicine for the mother to be taken postpartum: (i) AZT/3TC 7-day "tail" in fixed dose combination for HIV-positive women on ARV prophylaxis; (ii) vitamin A for all women. (6) Pink inner box containing medicine for the infant through six weeks of age: (i) NVP syrup and syringe for administration for HIV-positive women on prophylaxis and treatment [66].

Since the transition to Option B+, only the MBP packs for HIV-negative women on ART are administered. For HIV-positive women, they are offered the packs and they received on-going adherence counseling while on ART.

The MBP1 includes medications provided to all pregnant women, including the following:

- Ferrous sulfate 200 mg daily until 6 weeks post-partum
- Folic acid 5 mg daily until 6 weeks post-partum
- Vitamin B complex 2 tablets daily until 6 weeks post-partum [54]
- Vitamin A 200,000 IU once

A modified MBP3 is offered to HIV-infected pregnant women which contains a one-month dose of combination antiretroviral therapy and postnatal nevirapine prophylaxis for the infant. Mothers are instructed to bring the MBP with them to every ANC visit to allow for assessment of adherence, to collect the next months' ART, and to return with her newborn 7 days after delivery for a postnatal visit [54, 66]. In addition, mothers are instructed to return to the clinic with their infants at six weeks postpartum for HIV testing of the infant.

The following ARVs are available for second-line use: tenofovir, abacavir, zidovudine, lopinavir/ritonavir and atazanavir/ritonavir. The recommended third-line regimen is raltegravir, darunavir/ritonavir and etravirine [67,68]. In 2019, Lesotho transitioned to dolutegravir-based first-line treatment. The national PMTCT Technical Working Group is working on the details of the various lines of therapy.

The focus of the work of this thesis is in the full third and fourth prongs of PMTCT. As shown in Figure 13, the PMTCT services offered in Lesotho are a continuum and include ANC, intrapartum care, postnatal care for the mothers and the infants. In the ANC, pregnant women are offered HIV testing and counselling. HIV-negative women are given health education on the how to prevent HIV infection. This includes the use of condoms, and for those at higher risk of acquiring HIV, initiation of pre-exposure prophylaxis (PrEP) [67, 69]. High risk is defined as pregnant women in sero-discordant relationship where the sexual partner is HIV-positive while the women is HIV-negative or the partner does not know his HIV status [69]. Women found to be HIV-positive are enrolled into care in accordance with national guidelines [54, 67]. The following interventions are offered to the woman:

1. Clinical evaluation and WHO clinical staging. Although not mandatory, when available, women can have their CD4+ cells count done.
2. Initiation on ART if she was not yet on ART before she became pregnant. Details of ART regimens have been discussed above.
3. Safe obstetric practices.
4. Psychosocial support for HIV-infected pregnant woman including healthy living, care of HEI (infant feeding).

All HIV-positive pregnant women who are WHO clinical stage 3 or 4, or have CD4+<350 cells/ml are initiated on cotrimoxazole [54, 67].

For HIV-positive women, during the six-week postpartum visit by the mother-infant, the health care workers collect a DBS sample for PCR testing to determine the child's HIV status.

The WHO recommends that evaluations measure transmission at 6 weeks of age to indicate intrauterine and peripartum transmission, and at 18 months of age to indicate breastfeeding-associated transmission [70].

In summary, Lesotho PMTCT guidelines recommend rapid initiation of HIV-positive women on ART irrespective of their CD4+ cell count or WHO clinical staging. The Lesotho PMTCT guidelines recommend the following treatment options:

(a) First-line: tenofovir, lamivudine and dolutegravir [TLD]. The alternate regimen is tenofovir, lamivudine, and efavirenz [TLE] or zidovudine, lamivudine and efavirenz.

(b) Second-line regimen: zidovudine, lamivudine, and lopinavir boosted with ritonavir. The alternate regimen is tenofovir, lamivudine, and dolutegravir or lopinavir booted with ritonavir

(c) Third line: TLD with darunavir boosted with ritonavir [67, 68].

Treatment success is measured by VL testing. VL testing in Lesotho is done using the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 test quantitative test (v2.0) for which the limit of detectability is 20 copies/ml [54, 71-74].

1.10 **HIV testing and counselling**

HIV testing and counselling constitutes the entry point for all pregnant women into the HIV care services cascade [53, 68, 75, 76]. Irrespective of where the clients take an HIV test (facility or community), it is important to link them to appropriate HIV care services if they are found to be HIV-positive. Currently in Lesotho, patients are offered group pre-test information, then those who accept to undergo HIV testing are offered the test and those who are found to be HIV-positive are offered additional counselling with focus on positive health, dignity and prevention (PHDP) [75, 77, 78].

In PMTCT, Lesotho introduced routine HIV testing for pregnant women in 2005, and in 2010, the country chose the "opt out" strategy in which all clients presenting in ANC are offered HIV testing as part of package of services unless they explicitly decline to be tested. This has increased HIV testing with more than 97% of women in Lesotho accepting to be tested for HIV in ANC [68, 79-81]. Currently, the first test performed is the Determine™ HIV-1/2. Participants with a negative Determine assay are told their HIV-negative status. All participants who test positive will undergo a second test (with a new finger prick sample) using Uni-Gold™ Recombigen® HIV-1/2 and will be told their HIV-positive status if the second test is also positive [54, 68, 75, 82-85]. Those who test HIV-positive by the two initial tests will have a confirmatory test before being initiated on ART [82-85]. Figure 15 shows steps from HIV testing to linkage to treatment in Lesotho [68, 75].

1.11 **Postnatal care for HEI in Lesotho and monitoring of HIV-positive women**

Infants born to known HIV-positive mothers are identified as being HIV-exposed on the Child Health Card that is given to the mother during delivery. In the event that an infant or child attending the under-5 clinic does not have a known HIV exposure status, the national PMTCT guidelines recommend that the mother undergoes HIV testing to determine the

infant's exposure status. Should the mother decline HIV testing, the infant should be tested for HIV antibodies to determine HIV exposure status. The revised 2013 guidelines recommend that all HEI be given NVP from birth or as soon as possible after birth for six weeks. In addition, HEI are given Cotrimoxazole from four to six weeks of age until HIV infection has been definitively excluded [54].

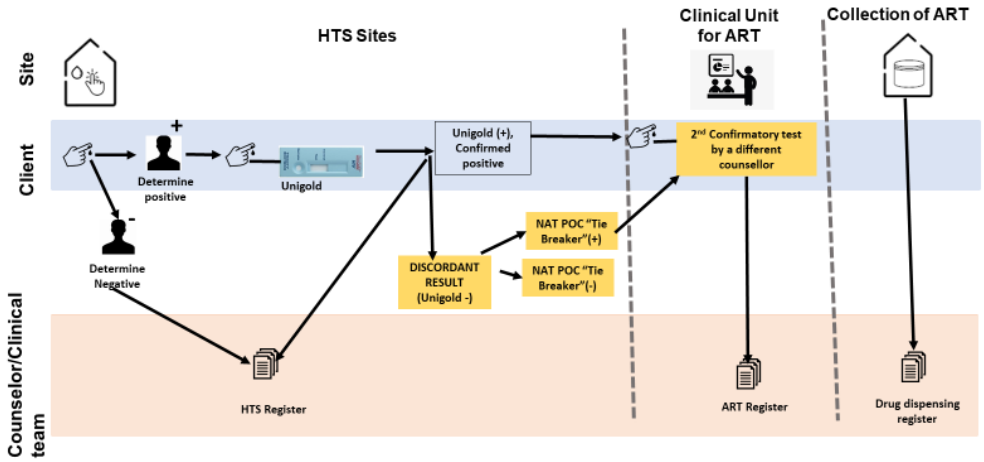


Figure 15. HIV testing and linkage to ART (Adapted from [68] and [75]).

Exclusion of HIV infection among infants requires direct viral testing, as HIV antibody testing by rapid tests is positive among HEI for a duration of time following birth due to the presence of maternal antibodies [54, 85, 86]. DNA PCR testing is recommended for use among infants less than nine months of age in Lesotho. HEI are tested for HIV by DNAPCR at 6 weeks and 14 weeks; and HIV antibody testing at 9 months and 18 months [54, 85-90]. Infants with a positive HIV test result are initiated on ART along while also undergoing a confirmatory HIV DNA PCR testing to confirm the initial positive result [54, 68, 89-92]. Final documentation of the infant's HIV status is conducted six weeks following cessation of breastfeeding using HIV DNA PCR testing among infants below nine months of age, or HIV rapid testing among infants above nine months of age [89, 90]. Children who test HIV-positive with rapid test undergo blood sample collection for HIV DNA PCR testing during the same visit.

The schedule of services for HEI also includes routine medical care such as growth monitoring, administration of childhood immunizations, and de-worming. Follow-up visits are recommended for all children at 7 days, 6 weeks, 10 weeks, 14 weeks, 6 months, 9 months, 12 months, and 18 months. In addition, HEI are expected to be followed on a monthly basis until 12 months of age, and every 2-3 months after the age of 12 months until final HIV status has been verified 6 weeks following breastfeeding cessation [54]. In addition, the EID cascade follows a complex pathway that may have bearing on the retention of HEI in care [54, 92-94]. The cascade is summarized in Figure 16 [95].

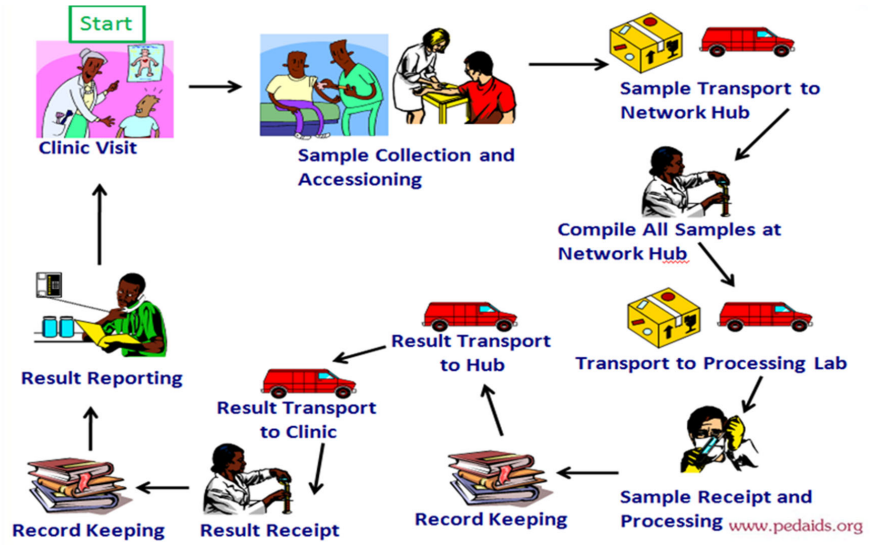


Figure 16. Step-by-step DNA PCR testing in Lesotho [95]

In December 2016, Lesotho launched the point-of-care (POC) EID with outstanding impact on rapid turnaround time of results and early initiation of HIV-infected children on treatment [96-100].

1.12 **Rationale and focus of the studies**

For more than 35 years, HIV has been a public health challenge and MTCT is the cause of more than 95% of pediatric HIV in the world [101-103]. In Lesotho, despite all the efforts, MTCT of HIV-1 contributes an important share of new cases of HIV. Of the 13,000 new HIV infections in Lesotho in 2018, it is estimated that 10% were from MTCT [26, 27, 55, 104]. The Lesotho PMTCT program has been fully scaled up for more than ten years but has not yet reached elimination of MTCT of HIV-1 [104, 105]. While Lesotho has been forward thinking in developing policies and guidelines in line with international standards, there is a need for a continued collaboration between the health system and the community to facilitate access for pregnant women to effective care with optimal results. For example, any obstruction in the EID cascade will impact negatively in timely management of HIV-infected children ultimately leading to high mortality [53, 104, 106, 107]. As recently as 2012, collected dry blood spot (DBS) samples for EID from six to eight weeks old, HEI had to be transported from the health facility to the district laboratory and then to the central laboratory in Lesotho or outside the country. Once processed, test results had to be delivered to the district laboratory and then physically returned back to health facility making the full process quite complex. A detailed analysis of the pathway of EID using the traditional approach of collecting blood through the peripheral facilities and transporting them to a central laboratory and returning the results is important. This is important because we have to identify the bottlenecks in the health system and them in order to effectively and efficiently optimize EID cascade. Furthermore, there has been a lot of focus on the HIV transmission and treatment of HIV-infected children. A lot of progress has been made in this regard, and countries like South Africa are offering HIV testing for infants at birth and immediate initiation of HIV-infected infants on ART [108-111]. However, based on emerging data assessing the impact of HIV exposure in utero on HEI in term of birth outcomes, there is need for continuous generation of evidence especially for HIV-exposed children in the era of lifelong therapy [112-116]. It is important to elucidate whether the birth outcomes and early infant survival is different between HEI and HUI. Where feasible,

it will be ideal to further explore if HIV exposure increases the developmental risk, morbidity and mortality to HEI compared to HUI. In addition, studies have reported variable treatment suppression for HIV-positive women on ART and have cited HIV viremia during pregnancy, intrapartum and during breastfeeding as one of the key factors contributing the MTCT [117-119]. After introduction of lifelong ART (Option B+), and the VL monitoring of HIV-positive pregnant and breastfeeding women in Lesotho, the quality of care for this important priority population has continued to improve. There are remaining gaps that go beyond the use of ART for treatment success: women's adherence has to be optimal and they have to be retained in care. One of the WHO-recommended methods for measuring PMTCT program effectiveness is the conduct of household surveys to document estimated transmission rates, HIV prevalence among children, and HIV-free survival [120, 121]. While literature the implementation Option B+ is limited, findings from Malawi suggest that women are more likely to be LTFU when starting on ART under Option B+ versus starting on ART for their own health [122, 123]. Household surveys from a three-country study found that 58.7% of breastfeeding women who did not know their HIV-positive status had VL levels above the suppression threshold of 1,000 copies/mL, increasing the likelihood of MTCT. Over half of these women had sought facility care during their pregnancy, tested HIV-negative in ANC, and had since sero-converted [124]. This incidental finding of HIV-positive pregnant and breastfeeding women poses a greater risk of HIV transmission to children thereby increasing the risk of HIV-related morbidity and mortality. Therefore, community household surveys may be a good approach to assess the effectiveness of PMTCT services in a holistic way.

Several studies in Lesotho have focused on the VL monitoring in the general adult population or among children, and have reported results which may not necessarily be applicable to pregnant and breastfeeding women [125-127]. There is need to evaluate VL monitoring of pregnant and breastfeeding women in the context of lifelong therapy.

2.0 STUDY AIM AND OBJECTIVES

2.1 Aim

The overall aim of this research was to assess the effectiveness of PMTCT approaches in resource-limited settings with focus on Lesotho and the effectiveness of the early infant cascade, outcomes of HEI in the context of lifelong ART and to assess treatment success of HIV-infected pregnant and breastfeeding women as measured by viral suppression.

2.2 Specific objectives

1. To identify delays in the EID process from the time HIV specimens are collected at six to eight weeks of age to when caregivers receive the results and HIV-infected infants are initiated on ART at study sites (Paper I).
2. To determine the effect of roll-out of lifelong ART on birth outcomes and the survival of infants born to HIV-positive mothers compared with a similar cohort of HIV-negative mothers and their HUI (Paper II).
3. To estimate MTCT rates, mortality, HIV prevalence, and HIV-free survival among children through a community-based survey among households with children born in the previous 18-24 months (Paper III).

3.0 SUBJECTS AND METHODS

This thesis is based on three different studies, all focusing on the remaining challenges in the PMTCT program after the introduction of lifelong ART for all HIV-infected individuals.

1. Paper I focuses on *early infant diagnosis* as a key step to measure PMTCT program performance.
2. Paper II focuses on the *birth outcomes of HEIs* compared to HUIs.
3. Paper III focuses on *overall transmission rates and mortality in children 18-24 months* old.

3.1 Study designs and procedures

An overview of the methodological aspects of each study is provided in Table 8. Below we present the designs and the procedures for each paper.

Paper I. Turnaround time for EID samples

We used a retrospective cohort design to analyze secondary data from health facility records using a step-by-step review of the pathway of samples for EID in order to understand the bottlenecks and calculate turnaround time (TAT) – the time from when the sample is drawn to when the results reach the mother.

We studied samples from HEI at six to eight weeks of age who had a DBS sample taken for a DNA PCR test at the selected study sites between January 1st, 2011 and December 31st, 2011, as identified in the EID laboratory records and by their mothers. In addition to the EID laboratory records, data on study women and infants were captured from routine PMTCT registers. A total of 1187 HEI records were reviewed for demographic characteristics including age, and sex of the child, use of ARVs for PMTCT, and maternal demographics. Then, these data were linked to mothers' PMTCT records by their ANC identification number to capture demographics and PMTCT-related information, including ARV regimen received.

Table 9. Summary of the methods used in the different papers.

Paper	Paper I	Paper II	Paper III
Objective	To describe the EID process and identify the barriers and delays in the pathway	To compare birth outcomes and six-week HIV-free survival of HEI with that of other infants	To assess the effectiveness of Option B+ approximately two years after implementation
Type	Quantitative study	Quantitative study	Quantitative study
Design	Retrospective cohort	Observational prospective cohort	Cross-sectional community-based survey
Coverage	All 10 districts	3 districts	4 districts
No of health facilities	25 health facilities	14 health facilities	The catchment areas of 25 health facilities
Study population	Files/clinical records of infants	HIV-negative and HIV-positive pregnant women, HIV exposed and HIV unexposed infants	HEI and HUI born in the previous 18-24 months and their mothers/caregivers
Sample size	n=1,187 samples from HEI	n=941 HIV-negative mother-infant pairs n=653 HIV-positive mother-infant pairs	n=1,852 mothers/caregivers; n=1,884 children
Data collection	Abstraction of clinical records from the electronic database	Interviews with participants and abstraction of clinical records from the electronic database	Interviews with participants and abstraction of clinical records and HIV test results from the electronic database
Time of data collection	Data from Jan 1 - Dec 31, 2011 collected in March-April 2012	Enrollment from Jun. 2014 – Feb. 2016 and follow up through pregnancy, delivery and six weeks postpartum	Data collected from Nov 2015 – Dec 2016
Data analysis	*Exploratory data analysis*: Descriptive statistics numerical and graphical display of important features of the data	*HIV-free survival* estimated as the proportion of children alive and HIV-negative among all exposed children. *Kaplan Meier* curves were used to graphically display infant mortality, infection, and HIV free survival	*HIV-free survival* estimated as the proportion of children alive and HIV-negative among all HIV-exposed children. *Log-rank test* used to determine differences between subgroups *Multivariate logistic regression* models to test the independent associations

Information about initiation of HEI on NVP suspension was extracted from improvised registers. The database and registers were checked against each other throughout data collection as needed. We entered abstracted data into a paper-based standardized data collection tool. Once records had been matched, a study identification number was created using a code for the site and a non-identifiable numeric code that was not linked to the original patient number. The link between the patient identification number and the study identification number was recorded in a study enrollment log. The log and the completed data collection forms were kept at the facility in a locked location with controlled access. Data from the paper-based tool were entered into a password-protected database using the generated study identification number. As the principal investigator, I checked all data for completeness.

Paper II. Six-week PMTCT outcomes for HEI and HUI

We conducted a prospective observational cohort study with the aim to assess the effectiveness of the Lesotho MOH PMTCT guidelines to reach the goal of the virtual elimination of new pediatric HIV infections in selected sites in Lesotho as measured by HIV transmission rates and HIV-free survival among HEI.

In this evaluation, we followed HIV-positive and HIV-negative pregnant women attending ANC during pregnancy and breastfeeding period and with their infants from June 2014 to February 2016. The mother-infant pairs were scheduled to come at least every three months for HIV-positive mothers and at least every 3-6 months among HIV-negative mothers and outcome for this analysis done at 6 weeks. HIV testing in HEI was conducted at four to six weeks of age, to assess if there had been any HIV transmission around birth. This provided an estimate of how effective the PMTCT program is at preventing HIV acquisition during pregnancy, delivery, and in the early postpartum period and whether there was any difference in birth outcomes between HEI and HUI. It allowed for determination of HIV-free survival among HEI.

The study population was pregnant women who attended ANC at a study facility, resided in the facility catchment area, and were willing to provide informed consent. We excluded pregnant women who were temporarily attending care at the study facility.

We used sampling proportional to population to estimate the target number of participants at each facility. All pregnant women attending ANC were eligible for study enrollment, either into the HIV-positive cohort or the HIV-negative cohort. Informed consent was obtained from all participants using an informed consent document translated into the local language (Sesotho). Consent included permission to follow the participant in the community for study follow-up, including home visits and phone calls or SMS messages to check on the mother and baby (i.e., in-between visits) or if the mother-infant pair did not return for scheduled routine (or study) health care visits. Phone calls and SMS messages were made to a phone number of their choice, either shared or individually owned. Only neutral SMS messages were sent, without reference to HIV. Lack of access to a phone did not prohibit women from participating in the study. Participants were consecutively enrolled until the predetermined sample size is reached.

Through interviews with the participants and extraction of information from clinical records during pregnancy, birth and six weeks postpartum, we collected socio-demographic data and medical information.

We entered abstracted data directly onto tablets. We used a range of source documents: Maternal bukana (health booklet) and infant health cards; ANC registers; delivery registers; under-five registers; laboratory results registers; ART registers and pharmacy registers/dispensing logs. We recorded the following: enrollment site, unique study identification number and mother/infant register numbers, and mother's ANC number as patient identifiers in a study enrollment log and database to allow for linkage of patient-level data across routinely used registers, logs, and forms. We entered all data on tablets by direct data entry into a study-specific database and stored on a secure, web-based server. Built-in data checks ensured that data were within a feasible range. Any out of range values were verified by looking through the source document. We reviewed all collected data for

completion. All electronic tablets were password protected, with access to only key study personnel. Tablets were maintained in a locked cabinet within a locked room when not in use by the appointed by study personnel, separate from any locator information. As the principal investigator, I carried out regular data collection monitoring and reviewed all procedures on site.

As part of this study, we collected DBS from infants by heel prick, and spotted directly onto filter paper for HIV DNA PCR testing. We also collected blood samples (finger prick or venipuncture) of pregnant and breastfeeding women as per routine clinical practices that were part of the standard of care. All used lancets/needles were placed in a designated, labeled biohazards container that is approved for this use for disposal per routine procedures. All laboratory tests were processed per routine Lesotho MOH protocols for HIV antibody testing, HIV DNA PCR and HIV VL. These included infant diagnosis using the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 qualitative test (v2.0) and VL measurement with the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 test quantitative test (v2.0).

Paper III. HIV-free survival at 18-24 months of age

We conducted cross-sectional community-based household survey with the aim to assess the population-based effectiveness of the Lesotho MOH PMTCT program to reach the goal of the virtual elimination of new pediatric HIV infections as measured by HIV transmission rates and HIV-free survival among HEI in selected districts. Paper III describes estimated MTCT rates, mortality, HIV prevalence, and HIV-free survival among children aged 18-24 months.

In this evaluation, we determined mortality and HIV infection as outcomes of the PMTCT program among HEI aged 18-24 months, who were born after the introduction of universal, lifelong ART for pregnant and breastfeeding women. This helped us to understand the current HIV mortality and infection rates among HEI at the near end or after completion

of all breastfeeding. The community-based approach was utilized, as this age group is less likely to be seen at health facilities compared to younger infants.

We selected the study population using a multi-stage process beginning with the selection of study facility catchment areas, followed by the selection of study communities, households, and finally study participants, Figure 17.

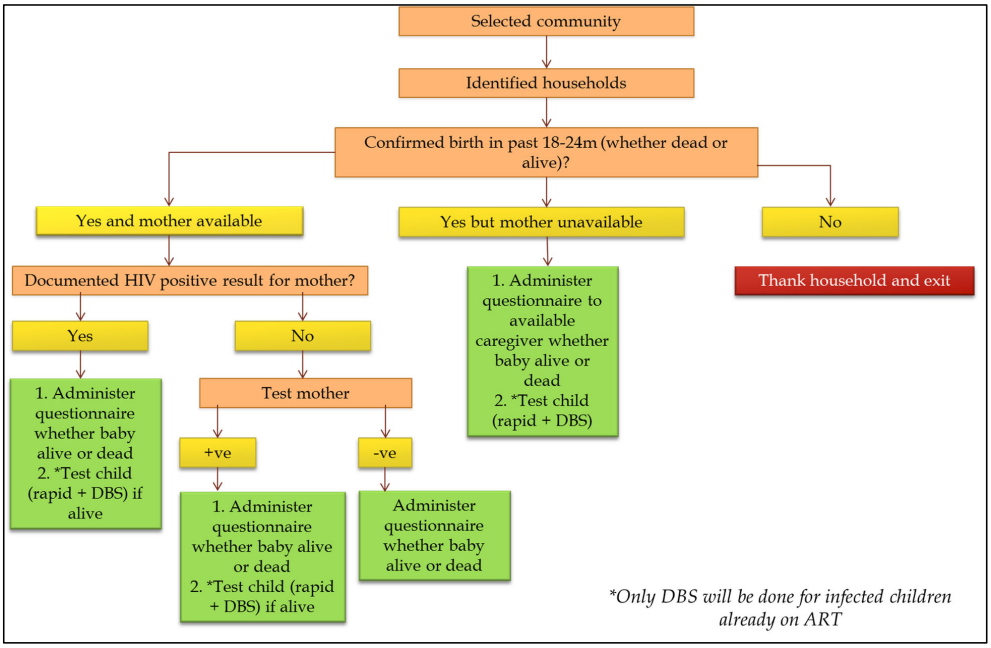


Figure 17. Paper III study flow chart.

In this study, we included mothers/caregivers and their infants aged 18-24 months and collected data from November 2015 and December 2016. We interviewed the mothers/caregivers in their language of choice using a structured data collection tool loaded on an electronic device with direct electronic data capture/entry. Once the child's HIV exposure status was determined, the appropriate study activities was carried out. For women with documented HIV-negative status only the questionnaire was completed. More detailed information was collected for women determined to be HIV-positive or who had

unknown HIV status. We collected data about demographics, use of health facilities for maternal and child health (MCH) and HIV services, maternal HIV status, maternal and infant receipt and use of ARVs during pregnancy and after delivery, infant feeding practices, and general well-being including growth and development. Information from the child health card and/or maternal health card was reviewed if available. The electronic devices for data collection had built-in data checks to ensure that data were within a feasible range. Data were stored temporarily on the device and regularly uploaded to the web-based server using a mobile internet connection. We reviewed all collected data for completion.

3.2 Study sites and description of the geographical setting

In Paper I, we included 25 sites from all the 10 districts. Eleven of the sites were defined as hard-to-reach with long TATs in the range of six to eight weeks and they were from six districts: Butha Buthe, Qacha's Nek, Thaba Tseka, Mokhotlong, Maseru, and Quthing. An additional 14 sites were selected representing all 10 districts, the majority with shorter TATs of two to four weeks. The total number of sites comprised approximately 10% of the Foundation's PMTCT coverage in the country.

Table 10. Study Facilities for Paper II

District	Health Facility Name	Facility Type (Government versus CHAL)	Patient Volume
Butha Buthe (Foothills)	Butha Buthe Hospital	Government	High
	Seboche Hospital	CHAL	High
	St Paul's Health Center	CHAL	High
	St Peters Health Center	CHAL	High
Mohale's Hoek (Lowlands)	Bethel Health Center	CHAL	Medium
	Holy Cross Health Center	CHAL	Medium
	Mootsinyane Health Center	Government	Medium
	Ntsekhe Hospital	Government	High
	Tsepo Health Center	Government	High
	Mofumali ha Rosalia	CHAL	Medium
Thaba Tseka (Highlands)	Lephoi Health Center	Government	Medium
	Paray Hospital	CHAL	High
	St. James Hospital	CHAL	High
	St Theresa Health Center	CHAL	High

The data for paper II were collected in 3 of the 10 districts of Lesotho, namely Butha Buthe, Mohale's Hoek, and Thaba Tseka. These districts were selected to ensure representation of PMTCT service delivery coverage, and heterogeneity in health-seeking behavior due to variances in terrain. We included all five hospitals in these districts and randomly selected nine medium volume health centers, Table 10.

For paper III, 10 facilities from Maseru District and 5 facilities each from the districts of Butha-Buthe, Mohale's Hoek and Thaba-Tseka were randomly selected, a total of 25 facilities, Table 11.

The four selected districts (Butha Buthe, Maseru, Mohale's Hoek and Thaba Tseka) are representative of the variation in overall PMTCT service delivery coverage, and the heterogeneity in health care seeking behavior due to the varying terrain. The seven hospitals from the four districts included in the study covered a representative part of the health system. With a total population 940,000, the four districts account for nearly half of the total population of Lesotho. Between the four districts, there is a large variation of daily activities for a living. While in the mountainous districts of Thaba Tseka and Mohale's Hoek the population are farmers, in the lowlands they tend to work in the textile factories. Health centers were classified into high, medium and low volume sites based on 2012 ANC attendance numbers. Low volume sites were excluded from the study because of the difficulty of recruiting enough women within the enrollment period.

3.3 **Sample size, study outcomes and statistical analysis**

In paper I, the sample size for this study was based on feasibility and convenience since the aim was to describe the current EID process and MTCT infection rates and no intervention was introduced. From the national routinely collected baseline data, the available sample of infants who received EID HIV testing around six weeks of age in one year at the study sites was estimated to be approximately 2,675. The key outcomes were (a) proportion of HEI caretakers who received DNA PCR results within two, four, six, or

eight weeks or more; (b) Mean TAT from the time the DNA PCR sample is taken to the time the caregiver receives the test result among those who receive results; and (c) proportion of HIV-infected infants initiated on ART.

Table 11. Selected study facilities by district for paper III

District	Selected facilities	Projected no of live births	Expected no of exposed infants	Distribution, among facilities of targeted sample size
Maseru	Likotsi Filter Clinic	923	235	52
	Qoaling Filter Clinic	1438	366	80
	Domiciliary HC	281	72	16
	St. Leo HC	337	86	19
	Thaba-Bosiu HC	356	91	20
	St Bernard HC	262	67	15
	St Joseph Hospital	704	179	39
	Koro-koro HC	168	43	9
	St Barnabas HC	464	118	26
	St Rodrique HC	244	62	14
Botha-Bothe	Botha-Bothe Hospital	307	78	17
	St Paul HC	292	75	17
	Ngoajane HC	141	36	8
	Tsime HC	197	51	11
	Linakeng HC	573	146	32
Mohale's Hoek	Ntšekhe Hospital	807	205	45
	Mofumahali-oa-Rosari HC	385	98	22
	Ha Tšepo HC	380	97	21
	Morifi HC	275	70	15
	Mpharane HC	480	122	27
Thaba Tseka	Thaba-Tseka HC	197	51	11
	St James Hospital	702	179	39
	Montmartre HC	275	70	15
	Mohlanapeng HC	249	64	14
	St Theresa HC	281	72	16
Total		10718	2733	600

We used SAS (SAS Institute, Cary, North Carolina, USA) for data analysis. Exploratory data analysis was done in order to allow us highlight general features of the data to direct future analyses and identify problem areas in the data. The average – geometric mean (95%

CI) – time intervals (in days) were stratified by relevant independent variables. These were calculated with linear mixed models with the assumption of a compound symmetry working correlation structure to account for the clustering of women in facilities in order to determine the stages with the longest days (see Paper I for details).

For paper II, we estimated the *in-utero* and peripartum HIV transmission rate to be approximately 4% at six to eight weeks under Option A based on our analysis of EID test results. As most HEI in Lesotho are breastfed, without ongoing prophylaxis throughout the breastfeeding period and depending on the type and timing of access to ARVs or even maternal HIV seroconversion during breastfeeding, an additional 0.16-1.57% of infants would acquire HIV infection per month during ongoing exposure to HIV through breastmilk [123]. At the time of the Option A implementation, the 2012 reported estimate of prophylaxis uptake during breastfeeding among mother-infant pairs was 19%, and approximately 45% of women who were eligible for treatment for their own health and were thought to have initiated ART in 2011. We used this to determine a range of transmission estimates and the required sample size [101, 123]. This sample size was derived to provide an estimate of the transmission rate across all three districts. Based on the number of pregnancies, HIV prevalence, and number of infants in the proposed districts, meeting the enrollment was considered feasible within approximately 12 months for the prospective cohorts. The estimated sample size was determined for the cohorts of HIV-positive and HIV-negative women based on the sample size necessary to measure the effect of the intervention on the primary endpoints. The sample selected was meant to allow us to determine the effect of the intervention with 80% power at 0.05 significance level, including adjustment for a design effect of 1.5 and a study LTFU of 20% (nQuery Advisor 4.0).

We enrolled 653 HIV-positive women and 941 HIV-negative women. Outcomes included: (a) birth outcomes including maturity, birth weight, and gross birth defects; (b) mortality; (c) HIV transmission; and (d) HIV-free survival at six weeks. For paper II we used Stata (StataCorp LLC, College Station, Texas, USA) for analysis. Categorical variables were

summarized using frequencies and percentages of participants while continuous variables using means (+/- standard deviation). We stratified maternal baseline characteristics based on HIV status at the time of enrollment into the study. Birth outcomes between HEI and HUI were compared. We estimated HIV-free survival as the proportion of children alive and HIV-negative among all HEI. The precision around survival estimates was assessed by 95% confidence intervals. Kaplan Meier curves were used to graphically display infant mortality, infection, and HIV free survival. For paper II specifically, the infection rate was calculated as the proportion of infants with a positive HIV test result by six weeks among HEI. Confidence intervals were estimated for both infection proportion and infection rate.

For paper III HIV-free survival among 9-24-month-old infants was estimated to be 91.9% with a precision of 1.4%. Assuming HIV-free survival among 18-24-month-old HIV-exposed infants was lower than the estimate from Rwanda, due to less extensive PMTCT coverage in Lesotho in comparison to Rwanda, in order to determine HIV-free survival with 3% precision around the point estimate, we needed to identify 545 HEI [124]. Adjusting the sample size by 10% for miscellaneous events (e.g., missing data, consent to some but not all study activities) we estimated a total sample size target of 600 HEI. Given the HIV prevalence of approximately 25.4% among pregnant women in ANC, we would need to visit or approach 2,363 households of infants or mother-infant pairs within the 18-24-month age range to identify 600 HEI. To account for approximately 15% refusals to participate in this component of the study and interviews with non-maternal caregivers of HIV-exposed children (who may not be able to adequately answer questions needed to address the research aims) we needed to visit approximately 2,700 households to reach the required sample of HEI and their mothers. We used Stata (StataCorp LLC, College Station, Texas, USA) for analysis. Categorical variables summarized using frequencies and percentages; continuous variables were summarized using means (standard deviations), or medians (interquartile ranges) as appropriate. HIV-free survival was estimated as the proportion of children alive and HIV-negative among all HIV-exposed children with a precision of 95% confidence interval. Log-rank test was used to determine whether there

were differences in HIV-free survival between subgroups. Multivariate logistic regression models to test the independent association of ANC attendance, mode and place of delivery, gestational age at birth, birth weight, maternal vital status, adherence to and timing of initiation of antiretroviral drugs, infant feeding method and nutritional status with HIV-free survival among HIV-exposed children.

3.4 Ethical considerations

Ethical approval was sought and obtained from the Lesotho Ministry of Health Research and Ethics Committee (REC), the Baylor College of Medicine Children's Foundation Lesotho Institutional Review Board (IRB), the George Washington University Committee on Human Research IRB and the Regional Committees for Medical and Health Research Ethics in Norway.

Waiver of consent was obtained for abstraction of data that contributed to paper I, and written consent was obtained from participants who participated in the studies for paper II and some data for paper III.

4.0 SUMMARY OF RESULTS

4.1 Paper I Turnaround time for samples for EID

Out of the 1187 HEI, 92.8% of the mothers had ART for PMTCT or treatment and at the time of review, the maternal mortality was 6.9% (82/1187). The mean infant age at the time of the sample was taken for DBS was 46.9 (±3.4) days. Of the 47 (3.9%) HIV-infected children, 36 (76.6%) were initiated on ART. The children were initiated on ART at an average of 1.3 days (95%CI: 0.3–5.7; range: 10–56) after receiving results. Considering the fact that some of the results were communicated by phone, some children were initiated on treatment before the parents physically received the results. The mortality rate among children at the time of review was 1.4% (17/1187) with the status of children who died not known.

The mean total turnaround time was 61.7 days (95%CI: 55.3– 68.7), Figure 18. The longest time spent by the specimen and results occurred at stage 3, which is the time from receipt of specimen at the central laboratory to receipt of results at district hospital, and it was 23.2 days (95% CI: 18.7–28.9). The average stage 1 and stage 3 time-intervals were significantly shorter in the Lowlands Region (0.9 and 16.2 days), compared to Highlands Region (6 days [P = 0.03] and 34.3 days [P < 0.01] (See Figure 19 below). The mean turnaround time was 47.4 (95% CI: 39.0–57.7) and 62.4 (95% CI: 55.6–69.9) for HIV-infected and HIV-uninfected children, respectively. The mean (95% CI) turnaround time (in days) calculated from a linear mixed model including test result, region and facility level, was found to be significantly shorter for HEI compared to HUI

($p<0.01$).

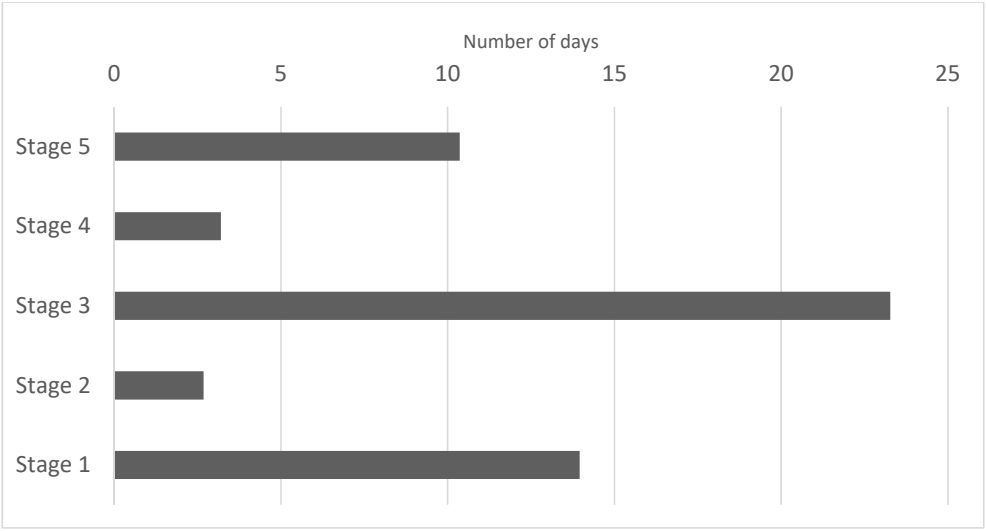


Figure 18. Mean turnaround time (TAT) in days per stage

- Stage 1 (bottom of the figure): Time from specimen collection to transfer to the district laboratory: 14.0 days (95% CI: 12.1- 16.1)
- Stage 2: Time from specimen transfer from the district laboratory to the central laboratory for testing: 2.0 days (95% CI: 1.5-4.9)
- Stage 3: Time from receipt of specimens at the central laboratory to the time results transferred from central laboratory to receipt of results at district hospital: 23.2 days (95% CI: 18.7-28.9)
- Stage 4: Time from receipt of results at the district hospital to receipt at health facility: 3.3 days (95% CI: 1.9-5.5)
- Stage 5: Time from receipt of results at health facility to results receipt by caregiver: 10.4 days (95% CI: 7.9-13.5)
- Total time: 61.7 days (95% CI: 55.3, 68.7)

4.2 Paper II 6-week PMTCT outcomes for HIV-exposed and HIV-unexposed infants

Of the 653 HIV-positive women enrolled, 623 HIV-positive women gave birth to 631 HEI while 868 HIV-negative women of the 941 HIV-negative pregnant women enrolled gave birth to 879 HUI, Figure 19. HIV-positive women were significantly older than HIV-negative women with a mean age of 28.7 (+/- 5.5) compared to 24.4 (+/- 5.7) years and they presented for ANC earlier than the HIV-negative women at a mean gestational age of

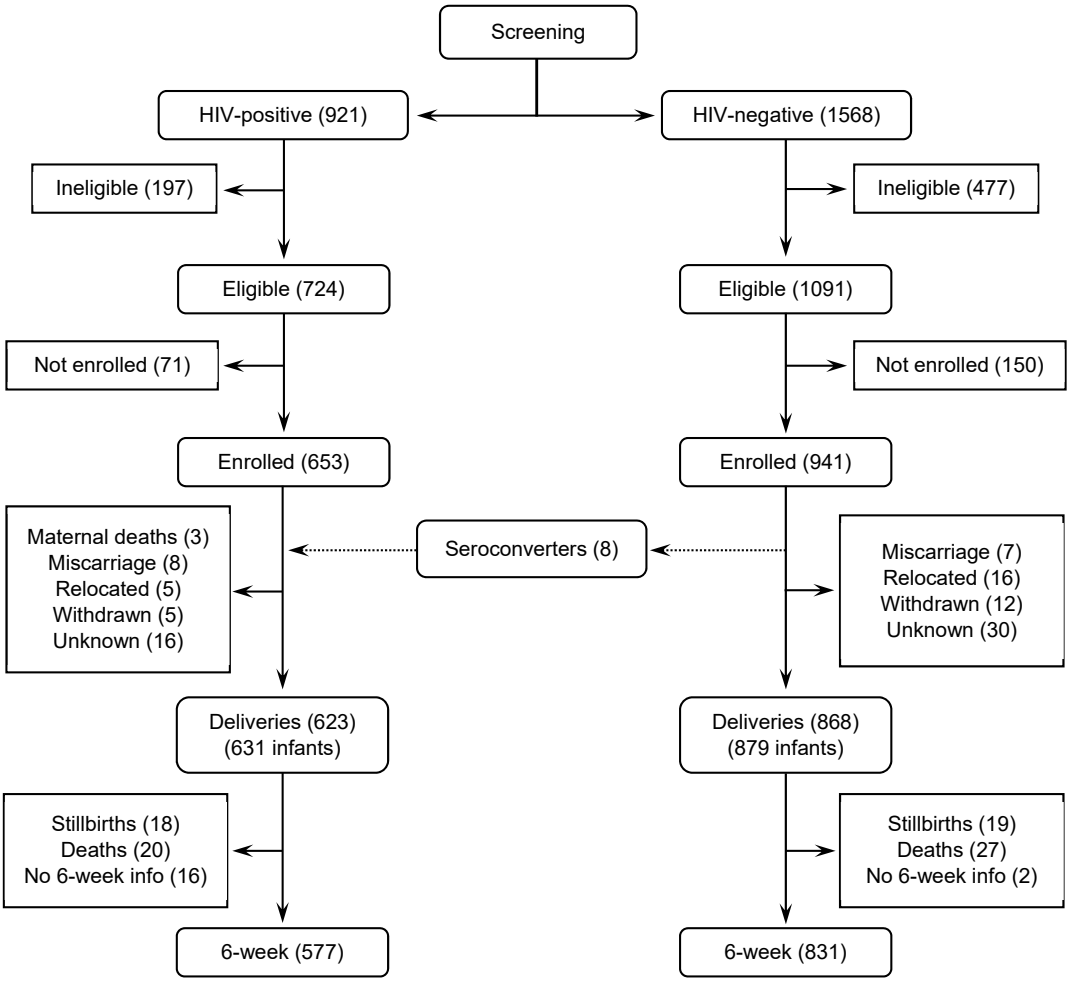


Figure 19. Study profile for Paper II.

23.0 (+/- 8.7) weeks compared to 25.3 (+/- 8.2) weeks. 97.8% of HIV-positive women were receiving ART at enrollment 40.2% of whom initiated ART before conception and 59.8% initiated ART after conception.

Concerning birth outcomes, 91.6% of study women delivered in a health facility with 96.8% of infants born alive. Although not statistically different, HIV-positive women were more likely to have had a macerated stillbirth, compared to HIV-negative women who were more likely to have had an intrapartum death. The risk of a preterm birth was twice as high among HEI compared to HUI (7.8% vs. 3.6%) while the risk of very preterm birth (gestational age <32 weeks) was considerably higher among HEI compared to HIU (2.2% vs 0.4%). Low birthweight (<2.5 kg) was 11.6% among women who initiated ART before conception compared to 11.9% among women who initiated ART after conception. Furthermore, very low birthweight (<1.5 kg) was 1.7% among women who initiated ART before conception compared to 1.2% among women who initiated ART after conception.

The estimated survival rates were 94.8% (95% CI: 93.1–96.1) among HUI and 94.0% (95% CI: 91.8–95.7) among HEI when including stillbirths as deaths. There was no difference in survival rates at 6 weeks of age by infant HIV exposure status. When considering postnatal deaths alone (excluding stillbirths), the estimated survival rates were 96.8% (95% CI: 95.4–97.9) and 96.7% (95% CI: 95.0–98.0) for HUI and HEI, respectively. Adjusting for maternal mortality and gestational age at first ANC visit, infant HIV exposure status was not associated with early infant mortality (aOR = 1.06, 95% CI: 0.56–1.99).

Focusing on HEI, the estimated HIV transmission rate among those tested at birth was 0.9% (95% CI: 0.25–2.36) and by six weeks the overall HIV transmission was 1.0% (95% CI: 0.38–2.23). The estimated HIV-free survival including stillbirths was 92.8% (95% CI: 90.5–94.8), and 95.6% (95% CI: 93.7–97.1) when stillbirths were excluded. At six weeks, the mortality rate was much higher among preterm babies, 24.6% (17/69) compared to term infants 2.1% (29/1391). The mortality at six to eight weeks was much higher among low birthweight infants compared to normal birth weight infants (7.0% vs. 1.9%). Very low birthweight infants had higher risk of death within six to eight weeks compared to infants with a birthweight of at least ≥1.5 kg (30% vs. 2.2%).

4.3 Paper III HIV-free survival at 18-24 months of age

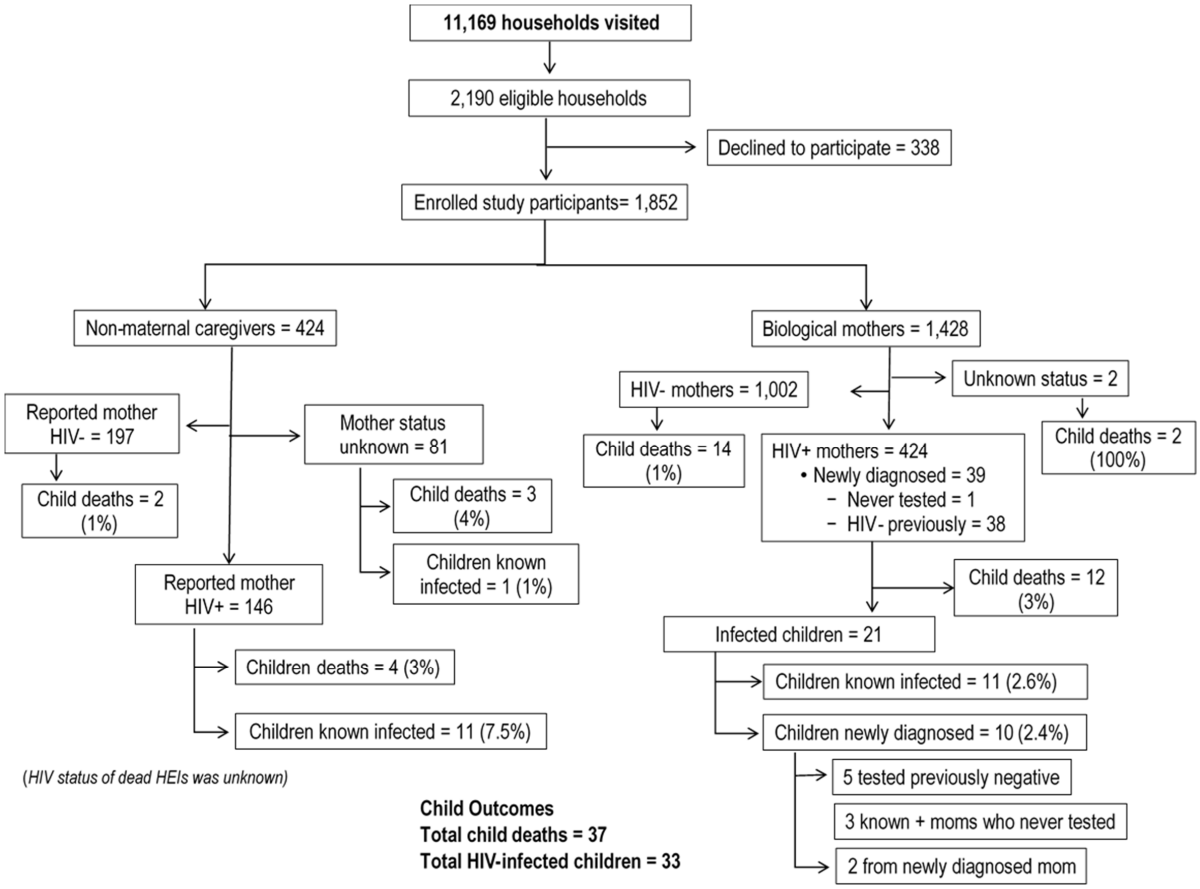


Figure 20. Study profile for Paper III.

Of the 11,169 households screened, 2,190 were eligible in which 1,852 mothers/caregivers consented to participate in the study. There were 1,882 deliveries including 64 twin births in the households which met the inclusion criteria (born 18-24 months prior to the time of data collection), Figure 19. Of the biological mothers, 424 were known to be HIV-positive while 39 additional women were diagnosed as HIV positive during the study of whom 38 had tested HIV negative during pregnancy. Overall, 95.8% and 80% of mothers had attended at least one ANC visit and had delivered in a health facility, respectively. Of the

HIV-positive women, 94.7% had disclosed their HIV status to someone and 59.9% had disclosed to their sexual partner. Note: Of the 338 households that declined to participate, the following reasons were provided: caregiver, child or both were unavailable (n=178), (157 of whom were at work); lack of time to participate (n=71); no incentive provided (n=25); not comfortable to participate (n=16); caregiver did not feel they had enough information to answer questions (n=15); mother needed husband's permission (n=9); caregiver was below 18 years of age (n=6); and other reasons (n=18).

The estimated HIV transmission was 5.7% (95% CI: 4.0-8.0). The estimated child mortality was 2.6% (95% CI: 1.6-4.2) among HIV-exposed children compared to 1.4% (95% CI: 0.9 – 2.3) among HIV-unexposed children. The majority of child deaths occurred after the neonatal period. Among the HIV-exposed children, HIV-free survival was 91.8% (95% CI: 89.2-93.8). Disclosure of mother's HIV status (aOR = 4.9, 95% CI: 1.3-18.2) and initiation of cotrimoxazole prophylaxis to the child (aOR = 3.9, 95% CI: 1.2-12.6) were independently associated with increased HIV-free survival while child growth problems (aOR = 0.2, 95%CI: 0.09 – 0.5) was independently associated with reduced HIV-free survival.

Of the children, 9.1% were reported to have been preterm. HIV-exposed children had a slightly higher rate of preterm births compared to HIV-unexposed (9.9% vs. 9.0%). The mean (±SD) birthweight was 3.02 (0.5) kg. HIV-exposed infants had marginally lower birth weight compared to HIV-unexposed, 2.9 (0.6) vs. 3.05 (0.5) kg. Of the children, 95.2% were breastfed with an overall median duration (IQR) of 12 (7-18) months. The HIV-exposed children had a shorter duration of breastfeeding compared to the HIV-unexposed, 11 (6-12) months vs. 15 (10-18) months. Fewer HIV-exposed children breastfed for more than 18 months compared to the HIV-unexposed (9.1% vs. 17.1%). Of the 37 children who were reported dead, 16 were born to HIV-positive women.

There was no difference in timing of deaths between HEI and HUI among the 28/37 children whose mothers/caregivers reported timing of death, Table 12. Half of the children died in the neonatal period and the other half after.

Table 12. Timing of death by HIV exposure for 28 children who had died in Paper III.

Time of death	HIV-unexposed n	HIV-exposed n	Unknown maternal HIV status n	Total
Within first day	5	1	2	8
Within 28 days	3	3	0	6
After 28 days	7	7	0	14
Total	15	11	2	28

Table 13. Timing of HIV diagnosis for the 33 HIV positive children in paper III.

HIV infections	Known HIV positive mothers	Unknown status for mothers	Newly diagnosed HIV positive mothers	Total
Known HIV-positive children before 18-24 months	22	1	-	23
Initially HIV-negative, but HIV-positive at 18-24 months	5	-	-	5
Newly diagnosed HIV-positive at 18-24 months	3	-	2	5
Total	30	1	2	33

Of the 33 children who were HIV-positive, 10 were diagnosed during our study, Table 13. Of the children newly diagnosed with HIV, two were from women who tested HIV-positive during the study, three were born to known HIV-positive women, but had never been tested, while five who were also born to known HIV-positive women who had initially tested HIV-negative as part of their care but had not had their confirmatory tests done at the time of the study. Of the HIV-positive women, 86.8% used ART during pregnancy and 37.6% of mothers of the children who were HIV-infected started ART either during or after the breastfeeding period compared to 9.6% of mothers of HIV-uninfected children. Among HIV-positive women, 88.0% reported that they were still taking their ARVs at the time of the study. 89.3% of HIV-exposed children had received nevirapine prophylaxis. Twelve of the thirteen HIV-infected children with records of their ART status were on treatment.

5.0 Discussion

The results presented in this thesis shows that the prevention of mother-to-child transmission of HIV goes beyond provision of ARVs. It involves health systems, women's participation in their own health and that of their children, appropriate monitoring of HEI to diagnose HIV and improve health outcome and accurate methodologies in measuring MTCT rates.

The first study showed the need to optimize EID to provide early initiation of treatment for children who are HIV infected (paper I). It is not enough that the child is HIV negative after birth, which has been the focus of many PMTCT programs in the past. The child has to remain alive while free of HIV. This starts with tracking the birth outcomes of HIV-exposed infants and ensuring their survival beyond the neonatal period (paper II). Building on the successes of ART scale-up among HIV-positive pregnant women and in line with the WHO recommendations, the effectiveness of a PMTCT program needs to be assessed by measuring HIV-free survival at the end of breastfeeding period when HEI is no longer exposed to the virus. Assessing HIV-free survival at 18-24 months of life is a good approach to achieve this (paper III).

5.1 Discussion of results

In the first study, we found that, although children presented to care for HIV testing on time, the overall TAT until the caregivers received the results was over two months. The longest delay – more than three weeks – was from the time of receipt of specimen at the central laboratory to receipt of results at the district hospital. Importantly, the overall TAT was significantly shorter for HIV-infected children compared to HIV-uninfected children. In addition, the second study showed that pregnant women in Lesotho continue to present late in the second trimester with HIV-positive pregnant women presenting earlier than the HIV-negative women. The rate of preterm birth was higher among HEI compared to HUI. There was no difference in rates of congenital anomalies between HEI and HUI. HIV transmission was 0.9% at birth and cumulative transmission of 1.0% at six weeks. The overall mortality at six weeks, including stillbirths, was 6.0% and 5.2% for HEI and HUI,

respectively. Preterm and low birth weight babies were more likely to die than term and normal weight babies, respectively. Among liveborn infants, six-week HIV-free survival for HEI was comparable to survival among HUI. After adjusting for maternal mortality and gestational age at first ANC visit, infant HIV exposure status was not associated with early infant mortality. Finally, in the third study, we found that the MTCT HIV-1 rate was 5.7% in birth whose mothers were initiated in care in the era of lifelong ART including those who sero-converted after initially testing HIV-negative. The mortality rate was almost as twice as high among HIV-exposed children compared to HIV-unexposed children. HIV-free survival was 91.8% [95% CI: 89.2-93.8] among HEI. Disclosure of mother's HIV status and initiation of cotrimoxazole prophylaxis in the child were independently associated with increased HIV-free survival while child growth problems were independently associated with reduced HIV-free survival.

Looking at Lesotho, a country with the second highest HIV prevalence in the world, it is important to ensure children who are HIV-exposed receive HIV testing on time, while child survival strategies are in place to improve birth outcomes and HIV survival beyond the neonatal period. It is sad to see that there has been no reduction at all in the adult HIV-prevalence over 10 years, which means we are continuously allowing new generations of adolescents to become infected with HIV.

5.1.1 EID as a key step in the care of HIV-exposed infant

At the time of the study for paper I, Lesotho, like many countries in sub-Sahara Africa, continued to see the age of children who get the first DNA PCR test to be more than six months of life [128-132]. Paper I described the comprehensive process from a heel-prick to the results getting back to caregivers and eventually the HIV-infected child being initiated on ART. This was the first time this kind of analysis was done in Lesotho. In this study, based on the conventional system of processing DNA PCR in a centrally located laboratory, we found that major delay occurred at the central laboratory (fig 19). This is a major issue because the delay in the health system impeded directly in the decision to care for HIV-infected children, knowing fully well that any delay in initiating these children in

care may cause up to half of them to die by their second birthday [133]. This is still the case in several countries in Africa [130, 131, 134]. In our study, the children generally presented on time for the EID test and the delay was mainly in the health system. This is contrary to the situation reported from Tanzania, where late presentation of the children was reported as the main cause of delay in early infant diagnosis [135]. There is, therefore, a need to rethink the health system for EID, if we are serious in wanting to reduce the infant mortality linked to HIV infection. In a country like Lesotho, considering the impact of geographic location of health facilities in the TAT, it will be key for the MOH to either a) improve transport of specimens and return of results in a timely manner, or b) decentralize the capacity to process DNA PCR in the district laboratory or c) introduce POC diagnosis for nucleic acid testing. Recent studies have shown that the introduction of POC technology reduced the TAT for EID significantly compared to conventional techniques [136-138]. This has been a great development but it brings a new layer of challenges, which are related to supply and distribution of cartridges, storage requiring refrigeration, and staffing and technical maintenance of the machine.

The other important finding was the fact that HIV-infected children received their results earlier and most of those who had a record about their ART initiation were rapidly initiated on treatment. Further exploration showed that the health workers had an understanding that HIV-infected children had higher rates of morbidity and mortality. Therefore, they instituted initiatives to contact caregivers of HIV-infected children over the phone. This appeared to be successful, however, health care workers did not do the same for HEI who had a DNA-PCR negative result. Of course, various studies have reported mixed reactions from health care workers about contacting caregivers in relation to children's EID results [139, 140]. Considering the fact that there is HIV-related stigma in Lesotho, health workers calling caregivers may indirectly disclose the status of the mother to whoever receives the phone call. In addition, several caregivers prefer to come back to health facilities to collect their child's results during their own ART collection appointment [141-144]. Considering the number of community workers who support the HIV program in Lesotho and other

countries in the region, they could serve as a liaison to reach out to caregivers once their child's results are ready in countries where conventional EID is still in use.

5.1.2. Maternal and child health services: the platform for successful PMTCT

The integration of HIV testing services in ANC has over the years revolutionized HIV care for pregnant and breastfeeding women. Once the HIV status is known, documented and the HIV-positive woman is appropriately counselled; the earlier she is initiated on ART, the better the woman's health outcome, and the lower the VL at the time of delivery, thereby reducing the risk of HIV transmission to her HEI.

The importance of outcomes among HIV-exposed children has been a topic of high interest in recent times. In our second study, we felt that the first step should be to consider birth outcomes and outcomes during and immediately after the neonatal period.

We found similar birth outcomes for HEI and HUI, except that the frequency of preterm delivery was higher among HEIs. This is an important finding because of the additional care required for preterm infants is causing additional strain on the family and the health system [145, 146]. This further compound the health challenge related to HIV-infection itself and the chronic use of ART. These results call for additional monitoring of the HEI beyond the neonatal period where we consider health complications among preterm children as they grow [145]. While the risk for preterm birth among HEIs has been reported in other studies, there have been limited studies conducted in routine health systems that have large numbers of both HIV-negative and HIV-positive women in the era of lifelong ART with TDF-based ART regimens. In our study, low birth weight and preterm delivery did not statistically differ between women who initiated ART before conception and those who initiated after conception. This aligns with other studies that found no relationship between preterm delivery and preconception ART [146].

We also found a high rate of ANC attendance reported by the women participating in our study above the 77% reported in the 2014 Lesotho Demographic Health Survey. Similarly, a high proportion of study participants had a facility-based delivery. This was an important

finding because when pregnant women attend ANC and deliver in a health facility, it reduces the risk of pregnancy and delivery complications and HEI are more likely to be given antiretroviral prophylaxis. And even if there are complications, the health team then has time to plan a proper management of the woman and the baby including EID [147-149]. Not surprising, most of the women who had HIV-infected children at six weeks had unsuppressed VL.

In our study, we found that that although there was no difference in the proportion of infant stillbirths among HIV-positive women compared to HIV-negative women, HIV-positive women were more likely to have had a macerated stillbirth versus fresh still birth which was found among HIV-negative women. The high number of macerated stillbirths is consistent with challenging quality of antenatal care in the country especially lack of optimal monitoring of HIV-positive pregnant women. This calls for close monitoring of the HIV-positive pregnant women as high risk pregnant during ANC, labor and delivery. Of note, in a study in India, there was higher intrauterine fetal deaths among HIV-positive pregnant women compared to HIV negative (AOR = 1.9; $p < 0.01$) [150-152].

5.1.3. Completion of PMTCT cascade is key to measure PMTCT effectiveness

In our third study, we found that 38 of the 39 women who newly tested HIV-positive had seroconverted since they tested HIV-negative during pregnancy. This calls for reinforcement of HIV re-testing for pregnant and breastfeeding women in countries with high adult HIV prevalence like Lesotho. Furthermore, this raises some questions whether these women knew their partners' HIV status [152]. Based on current guidelines, if these women were in a HIV sero-discordant relationship, PrEP should be proposed [153].

In addition, at the time of the study, we found that 88.0% of HIV-positive mothers reported currently taking ART with 89.3% of HEI receiving NVP prophylaxis within three days of delivery. At the time of the interview, more than 10% of women were no longer on ART and more than 10% of HEI did not receive NVP prophylaxis. This is an important finding because it has been reported that leaks along the PMTCT cascade contribute to high

transmission [154]. Although some leaks could be due to maternal or child mortality, there is a need to explore measures that can be used to retain at least 95% of patients in care in accordance to global target.

5.1.4. Late HIV diagnosis among HIV-exposed children

Timely diagnosis of children will foster earlier ART initiation, thereby reducing morbidity and mortality. In our Paper III, one-third of HIV-infected children were diagnosed during the study with half of those having tested HIV-negative earlier. This, further buttresses the need for subsequent repeat tests for HEI.

Furthermore, HIV-free survival is a better approach to measure the PMTCT effectiveness because it does not only measure the effectiveness of ART to reduce the MTCT rate, but it also shows how PMTCT services built on the MCH platform are able save young children's lives and measure early deaths among HEI [29, 38, 155-157].

5.2 Strengths and limitations of the methods and design

One strength in this thesis work is that we used the most appropriate methods for data collection for this setting. The retrospective cohort data abstraction collected data from a real-world setting. This method produced our baseline and identified the real challenges faced by the health system. The study participant interviews during the observational prospective cohort coupled with clinical record abstraction within research sites gave the opportunity to control for some of the challenges noted in the retrospective cohort. Finally, the community-based household interviews with community-based HIV testing provided a comprehensive assessment of the program. This method of data collection was used to ensure that women who did not attend ANC or were lost to follow up were assessed. And methods used for data collection operated in the real world to give results that are applicable to general program settings.

A second strength is that all the studies had large sample sizes, which made the descriptive analysis of factors associated with the outcomes stronger. For example, from a sample of

Table 14. Summary of strengths and weaknesses of the studies.

	Paper I	Paper II	Paper III
Strengths	- Retrospective cohort design - Large sample size - Inclusion of large number of sites from across the whole country - Comparison of HIV exposed infected and HIV-exposed uninfected - Data about mother-infant pairs - Low cost of the study - Rapid results	- Prospective cohort study design - Large sample size - Comparison of HEI and HUI - Geosocial organization of Lesotho was considered in the selection of study sites - Collected data on mother-infant pairs - Clinical records abstraction supplemented by interviews - Study introduced at the time of policy change	- Cross-sectional design - Large sample size - Study sites represent all geosocial structures in Lesotho - Community-based data collection - Reached women and children who may have missed ANC attendance - Able to test women and children during the study - Able to measure sero-conversion among women and children
Weaknesses	- Primary data collection tool not designed for research - Missing data - Exclusion of hard to reach sites - Analysis mainly descriptive	- Design: observational cohort so, no intervention - Bias: information limited to clinical setting - Limited number of districts	- Cross-sectional design measures event at one time point - Biological mother absent - Recall bias - Identifying dead children

1187 HEI, the TAT for the results of children who were HIV-positive was significantly shorter than those who were HIV-negative ($p<0.001$). In the observational cohort sub-study, although there was no difference in birth outcomes between HEI and HUI up to six to eight weeks, when followed up to 24 months, HEI had higher mortality than HUI (Log-rank $p<0.001$). Finally, in the large community sample, HIV-free survival among HEI was reliably assessed in Lesotho.

A third strength is that the study sites were drawn from several sites in Lesotho. For example, for paper I, the sites of the study were from all the ten districts. Although some hard to reach sites were excluded, the studies' findings could be generalized to the entire country based on the large sample size coupled with site selection drawn from across the

country. In addition, the studies for paper II and III took into consideration the geographical and social determinants of health-seeking behaviors in Lesotho. Sites were selected to represent the highlands, the foothills and the lowlands making the results applicable to these settings. A larger sample size in a quantitative study makes results more generalizable [158].

A retrospective cohort – also called a historical cohort – is a form of observational cohort. A major interest in this study was the assessment of the TAT of EID from sample collection to caregivers receiving results and initiating HIV-infected children on treatment. For us to get the true picture of the TAT of EID in Lesotho, it was essential to track each step DBS specimens went through. It would have been impossible to carry out this study prospectively and get the true picture without influencing the health system and the results, the so-called Hawthorne effect. Therefore, the best design was retrospective cohort in which we followed the circuit of a number of samples [159]. The advantages of the retrospective cohort design as stated by Sedgwick; minimized selection bias, minimized recall bias and possibility to estimate population at risk applied clearly in our study. The challenge observed in our study with this design was that, we used records that were not designed for the study, therefore the available data had issues with quality. There was limited data on potential confounding factors. Differential losses to follow-up can also bias retrospective cohort studies. In order to address this challenge, we used data from several sources so that each variable could be verified.

In addition, we had an interest to know whether birth and six-week outcomes among HEI was different from those observed among HUI. An observational prospective cohort indicates the sequence between exposure and outcome over time. By definition in this design, at the beginning of the study, it is known that subjects do not suffer from the disease. In our case, we had comparison group as HUI. The advantage is that cohort studies allow you to calculate relative risk or ratio which was important in our study. In addition, prospective cohort studies reduce the possibility that the results will be biased. In our study, the comparison group was HUI. One challenge with the prospective cohort design is the

cost and the risk of LTFU. Concerning the cost, the study was imbedded as part of a large project, which was well resourced. The challenge of potential loss to follow-up (LTFU) was mitigated by ensuring scrupulous implementation of the intervention to track mother-infant pairs according to national standards [160].

The cross-sectional design requires a single interaction between study participants and the data collection team. There are indeed reduced chances to of LTFU compared to cohort studies. Compared to other designs, the cross-sectional design is less expensive and is importantly faster to conduct. Since the sample is taken from the whole population it estimates the prevalence of the outcome of interest. It gives an opportunity to asses outcomes and risk factors. Our study for paper III used this design because it was the most adapted option in the context. The disadvantages include a difficulty to make causal inference and data represents only a snapshot in a timeframe that may change quite quickly. There is a prevalence-incidence bias (also called Neyman bias). By definition, prevalence-incidence bias occurs when the patients with severe or mild disease are excluded, resulting in an error in the estimated association between an exposure and an outcome. Excluding the patients who have died will make the disease condition look less severe than it should be while excluding the patients who will recover from the disease will make the condition look severe. And in the case of HIV being a chronic disease and the fact that our primary outcome was a composite outcome that included HIV transmission and death, results that included deaths might have been under-represented [160-162].

The studies also had limitations and we will touch on the most important ones here.

For paper I, while this study focused primarily on the health facility and laboratory delays with regards to EID processes, in-depth information on mother-infant pairs' access to PMTCT services and reasons for LTFU were not comprehensively captured. However, this study yielded important information on barriers to early diagnosis and treatment for HEI, such as transport and reporting systems and postnatal follow-up of HEI, and where best to target interventions to ensure children who test positive are promptly provided with life-saving treatment. There were documentation issues at several study sites, including missing

information in registers, missing files, and laboratory results that were not copied into records. This limitation motivated the design of the study for paper II which had the opportunity to put in place additional support to study sites.

For paper II, since the study measured birth outcomes and six-week HIV-free survival in a facility-based population, we may have missed women and children who did not seek care in health facilities and women who lost pregnancies early. That is why we carried out the third sub-study in community which informed the results presented in paper III.

For paper III, the limitation might have been the difficulty to accurately measure deaths in the community because of the discomfort of the parents may have felt to report death infants. However, when put together, the three papers bring wealth of data which presented the full picture of PMTCT in Lesotho with comprehensive results.

5.3 Conclusions

- Long TAT for EID have constituted a major bottleneck for timely initiation of HIV-positive children on ART. Innovative approaches, such as point of care testing, are currently being tested and is likely to be part of the solution to this problem.
- The implementation of universal ART in Lesotho continues to have a significant impact in reducing HIV transmission to children and was associated with low MTCT by six weeks of age, with no differences in congenital anomalies or early mortality between HEIs and HUIs.
- The community survey showed that Lesotho has achieved a high utilization of MCH services by pregnant women. The use of PMTCT services from testing to enrolment on ART is high, resulting in HIV-free survival for HIV-exposed children at 18-24 months comparable to other countries in the region.
- Of the children diagnosed with HIV at the age 18-24 months in the community survey, one-third were diagnosed for the first time during our survey, partly due to new infections in the adult population, which calls for:
    - reinforced efforts to control the adult epidemic
    - innovative ways to prevent postnatal MTCT
    - continued follow-up of these children until the final diagnosis is known

5.4 Recommendations to policy makers

- All countries with centralized EID system where TAT is counted in weeks or even months, need to consider the introduction of POC EID.
- The MOH needs to put in place a system to fast-track blood specimens and results to ensure timely return of results to patients for expedited care for HEI or infected children.
- Government needs to consider support strategies for intensive care for HIV-positive pregnant women as they should be classified as high-risk pregnancy.
- The MOH needs to consider reinforced efforts to control the adult HIV, also among women during pregnancy and breastfeeding.

5.5 Recommendations for research

- As the expansion of PMTCT services in the context of lifelong ART continues, we recommend the establishment of observational cohorts across many countries with regional data centers to measure long term health and survival of HEI in Africa beyond 24 months.
- Considering the importance of community household surveys, it is recommended that researchers repeat surveys sequentially at two-three-years-intervals to measure the true impact of PMTCT services over time.
- MTCT occurs even in women who tested negative during antenatal due to sero-conversion during pregnancy or breastfeeding period. As we implement strong PMTCT services, new infections in children will be driven by seroconversion among HIV negative pregnant and breastfeeding women, therefore, we need to identify additional interventions that reduce MTCT in this context.

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RESEARCH ARTICLE

Conventional early infant diagnosis in Lesotho from specimen collection to results usage to manage patients: Where are the bottlenecks?

Appolinaire Tiam[1,2]*, Michelle M. Gill[3☯], Heather J. Hoffman[4], Anthony Isavwa[2], Mafusi Mokone[2], Matokelo Foso[2], Jeffrey T. Safrit[5], Lynne M. Mofenson[3‡], Thorkild Tylleskär[1‡], Laura Guay[3,4☯]

1 Centre for International Health, University of Bergen, Bergen, Norway, **2** Medical and Scientific Affairs, Elizabeth Glaser Pediatric AIDS Foundation, Maseru, Lesotho, **3** Research, Elizabeth Glaser Pediatric AIDS Foundation, Washington DC, United States of America, **4** Milken Institute School of Public Health, George Washington University, Washington DC, United States of America, **5** Research Alliances, International AIDS Vaccine Initiative, New York, New York, United States of America

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* atiam@pedaids.org, Appolinaire.tiam@gmail.com

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Abstract

Introduction

Early infant diagnosis is an important step in identifying children infected with HIV during the perinatal period or in utero. Multiple factors contribute to delayed antiretroviral treatment initiation for HIV-infected children, including delays in the early infant HIV diagnosis cascade.

Methods

We conducted a retrospective study to evaluate early infant diagnosis turnaround times in Lesotho. Trained staff reviewed records of HIV-exposed infants (aged-6-8 weeks) who received an HIV test during 2011. Study sites were drawn from Highlands, Foothills and Lowlands regions of Lesotho. Central laboratory database data were linked to facility and laboratory register information. Turnaround time geometric means (with 95% CI) were calculated and compared by region using linear mixed models.

Results

1,187 individual infant records from 25 facilities were reviewed. Overall, early infant diagnosis turnaround time was 61.7 days (95%CI: 55.3–68.7). Mean time from specimen collection to district laboratory was 14 days (95%CI: 12.1–16.1); from district to central laboratory, 2 days (95%CI 0.8–5.2); results from central laboratory to district hospital, 23.3 days (95%CI: 18.7–29.0); from district hospital to health facility, 3.2 days (95%CI 1.9–5.5); and from health facility to caregiver, 10.4 days (95%CI, 7.9–13.5). Mean times from specimen transfer to the central laboratory and for result transfer from central laboratory to district hospital were significantly shorter in the Lowlands Region (0.9 and 16.2 days, respectively), compared to Highlands Region (6.0 [P = 0.030] and 34.3 days [P = 0.0099]. Turnaround time from blood

draw to receipt of results was significantly shorter for HIV infected infants compared to HIV uninfected infants [p = 0.0036] at an average of 47.1 days (95%CI: 38.9–56.9) and 62 days (95%CI: 55.9–68.7) respectively. Of 47 HIV-infected infants, 36 were initiated on antiretroviral therapy at an average of 1.3 days (95%CI: 0.3, 5.7) after caregiver received the result.

Conclusion

HIV-infected infants received results earlier and were rapidly initiated on antiretroviral therapy once the result was delivered to caregiver. However, average early infant diagnosis turnaround time was two months; the longest period of delay was transfer of results from central laboratory to district hospital. Turnaround time of results based on geographical regions or between hospitals and health centres varied but did not reach statistical significance.

Introduction

Multiple factors contribute to delayed antiretroviral treatment (ART) initiation for HIV-infected children, including service delivery gaps in early infant HIV diagnosis (EID) [1–3]. Despite advances made in the field of prevention of mother-to-child transmission (PMTCT) of HIV, the average age of initiation of treatment for HIV-infected children is approximately five years [3,4]. Though there are multiple reasons for this, poor access to infant HIV testing and diagnosis is one key barrier. While EID access has improved, only 51% of the 1.6 million HIV-exposed African children had access to EID testing in 2015[5]. Approximately 23% of Lesotho's population are living with HIV, making it among the highest per capita HIV infection rates in the world [4,5]. Of the approximately 310,000 individuals living with HIV in Lesotho, 13,000 are children $<$ 15 years of age [6,7].

The Lesotho Ministry of Health (MOH) guidelines for EID are consistent with the World Health Organization (WHO) guidelines, recommending that the first virological test for infants exposed to HIV should be conducted at or around 6 weeks following birth, and all infants diagnosed with HIV should be started on ART immediately irrespective of CD4 count [8,9]. However, there are still gaps in coverage for EID/Early Infant Treatment (EIT) services in Lesotho, especially in rural areas [10]. In a study in Uganda, it was found that EID coverage was 16% (101/636); 4.5% (8/179) and 20.3% (93/457) in rural and urban health facilities respectively [11]. Similar gaps were reported in South Africa [12].

As of 2013, more than 50% of exposed/infected children did not receive their results within 2–4 weeks and approximately 30% of infants were lost to follow-up (either not enrolled into care or retained in care) in Lesotho [9]. The pathway from sample collection to results received was multifaceted and a delay in one point influenced the overall efficiency and TAT of the process. At the time of the study, the utilized pathway had several steps that could cause significant delays. While EID can be conducted using a variety of virologic assays, in Lesotho, EID is conducted using a DNA polymerase chain reaction (DNA-PCR) assay. At the six-week postpartum visit, HIV-exposed infants received a physical examination and immunizations and a dried blood spot (DBS) sample was taken for DNA-PCR testing to determine the child's HIV status. Collected DBS samples were transported from health facilities to the district laboratory (referred to as the district hub); for visits at district hospitals, the laboratory was on the same campus. The specimen was then sent to laboratory headquarters (central laboratory) for

personnel to decide whether the specimen should be processed at the central laboratory in Maseru or sent to another laboratory in South Africa for processing. This decision was based on the origin and quality of the sample. The national laboratory in South Africa operated an automated system while at the time of the study, the Lesotho National reference laboratory operated a manual system that was replaced by an automated system. For specimens to be sent to South Africa, the blood spot must be within the demarcated circle otherwise it had to be processed in Maseru. In addition, high volume facilities such as hospitals had their specimens sent to South Africa. At the time of the study, the proportion of specimens sent to South Africa for processing varied from one third to about half. Once processed, test results were delivered back to the district hub and then returned to the health facility. Caregivers were typically advised to return to the health facility after four weeks from the date of child's blood draw. However, when some caregivers returned and test results were not yet available, they might not return to the health facility again to collect their infants' test results. Additionally, some patients did not return to the health facility for other reasons and were then lost to follow-up. Furthermore, for children who were diagnosed as HIV-infected, test results were sent electronically to health facilities through 3G mobile internet and short message service (SMS). Community health workers actively track HIV-infected children back to the health facility within seven to ten days to enable them to be initiated on ART in accordance to national guidelines.

As the guidelines call for testing and treating all those living with HIV including children, there is an increasing need to scale up HIV diagnosis especially among infants and children [9]. Lesotho has made great strides in rolling out a national PMTCT program and the current mother to child transmission of HIV (MTCT) rate is estimated at 5.9% [7]. There are limited data that analyze the EID cascade to demonstrate barriers to efficient EID in sub-Saharan Africa, especially in a country with diversified topography like Lesotho. This paper describes the EID process and identifies bottlenecks within the EID pathway by tracking the length of time for each step in the cascade.

Materials and methods

Study design

We conducted a retrospective cohort study with the aim to describe the EID process in order to identify the barriers and delays within the EID pathway by tracking the length of time for each step. To determine where the delays existed between steps in the EID pathway, we estimated the average time intervals between the following time points:

- The 6-8-week HIV specimen is collected at the facility,
- The specimen is sent to the laboratory,
- The laboratory received the specimen,
- The laboratory processes the specimen and obtains results,
- The laboratory sends the results back to the facility,
- The facility receives the results from the laboratory,
- The facility contacts the caregiver,
- The caregiver receives the results,
- The HIV-infected infants are initiated on ART at study sites.

The variability for each time interval was estimated and compared between caregivers within sites (mean of the variances) and between sites (variance of the means). The characteristics of facilities were purposively predetermined based on topographic location as highlands, foothills and lowlands, which determine health seeking behaviour in Lesotho.[6] Unique characteristics of sites with the shorter time intervals were identified and compared to the sites with longer time intervals.

Study population

Trained study staff abstracted data from the national laboratory database on all HIV-exposed infants who had a DNA-PCR EID test at 6–8 weeks of age from January to December 2011 in 25 sites from all 10 districts in Lesotho. Data abstracted from the database included infant age, test result, district hub and dates when the tracked specimen was received and processed. Using the child's name and other key information, infants' database records were linked to facility records from which their mothers received care. The records included the DNA-PCR EID test result, antenatal care (ANC), and ART registers and laboratory documentation. From these records, limited mother and infant demographics, mother and infant ARV regimens, and additional dates that documented the specimen and result along the EID pathway were abstracted. The documented dates reflected the physical receipt of the specimen and the results. During the study time, all sites were using 3G mobile internet and short message service (SMS) to communicate some of the results, especially results that were HIV-positive. The 3G mobile internet and the SMS systems were rolled out as part of project supported PEPFAR through the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF). Once the results were sent to health facilities, electronic records as email were kept in the EGPAF head office in Maseru. Study staff also completed a data collection form which documented information about each facility, including characteristics, such as distance to district hub and mode of transport for samples, and strategies for identification and follow-up of mother-exposed infant pairs.

Study sites

25 sites were included in this study. Of these 25 study sites, 11 were health centers that were purposively selected, and were defined as hard-to-reach.[6] Of these 11 hard-to-reach, 8 facilities were located in the highlands and presented a challenge during winter when roads are blocked with heavy snow fall. These 11 facilities were located in six districts, each representing the three geo-topographical areas of Lesotho as follows: Highlands (Mokhotlong, Qacha's Nek, Thaba Tseka); Foothills (Butha Buthe and Quthing); and Lowlands (Maseru). Program data showed that the hard-to-reach sites had longer TATs estimated at 6–8 weeks. An additional 14 sites were selected from all 10 districts of the country in order to represent more typical TATs. The topographical distribution of these 14 sites was as follows: Highlands (Mokhotlong, Qacha's Nek, Thaba Tseka) 3 sites; Foothills (Butha Buthe, Leribe, Mohale's hoek and Quthing) 6 sites; and Lowlands (Berea, Mafeteng, Maseru) 5 sites. The majority of these latter sites had estimated TATs of 2–4 weeks. Study sites included 16 health centers and nine hospitals.

Data collection and analysis

Data were collected in March–April 2012 and entered into MS Access (2007–2010). The ANC identification number was used to link laboratory and facility records. Once records had been matched, a study identification number was created using a code for the site and a non-identifiable numeric code that was not linked to the original patient number. The link between the

patient identification number and the study identification number was recorded in a study enrolment log. The log and the completed data collection forms were kept at the facility in a locked location with controlled access. Following study completion, these documents were destroyed. Data from the paper-based tool were entered into a password protected database using the generated study identification number, which were then imported into SAS for statistical analysis. Data were analysed at the EGPAF/Lesotho office in Maseru and EGPAF Global in Washington, DC.

Steps along the EID pathway were categorized into five stages. Stage 1 was the time from specimen collection to transfer to district laboratory. Stage 2 was the time from specimen receipt in the district hub to transfer to central laboratory for testing. Stage 3 was time from receipt of specimens at the central laboratory to receipt of results at district hospital. Stage 4 was the time from receipt of results at the district hospital to receipt at health facility. Stage 5 was the time from receipt of results at health facility to results receipt by caregiver.

For this study, we conducted an exploratory data analysis using numerical and graphical methods to display important features of the data, an analysis for each objective and its associated variables, and a descriptive data analysis. Exploratory data analysis allowed us to highlight general features of the data to direct future analyses and identify problem areas in the data.

The average–geometric mean (95% CI)–time intervals (in days) were stratified by relevant independent variables. They were calculated with linear mixed models assuming a compound symmetry working correlation structure to account for the clustering of women in facilities in order to determine the stages with the longest days.

In order to explore variations in TAT at study sites, for the purpose of this study, mean TATs (based on TAT for the caregiver) was categorized as long, (median 75.5–99 days), medium (62.5–67 days), or short (33.5–60 days).

Since this study was a retrospective review of existing records, there was no consent involved and the ethics committee waived the need for consent. This study received approval from the Baylor College of Medicine Children's Foundation, Lesotho institutional review board and the Lesotho national research and ethics committee.

Results

Characteristics of the study population (mothers and infants)

In this study, 1187 HIV-exposed infants' records were reviewed. The mean (standard deviation, SD) maternal age at the time of the delivery was 28 (±5.6) years.

Table 1 describes characteristics of the mothers and infants whose records were reviewed in this study. 92.8% of women received antiretroviral (ARV) drugs for PMTCT or treatment: 33.4% received antiretroviral therapy (ART) for their own health; 44.2% received zidovudine for prophylaxis against transmission; and 15.2% received an unknown ARV regimen. Maternal death rate at the time of review was 6.9% (82/1187).

The mean (SD) infant age at blood draw was 46.9 (3.4) days. The HIV transmission rate at 6–8 weeks in the study participants was 3.9%. Of the 47 children who were HIV-infected, 39 had records of ART status, with 36/47 (76.6%) initiated on ART. The 36 children were initiated on ART at an average of 1.3 days (95%CI: 0.3–5.7; range: 10–56) after result receipt (in some cases children who tested positive were tracked outside the conventional system and contacted by phone, with some initiated on ART before they received the physical result through the conventional system). Overall 17/1187 (1.4%) of children had died at the time of review. The HIV status of children who died was unknown.

Table 1. Mother and infant characteristics.

	Number of HIV exposed infants n (%) N = 1187
Facility location (region)	
Highlands	183 (15.4)
Foothills	528 (44.5)
Lowlands	476 (40.1)
Maternal ART regimens during pregnancy	
ARV for prophylaxis	525 (44.2)
ART prior to index pregnancy	161 (13.6)
ART initiated during index pregnancy	156 (13.1)
None	86 (7.2)
ART unknown when initiated	79 (6.7)
ARV regimen unknown	180 (15.2)
Mothers alive	
No	82 (6.9)
Yes	1023 (86.2)
Unknown	82 (6.9)
Infant EID results at 6–8 weeks of life	
Positive	47 (3.9)
Negative	1139 (95.9)
Unknown	1 (0.02)
Infant received ARV prophylaxis after birth	
Yes	1033 (87.0)
No	53 (4.5)
Unknown	101 (8.5)
Infant alive	
No	17 (1.4)
Yes	1089 (91.8)
Unknown	81 (6.8)
HIV-infected infant initiated on ART	N = 47
Yes	36 (76.6)
No	3 (6.4)
Unknown	8 (17.0)

Calculated interclass correlations (ICCs) for all variables and all were close to zero. These variables are not facility dependent. **Note:** ART: antiretroviral therapy; ARV: antiretroviral; EID: early infant diagnosis.

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Turnaround time analysis

S1 Fig shows mean times that specimens and results took at each stage of the EID cascade. Overall, the mean total turnaround time in this sample of sites was 61.7 days (95%CI: 55.3–68.7). The longest time spent by the specimen and results occurred at stage 3, which is time from receipt of specimen at the central laboratory to receipt of results at district hospital, and was 23.2 days (95%CI: 18.7–28.9). The average stage 1 and stage 3 time intervals were significantly shorter in the Lowlands Region (0.9 and 16.2 days), compared to Highlands Region (6 days [$P = 0.03$] and 34.3 days [$P = 0.00$].

The mean turnaround time was 47.4 (95%CI: 39.0–57.7) and 62.4 (95%CI: 55.6–69.9) for HIV-infected and HIV-uninfected children, respectively (Table 2). The mean (95% CI)

Table 2. Turnaround time (TAT) for the stages in the EID cascade calculated from linear mixed models.

Stage	Start time	Stop time	Infants HIV infected at 6–8 wks		Infants HIV uninfected at 6–8 wks		All infants at 6–8 wks	
			N (%)	Mean TAT in days (95%CI)	N (%)	Mean TAT in days (95%CI)	N	Mean TAT in days (95%CI)
1	Specimen collection	Specimen transfer to district laboratory	43 (4.0)	14.4 (11.3–18.4)	1045 (96.0)	13.9 (12.0–16.2)	1088	14.0 (12.1–16.1)
2	Specimen transfer from district laboratory	Specimen receipt at the central laboratory for testing	8 (3.2)	3.5 (0–15000)	239 (96.8)	2.0 (0.0–552.6)	247	2.0 (0.8–5.2)
3	Specimen transfer to central laboratory	Time results transferred from central laboratory to receipt of results at district hospital	16 (3.0)	20.6 (13.3–31.7)	510 (97.0)	23.2 (18.4–29.4)	526	23.3 (18.7–29.0)
4	Result receipt at district hospital	Receipt of results at health facility	19 (3.7)	3.0 (1.2–7.9)	494 (96.3)	3.2 (1.8–5.8)	513	3.2 (1.9–5.5)
5	Result receipt at health facility	Result receipt by caregiver	37 (4.2)	4.3 (2.4–7.5)	842 (95.8)	10.8 (8.2–14.2)	879	10.4 (7.9–13.5)
Overall	Specimen collection	Result receipt by caregiver	37 (4.2)	47.4 (39.0–57.7)	850 (95.8)	62.4 (55.6–69.9)	887	61.7 (55.3–68.7)

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turnaround time (in days) calculated from a linear mixed model including test result, region and facility level (Table 3), was found to be significantly shorter for HIV-infected infants compared to HIV-uninfected infants ($p<0.01$).

Overall, the turnaround time of results based on geographical regions (Highlands, Foothills and Lowlands) or between hospitals and health centers varied but did not reach statistical significance.

Discussion

In this study we found that the major contributor to health system delays in turnaround time in the EID cascade in Lesotho was the time from receipt of specimen at the central laboratory to return of results to district laboratory (Stage 3), followed by the time for transfer of specimens from health center to district laboratory hub (Stage 1). Although HIV-exposed infants are brought to the clinic at an average of seven weeks of life to have blood drawn for their 6-weeks DNA-PCR EID test, it took over two months for their caregivers to get the results back. In our step-by-step analysis, the longest delay occurred within the laboratory chain. In fact, even though it took two weeks to get the DBS samples from the collection point to the

Table 3. Turnaround time for 887 subjects with complete data calculated from a linear mixed model including test result, region and facility level.

Variable	Number N = 887 n (%)	Turnaround time (days) Geometric mean	95%CI	p-value
Test Result				0.0036
Positive	37 (4.2)	47.1	(39.0–57.0)	
Negative	850 (95.8)	62.0	(55.9–68.8)	
Region				0.057
Highlands	77 (8.7)	63.6	(51.8–78.1)	
Foothills	401 (45.2)	54.6	(46.0–64.8)	
Lowlands	409 (46.1)	45.5	(37.3–55.5)	
Facility Level				0.21
Hospital	710 (80.0)	57.7	(48.9–68.2)	
Health centre/filter clinic	177 (20.0)	50.6	(43.1–59.4)	

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national central laboratory, it took more than three weeks to get the results back to district laboratories. This may be caused by the system focusing primarily on getting blood specimens from HIV-exposed infants while neglecting that the EID cascade is only complete when results get back to the caregiver. The main bottleneck in the EID cascade was the central laboratory.

Our data are similar to the findings of a study in Uganda, where a median turnaround time of EID at 39 days was found [13]. Together these findings point out that scale up of HIV services is not always accompanied by health system strengthening to absorb the additional burden that may occur with scale-up, including the laboratory system [14,15]. The effectiveness of PMTCT can only be achieved if HIV-exposed infants' caregivers get results of their HIV status in a timely manner and children who are HIV-infected are initiated on ART [16–18]. In addition, children who are not HIV- infected at this early stage can continue to be monitored while they receive the much needed child survival package to reduce the risk of death from other childhood illnesses.

In our cohort of 6–8 weeks' old infants, the HIV prevalence was below the targeted HIV MTCT-elimination rate of 5%. This result can be attributed to significant coverage of interventions for PMTCT in this study population, with over 90% coverage of maternal and neonatal ARV prophylaxis or treatment. This is encouraging but must be interpreted with caution. In a population where majority of the women breastfeed their infants such as in Lesotho, it is recognized that a significant number of children may still get infected throughout breastfeeding [15,19,20]. In April 2013, Lesotho adopted lifelong ART for all HIV-infected pregnant and breastfeeding women regardless of CD4 cell count or WHO clinical staging, ahead of the June 2013 WHO guidelines for PMTCT [9, 21]. The gains of this kind of policy change can only become a reality if the health system, including the laboratory network, is strengthened to ensure timely collection and processing of EID specimens and timely return of results to caregivers [20,22].

Our data demonstrated that, when the infants' HIV status was considered, HIV-infected infants received their results in significantly shorter time. In addition, health care workers were requested to reach the caregivers of children who have a positive HIV DNA-PCR EID test result directly by phone. Children with positive results were required to be seen and initiated on treatment within seven days after communicating with the caregiver. Importantly, many of the children were initiated on treatment after the health facilities received their electronic results, well ahead of receiving the printed copies of the results. As part of overall program implementation, there was provision of mobile internet for electronic transfer of the positive DNA-PCR EID test results. There was no significant difference in turnaround time based on health facility level.

The limitations of this study included the fact that it was a retrospective cohort descriptive study. The use of existing medical records with incomplete information made data collection challenging.

Interventions to improve the delays have potential multiple benefits, which include improvement in the uptake of pediatric care and treatment for infants identified as infected, and ultimately contribute to reducing HIV morbidity and mortality in children. In addition, early knowledge of child's status may serve a psychological boost for the mother to maintain maternal adherence to ART leading to better maternal health and adherence to required health care visits for HIV-uninfected children.

Conclusion

In conclusion, the central laboratory and district laboratory were major contributors to the delays in TAT of initial EID in Lesotho. All geographical regions (highlands, foothills, and

lowlands) and hospitals, and health centers were affected in a similar way. As technologies evolve with the advent of point-of-care testing (POCT) EID assays becoming available, it will be essential to evaluate how the new POCT approach impacts on the EID cascade. Furthermore, countries with similar challenges should consider efforts to improve timely initiation of ART for HIV-infected children and delivery of negative results to caregivers or providers.

Supporting information

S1 Fig. Mean turnaround time (TAT) (days) per stage. Stage 1: Time from specimen collection to transfer to the district laboratory: 14.0 days (95%CI: 12.1–16.1) Stage 2: Time from specimen transfer from the district laboratory to the central laboratory for testing: 2.0days (95%CI: 1.5–4.9) Stage 3: Time from receipt of specimens at the central laboratory to the time results transferred from central laboratory to receipt of results at district hospital: 23.2 days (95%CI: 18.7–28.9) Stage 4: Time from receipt of results at the district hospital to receipt at health facility: 3.3 days (95%CI: 1.9–5.5) Stage 5: Time from receipt of results at health facility to results receipt by caregiver: 10.4 days (95%CI: 7.9–13.5) Total time: 61.7 days (95%CI: 55.3, 68.7).
(TIF)

S1 File. EID dataset.accdb.
(ZIP)

S2 File. Facility information.accdb.
(ZIP)

S3 File. Revised data collection tool for abstraction.
(DOCX)

S4 File. Revised HF Characteristics data collection tool.
(DOCX)

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Author Contributions

Conceptualization: Appolinaire Tiam, Michelle M. Gill, Heather J. Hoffman, Anthony Isavwa, Matokelo Foso, Jeffrey T. Safrit, Laura Guay.

Formal analysis: Appolinaire Tiam, Michelle M. Gill, Heather J. Hoffman.

Funding acquisition: Appolinaire Tiam, Jeffrey T. Safrit.

Investigation: Appolinaire Tiam.

Methodology: Appolinaire Tiam, Heather J. Hoffman, Anthony Isavwa, Mafusi Mokone, Matokelo Foso, Jeffrey T. Safrit, Laura Guay.

Project administration: Appolinaire Tiam.

Resources: Appolinaire Tiam, Laura Guay.

Supervision: Appolinaire Tiam.

Writing – original draft: Appolinaire Tiam.

Writing – review & editing: Michelle M. Gill, Heather J. Hoffman, Anthony Isavwa, Mafusi Mokone, Matokelo Foso, Jeffrey T. Safrit, Lynne M. Mofenson, Thorkild Tylleskär, Laura Guay.

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RESEARCH ARTICLE

Comparison of 6-week PMTCT outcomes for HIV-exposed and HIV-unexposed infants in the era of lifelong ART: Results from an observational prospective cohort study

Appolinaire Tiam[1,2]*, Seble G. Kassaye[3], Rhoderick Machekano[1], Vincent Tukei[4], Michelle M. Gill[1], Majoalane Mokone[4], Mosilinyane Letsie[5], Mots'oane Tsietso[5], Irene Seipati[5], Janety Barasa[4], Anthony Isavwa[4], Thorkild Tylleskär[2], Laura Guay[1,6]

1 Elizabeth Glaser Pediatric AIDS Foundation, Washington D.C., United States of America, **2** Centre for International Health, University of Bergen, Bergen, Norway, **3** Department of Medicine Georgetown University School of Medicine, Washington D.C., United States of America, **4** Elizabeth Glaser Pediatric AIDS Foundation, Maseru, Lesotho, **5** Ministry of Health, Maseru, Lesotho, **6** Department of Epidemiology and Biostatistics, George Washington University Milken Institute School of Public Health, Washington D.C., United States of America

* atiam@pedaids.org

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Abstract

Background

Lifelong antiretroviral therapy (ART) reduces mother-to-child HIV transmission (MTCT) and improves maternal health. Data on the outcomes of HIV-exposed infants (HEI) compared to their unexposed counterparts in the era of universal ART is limited. We compared birth and 6-week outcomes among infants born to HIV-positive and HIV-negative women in Lesotho.

Methods

941 HIV-negative and 653 HIV-positive pregnant women were enrolled in an observational cohort to evaluate the effectiveness of prevention of mother-to-child HIV transmission (PMTCT) program after implementation of universal maternal ART in 14 health facilities. Pregnancy, delivery, birth, and 6-week data were collected through participant interviews and medical record review. DNA PCR testing for HEI was conducted within 2 weeks of birth and at around 6 weeks of age. Data were analysed to estimate the distribution of birth outcomes, mortality, HIV transmission and HIV-free survival at 6 weeks.

Results

HIV-positive women were older (mean age of 28.7 vs. 24.4 years) and presented for antenatal care earlier (mean gestational age of 23.0 weeks vs 25.3 weeks) than HIV-negative women. Prematurity was more frequent among HEI, 7.8% vs. 3.6%. There was no difference in rates of congenital anomalies between HEI (1.0%) and HIV-unexposed infants (HUI) (0.6%). Cumulative HIV transmission was 0.9% (N = 4/431) (95% CI:0.25–2.36) at birth and 1.0% (N = 6/583) (95% CI:0.38–2.23) at 6 weeks. Overall mortality, including

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stillbirths, was 5.2% and 6.0% by 6 weeks for HUI and HEI respectively. Among liveborn infants, 6-week HIV-free survival for HEI was 95.6% (95% CI:93.7–97.1) compared to 96.8% (95% CI:95.4–97.9) survival for HUI.

Conclusions

Implementation of universal maternal ART lowers MTCT at 6 weeks of age with no differences in congenital anomalies or early mortality between HIV exposed Infants and HIV unexposed infants. However, HIV exposed infants continue to have high rates of prematurity despite improved maternal health on ART.

Introduction

Effective prevention of mother to child transmission (PMTCT) programs offering universal life-long antiretroviral therapy (ART) reduce HIV transmission to children from their mother and improve maternal health [1,2]. A critical question remains as to whether the reduction in MTCT rates and improvement in maternal health are coupled with improvement in birth outcomes and survival among HIV-exposed infants (HEI) to match those of HIV-unexposed infants (HUI).

There have been conflicting data reported on birth outcomes among HIV-positive women who are on lifelong ART compared to HIV negative women. Some studies found that adverse birth outcomes, such as increased preterm deliveries, stillbirths and low birth weight, occurred more frequently among HEI [3–8]. Other studies found no association between use of ART and adverse birth outcomes [9,10].

Intrauterine and perinatal HIV transmission measurement in the era of lifelong ART is limited. In Rwanda, a study measuring HIV-free survival in a cohort of HEI, found a 6-week Mother to child transmission rate of 0.5% (95% CI:0.2–1.6) demonstrating the effectiveness of lifelong ART for HIV-positive pregnant women in preventing perinatal HIV transmission [11]. UNAIDS spectrum data in Lesotho reported an estimated 6-week transmission of 6.9% in 2016 [1].

Lesotho is a mountainous country in southern Africa with one of the highest HIV prevalence documented in the world. The Lesotho Ministry of Health implemented universal ART for all HIV-positive pregnant women in 2013 using a once-daily fixed dose combination of tenofovir disoproxil fumarate (TDF), lamivudine (3TC) and efavirenz (EFZ) [12,13]. HIV-exposed infants are given nevirapine (NVP) for 6 weeks after birth.

The roll out of lifelong ART for all pregnant women in Lesotho provided a unique opportunity to determine its effect on birth outcomes and survival of infants born to HIV-positive mothers compared with a similar cohort of HIV-negative mothers and their HIV-unexposed infants. The study also assessed the effectiveness of the Lesotho PMTCT program in reaching the goal of the virtual elimination of new pediatric HIV infections in selected sites in Lesotho.

Methods

Design

HIV-positive and HIV-negative pregnant women attending routine antenatal care (ANC) services were enrolled in a prospective observational cohort study from June 2014 to February 2016. Study personnel captured demographic, social, and medical information through

participant interviews and extraction of medical record information during pregnancy, and at delivery, birth and 6 weeks postpartum.

Setting

The study was conducted in 3 districts in Lesotho–Botha-Bothe, Thaba-Tseka and Mohale's Hoek. These districts were selected to include areas with varying levels of PMTCT service delivery coverage, and heterogeneity in health-seeking behavior due to variances in terrain (lowlands, foothills, mountains). We included all 5 hospitals in these districts and randomly selected 9 medium volume (100–200 ANC women/year) or high (>200 ANC women/year) health centers to be included in the study.

Population and enrollment

Eligible HIV-positive and HIV-negative pregnant women attending ANC in the study health facilities were recruited for this study. They were eligible for study enrollment if they resided in the area, planned to continue to receive services at the facility following delivery, were willing to have their infant co-enrolled after birth, and were willing to provide written informed consent. Population proportional sampling was used to estimate the enrollment target at each of the 14 study facilities. The sample size for HIV-positive women was calculated based on a 4% estimated HIV transmission at 6–8 weeks in Lesotho at the time of study initiation with a precision of 1.4% [12]. Consecutive consenting women were enrolled until the sample size was reached. HIV-negative women were enrolled into an HIV seroincidence cohort study with scheduled repeat HIV testing at 36 weeks gestation, delivery, and every 3–6 months postpartum until 24 months postpartum [14].

Data collection

Study data were collected by trained study nurses through structured interviews and by abstraction of data from clinical and laboratory records. Mother-infant pair information such as demographic variables, date of last menstrual cycle, HIV status of spouse, disclosure of HIV status was collected through structured interviews with the pregnant women. General health and clinical history, ARV use and toxicity, adherence, retention in care, family planning, infant feeding practices and infant growth were extracted from the women medical charts and clinic registers. Electronic tablets were used to enter data directly into a web-based database (SurveyCTO).

Gestational age at birth was estimated by the time between the date of last menstrual period given by the women at first ANC and the date of birth. Very preterm birth was defined as infant born at a gestational age of 28–32 weeks while preterm birth was infant born after 32 weeks but before 37 weeks [15]. In addition, miscarriage was defined as loss of pregnancy before the gestational age of 28 weeks and stillbirth was considered when the pregnancy was lost after 28 weeks [16].

Laboratory methods

Blood samples were collected from a subset of HEI at or within 2 weeks of birth and from all HEI at 6–8 weeks of age to determine their HIV infection status. Trained nurses obtained blood from infants by heel prick, which was spotted directly onto filter paper to create a dried blood spot for HIV DNA PCR testing as per the standard Ministry of Health (MOH) protocol. HIV testing was performed by the national reference laboratory using the Roche COBAS

Ampliprep/COBAS TaqMan HIV-1 qualitative test (v2.0). Test results were obtained directly from the laboratory and entered into the study database.

Statistical analysis

Quantitative data analysis was performed using STATA version 15.1 (College Station, TX, USA). We summarized categorical variables using frequencies and percentages of participants and continuous variables using means (+/- standard deviation). Maternal baseline characteristics were stratified by HIV status at enrollment. We compared birth outcomes between HIV-exposed and HIV-unexposed infants. HIV-free survival was estimated as the proportion of children alive and HIV-negative among all exposed children. The precision around survival estimates was assessed by 95% confidence intervals. We used the Kaplan Meier curves to graphically display infant mortality, infection, and HIV free survival. We performed complete case analysis, and missing data were not imputed.

Ethical considerations

The study was approved by the Lesotho Ministry of Health Research and Ethics Committee, the Baylor College of Medicine Children's Foundation Lesotho Institutional Review Board (IRB), and the George Washington University Committee on Human Research IRB. All women were informed of the study protocol, requirements, and risks and benefits, and provided written informed consent to participate in the study.

Results

Participant characteristics

A total of 1594 pregnant women (941 HIV-negative and 653 HIV-positive) were enrolled in the study with their infants (Fig 1). Eight HIV negative women seroconverted before delivery. Delivery information was available for 95.4% of HIV positive women (623/653 and 92.2% of HIV negative women (868/941). 623 HIV positive women gave birth to 631 HIV exposed infants (HEI) and 868 HIV negative women gave birth to 879 HIV unexposed infants (HUI). Six week-follow-up information was available for 577 and 831 HEI and HUI respectively.

Characteristics of HIV-positive and HIV-negative study women at enrollment are presented in Table 1. HIV-positive pregnant women were older than their HIV-negative counterparts with a mean age of 28.7 (+/- 5.5) compared to 24.4 (+/- 5.7) years. HIV-positive women also presented earlier for ANC at a mean gestational age of 23.0 (+/- 8.7) weeks compared to 25.3 (+/- 8.2) weeks. Overall, 83.5% of the women were married, and 59.3% had disclosed their HIV-status to their partner/husband. Concerning HIV status of spouses, 4.2% of HIV negative women had HIV positive partners while 29.6% of HIV positive women had an HIV negative partner.

Among HIV-positive women, 97.8% were receiving ART at enrollment with 84.2% receiving the TDF/3TC/EFV first-line regimen. Among the 619 women with data available on the timing of ART, 249 (40.2%) initiated ART before conception and 370 (59.8%) initiated ART after conception.

Birth outcomes

Overall, 91.6% of study women delivered in a health facility and 96.8% (n = 1443) of infants were born alive (Table 2). There was no difference in the proportion of infant stillbirths, however, HIV-positive women were more likely to have had a macerated stillbirth (consistent with antepartum death), while HIV-negative women were more likely to have had an intrapartum

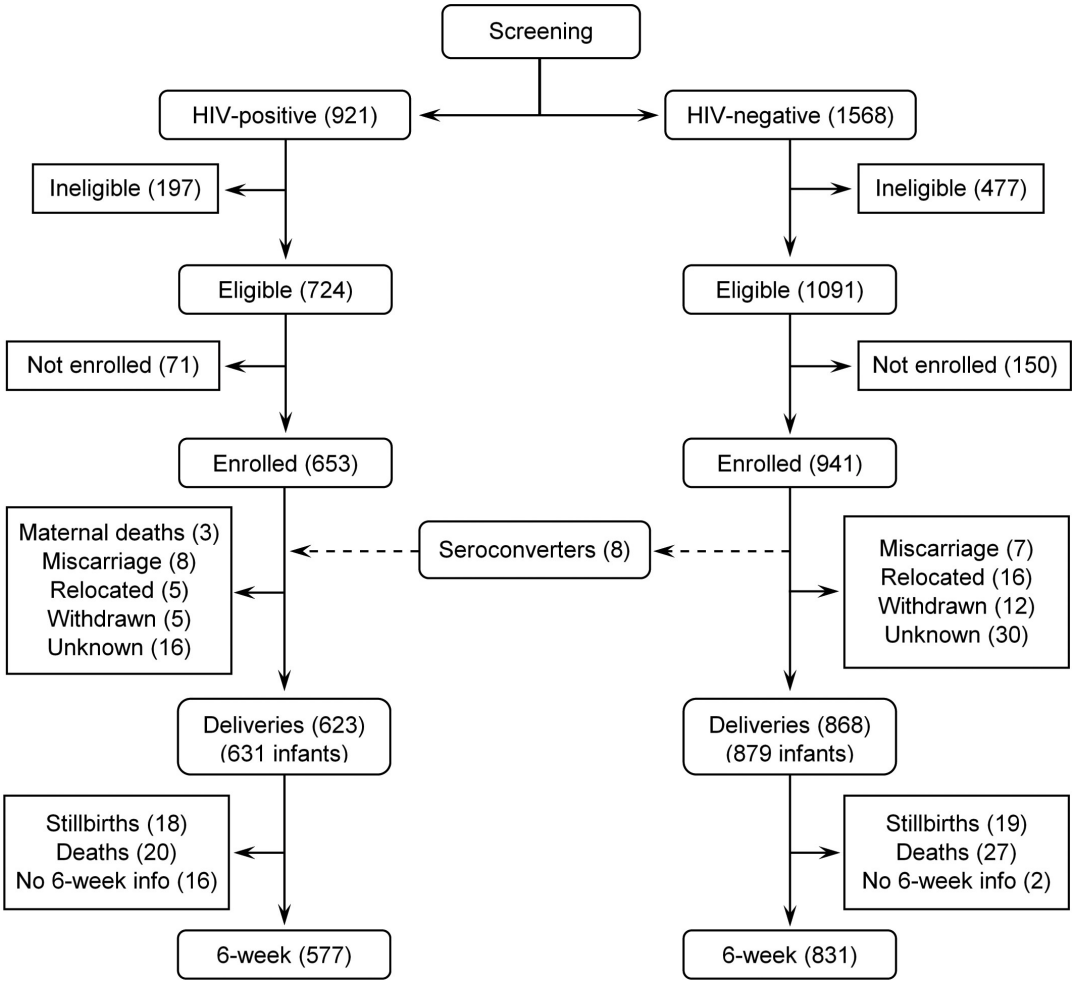

Fig 1. Study enrollment for comparison of 6-week PMTCT outcomes for HEI and HUI in the era of lifelong ART.

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death (fresh stillbirth or a liveborn infant who died within two hours of delivery). The risk of premature birth in HEI was more than double the risk of prematurity in HUI (7.8% vs 3.6%). The risk of very premature births (<32 weeks gestational age) was also substantially higher in HEI (2.2%) compared to HUI (0.4%).

Of the 249 women who initiated ART pre-conception, 24 (9.6%) had premature babies compared to 27 (7.3%) among the 370 women who initiated ART post-conception. The rates of very low weight (<1.5kgs) and low birth weight (<2.5 kgs) among women who initiated ART before conception were 1.7% and 11.6% respectively compared to 1.2% and 11.9% among women who initiated ART after conception.

Table 1. Characteristics of study women at enrollment.

	Maternal HIV Status		Total Mothers
	HIV-negative (N = 941)	HIV-positive (N = 653*)	Total (N = 1593)
	n (%)	n (%)	n (%)
Maternal age in years (mean +/- SD)	24.4 +/- 5.7	28.7 +/- 5.5	26.0 +/- 6
Gestational age at first ANC in weeks (mean, SD)	25.3 +/- 8.2	23.0 +/- 8.7	24.4 +/- 8.5
Marital Status: Married	801 (85.1)	528 (81.0)	1329 (83.4)
Mother disclosed HIV status to husband/partner	521 (55.4)	422 (64.9)	943 (59.3)
Missing Data	0	3	3
Maternal ARV Regimen at enrollment			
TDF+3TC+EFV	N/A	548 (84.2)	548 (84.2)
TDF+3TC+NVP	N/A	25 (3.8)	25 (3.8)
AZT+3TC+EFV	N/A	28 (4.3)	28 (4.3)
AZT+3TC+NVP	N/A	29 (4.5)	29 (4.5)
ART-other regimens	N/A	7 (1.1)	7 (1.1)
None	N/A	14 (2.2)	14 (2.2)
Missing data	N/A	1	1
Husband/Partner's HIV Status			
Positive	19 (4.2)	230 (68.9)	249 (31.8)
Negative	422 (94.0)	99 (29.6)	521 (66.5)
Unknown	8 (1.8)	5 (1.5)	13 (1.7)
Not tested	492	318	810
Husband/Partner Taking ARVs			
Yes	14 (73.7)	166 (72.2)	180 (72.3)
No	5 (26.3)	63 (27.4)	68 (27.3)
Unknown	0	1 (0.6)	1 (0.4)

*One woman was enrolled but excluded from analysis due to missing enrolment questionnaire data

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The rate of congenital anomalies was 0.6% and 1% among HEI and HUI respectively.

Infant survival

There were no substantial differences in the rates of infant survival at 6 weeks of age by infant HIV exposure status (Fig 2 and Table 3). When including all deaths (liveborn plus stillbirths), the estimated survival rates were 94.8% (95% CI: 93.1–96.1) among HUI and 94.0% (95% CI: 91.8–95.7) among HEI. Analysis of postnatal deaths only (excluding stillbirths), yielded estimated survival rates of 96.8% (95% CI: 95.4–97.9) and 96.7% (95% CI: 95.0–98.0) for HUI and HEI respectively. Adjusting for maternal mortality and gestational age at first ANC visit, infant HIV exposure status was not associated with early infant mortality (aOR = 1.06, 95% CI: 0.56–1.99).

Mortality and HIV-free survival among HEI

Six infants were diagnosed with HIV infection by 6 weeks of age, including 4 diagnosed at birth, and 2 diagnosed at 6 weeks of age (Table 4). The estimated HIV transmission rate among those tested at birth was 0.9% (95% CI: 0.25–2.36) and by 6 weeks the overall HIV transmission was 1.0% (95%CI: 0.38–2.23). The estimated HIV-free survival including stillbirths was 92.8% (95% CI: 90.5–94.8), and 95.6% (95% CI: 93.7–97.1) when stillbirths were excluded.

Table 2. Birth outcomes by mother's HIV status at delivery.

	Mother's HIV Status at delivery		Total Mothers N = 1491 n (%)	P-value
	HIV-negative N = 868 n (%)	HIV-positive N = 623 n (%)		
Miscarriage*	7 (0.8)	10 (1.6)	17 (1.1)	0.16
Mode of Delivery				
Vaginal	748 (86.6)	525 (84.8)	1273(85.9)	0.11
Cesarean section	116 (13.4)	94 (15.2)	210(14.1)	
Place of delivery				
Health facility	786/858 (91.6)	560/620(90.3)	1346(91.1)	
Home	67 (7.8)	53 (8.5)	120 (8.1)	
Other	5 (0.5)	7 (1.1)	12 (0.8)	
Missing data	10	2	12	
Birth Outcome				
Liveborn†	840 (96.8)	603 (96.8)	1443 (96.8)	
Antepartum death	5 (0.6)	12 (1.9)	17 (1.1)	0.01
Intrapartum death	23 (2.6)	8 (1.3)	31 (2.1)	
Newborn Maturity				
Mature	835 (96.0)	574 (91.7)	1409 (94.2)	0.001
Premature	35 (4.0)	52 (8.3)	87 (4.8)	
Very Premature delivery (<32 wks)	3 (0.4)	13 (2.2)	16 (1.1)	0.001
Premature delivery (<37 wks)	31 (3.6)	48 (7.8)	79 (1.6)	<0.01
Missing data	5	1	6	
Newborn with congenital anomalies	9 (1.0)	4 (0.6)	13 (0.9	0.56
Birth Weight in kilograms				
Normal weight	736 (90.8)	514(88.3)	1250(89.7)	0.15
Low Birth Weight (<2.5 kg)	75 (9.2)	68(11.7)	143(10.3)	
Very Low Birth Weight (<1.5 kg)	7 (0.9)	8 (1.4)	15(1.1)	0.43

*There were no miscarriages or stillbirths recorded among the 8 women who seroconverted between enrollment and delivery

†This does not include babies who were born alive and died with two hours.

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Five of the 6 HIV-infected infants had mothers that were initiated on ART post-conception (Table 5). Five of the mothers of infected infants had records of viral load at delivery and of these, 4 women had viral loads above 100,000 copies/ml. All mothers were on a TDF/3TC/EFZ regimen as per the national guidelines.

At 6 weeks, the mortality rate was higher among premature babies 24.6% (17/69) compared to mature infants 2.1% (29/1391). There was also a difference in 6-8-week mortality between low birth weight infants compared to normal birth weight infants (7.0% vs. 1.9%). Very low birth weight infants had higher risk of death within 6–8 weeks compared to infants born weighing at least 1.5kgs or more (30% vs 2.2%).

Discussion

This is the first prospective cohort study in Lesotho comparing birth and 6-week outcomes between HEI and HUI in a large cohort of HIV-positive and negative women and their infants. We found that birth outcomes of HEI were similar to those of HUI except for the frequency of prematurity, which was found to be significantly higher among HEI. While prematurity among HEI has been reported in several other studies [17–19], there have been few studies

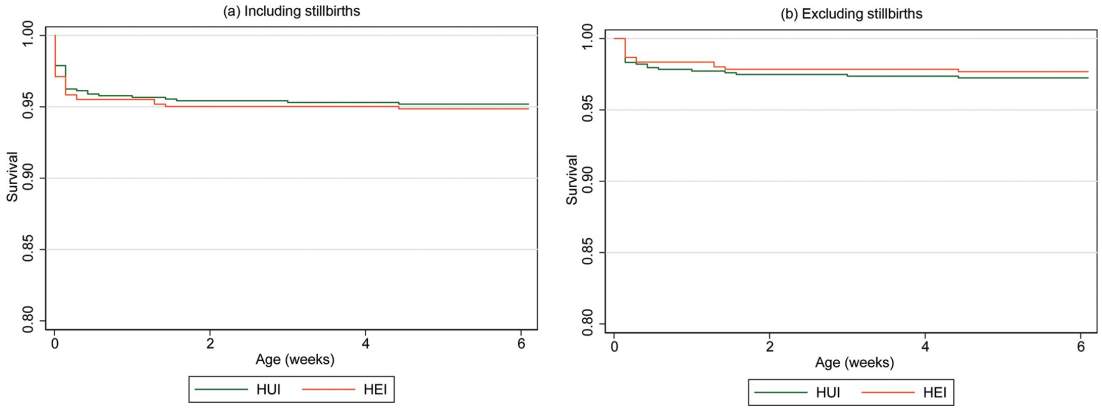

Fig 2. Survival of HUI and HEI at six weeks of age including and excluding stillbirths.

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conducted within routine health systems with large numbers of both HIV-positive and HIV-negative women in the era of lifelong ART for all pregnant women with TDF-based ART regimens. In our study, infant prematurity and low birth weight were not significantly different among women who initiated ART before conception compared to those who initiated ART after conception. This is consistent with other studies that reported no relationship between preconception ART and preterm delivery [17,18]. However, in a systematic review and meta-analysis of adverse pregnancy outcomes and timing of initiation of ART, Uthman et. al reported significantly higher risk of prematurity among HEI whose mothers initiated ART before conception compared to those who initiated ART after conception (pooled RR 1.20, 95%CI 1.01–1.44) [8].

As recommended for first-line treatment in Lesotho, 88% of HIV-positive women were on TDF-based ART regimens. In a systematic review and meta-analysis looking at safety of TDF-based regimens in pregnancy for HIV-positive women and their infants, the rates of prematurity and stillbirths were significantly lower among women on TDF-based ART compared to other ART regimens [20, 21]. However, even with the use of TDF regimens, our study showed that HEI still had a higher risk of prematurity compared to HUI.

We found that HEI were more likely to die antepartum (1.9% versus 0.6%), consistent with medical complications, while HUI were more likely to die during the intrapartum period (2.6% versus 1.3%), consistent with obstetrical complications. However, it may be important to note that these women were followed up only from their first ANC and we may have missed some of the antepartum deaths which occurred before women were enrolled in the study. A number of studies have explored causes of antepartum death among HEI. In South Africa and Botswana, maternal vascular malperfusion was more frequent among HIV-positive women

Table 3. Survival of HUI and HEI at six weeks of age.

	HIV-Unexposed		HIV-Exposed	
	Death (rate)	Survival (95% CI)	Death (rate)	Survival (95% CI)
Including stillbirths	46/877 (5.2%)	94.8% [93.1–96.1]	38/631 (6.0%)	94.0% [91.8–95.7]
Excluding stillbirths	27/858 (3.2%)	96.8% [95.4–97.9]	20/613 (3.3%)	96.7% [95.0–98.0]

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Table 4. HIV-free survival at six weeks of age.

	HIV-Exposed Infants			
	Death (rate)	HIV infection (rate)	Infected/Death (rate)	HIV-Free Survival (95% CI)
Including stillbirths	38/627 (6.1%)	6/581 (1.0%)	44/613 (7.2%)	92.8% [90.4–94.7]
Excluding stillbirths	20/609(3.3%)	6/581 (1.0%)	26/595(4.4%)	95.6% [93.7–97.1]

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and placenta insufficiency associated with hypertension accounted for most stillbirths [22,23]. In our study, postpartum infant mortality at 6 to 8 weeks was independently associated with gestational age, but not with HIV exposure status.

We found one of the lowest 6-week HIV transmission rates (1.0%) reported in Lesotho coupled with very high HIV-free survival among liveborn infants at 6 weeks of age. This is a significant improvement compared to the estimated 6-week transmission of 7% reported in Lesotho in 2016 and in other countries in the region [1, 24]. Two-thirds of the HIV-positive infants identified were infected in utero. Most women presented for their first ANC visit toward the end of the second trimester, contrary to the WHO recommendation for women to present during the first trimester. Early ANC visits are especially important for HIV-positive pregnant women because earlier ART initiation may further reduce MTCT in utero [12,25]. This was buttressed by the findings that almost all women who transmitted HIV to their infants had a high viral load despite being on ART. Therefore, we agree with Smith et. al that it is essential to maximize viral suppression for HIV-positive women on ART [25].

Our findings indicate that the current universal ART program within the setting of routine care is effective. Implementation of this program showed that high ANC utilization, and high uptake of ART during pregnancy, including a high proportion of facility-based deliveries, collectively led to improved pregnancy outcomes among HIV-positive women. Our study also demonstrates the importance of incorporating implementation research to document program effectiveness within routine, public health settings. Lesotho's experience shows that when PMTCT programs are well implemented, routine program setting can achieve high effectiveness, comparable to more controlled research settings.

A limitation of our study is that it measured birth outcomes and 6-week HIV-free survival in a facility-based population and may have missed women and children who did not seek care in health facilities. We may have also missed women who lost pregnancies early. In addition, our study may have potential systematic errors arising from estimation of gestational age with the potential of misclassifying baby's maturing at delivery. However, since ANC attendance in Lesotho is higher than in many African countries, we believe that the results are

Table 5. Timing of ART initiation and maternal viral load at delivery for HIV-infected infants.

Infant #	Maternal age (years)	Gestational age at first ANC (weeks)	Timing of ART initiation	Maternal duration on ART before delivery (months)	Infant maturity and weight (kg) at birth	Maternal ART regimen at enrollment +	Maternal VL at delivery (copies/ml)
1	23	32	Pre-conception	32.5	Mature—2.4	TLE	36,881
2	22	24	Post-conception	3.5	Mature—3.5	TLE	109,000
3	32	18	Post-conception	5.6	Mature—3.5	TLE	428,054
4	18	18	Post-conception	4.6	Mature—2.8	TLE	320,000
5	23	10	Post-conception	6.9	Mature—2.8	TLE	100,062
6	25	29	Post-conception	2.6	Mature—2.9	TLE	-

[+]All mothers were initiated on Tenofovir-Lamivudine-Efavirenz (TLE) after HIV diagnosis and remained on this regimen throughout the study.

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reflective of the Lesotho context [26, 27]. The successful reduction of perinatal HIV transmission that we report may not be comparable in areas with lower ANC attendance and low rates of facility-based deliveries. Additionally, study sites were purposively selected so the findings may not be generalizable to the whole country of Lesotho, especially to low volume facilities, which were not included in the study. However, the purposive selection of sites from the three geo-ecological settings in Lesotho (highlands, foothills, lowlands) does account for the variances in health-seeking behaviors.

Conclusion

Implementation of universal maternal ART was associated with low MTCT among infants at 6 weeks of age with no differences in congenital anomalies or early mortality between HEI and HUI. However, HEI continue to have increased rates of prematurity even in the era of lifelong combination ART.

Supporting information

S1 File. S3_File.excel birth outcomes data set.
(XLSX)

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Author Contributions

Conceptualization: Appolinaire Tiam, Seble G. Kassaye, Rhoderick Machekano, Michelle M. Gill, Majoalane Mokone, Mosilinyane Letsie, Mots'oane Tsietso, Irene Seipati, Anthony Isavwa, Laura Guay.

Data curation: Appolinaire Tiam, Seble G. Kassaye, Rhoderick Machekano, Vincent Tukei, Michelle M. Gill, Janety Barasa, Thorkild Tylleskär, Laura Guay.

Formal analysis: Appolinaire Tiam, Rhoderick Machekano.

Funding acquisition: Appolinaire Tiam.

Methodology: Appolinaire Tiam, Seble G. Kassaye, Rhoderick Machekano, Vincent Tukei, Michelle M. Gill, Majoalane Mokone, Mosilinyane Letsie, Mots'oane Tsietso, Irene Seipati, Anthony Isavwa, Thorkild Tylleskär, Laura Guay.

Project administration: Appolinaire Tiam, Vincent Tukei, Laura Guay.

Resources: Appolinaire Tiam, Majoalane Mokone.

Software: Rhoderick Machekano, Anthony Isavwa.

Supervision: Appolinaire Tiam, Vincent Tukei, Michelle M. Gill, Majoalane Mokone, Mots'oane Tsietso, Irene Seipati, Janety Barasa, Laura Guay.

Writing – original draft: Appolinaire Tiam.

Writing – review & editing: Seble G. Kassaye, Rhoderick Machekano, Vincent Tukei, Michelle M. Gill, Majoalane Mokone, Mosilinyane Letsie, Mots'oane Tsietso, Irene Seipati, Janety Barasa, Anthony Isavwa, Thorkild Tylleskär, Laura Guay.

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III

18-24-month HIV-free survival as measurement of the effectiveness of prevention of mother-to-child transmission in the context of lifelong antiretroviral therapy: results of a community-based survey.

Authors: Appolinaire Tiam[1]*, Michelle M. Gill[1], Rhoderick Machekano[1], Vincent Tukei[3], Majoalane Mokone[3], Shannon Viana[1], Mosilinyane Letsie[4], Mots'oane Tsietso[4], Irene Seipati[4], Cecilia Khachane[4], Marethabile Nei[3], Florence Mohai[3], Thorkild Tylleskär[5], Laura Guay[1,4]

1. Centre for International Health, University of Bergen, Bergen, Norway, Elizabeth Glaser Pediatric AIDS Foundation, Washington D.C. United State of America
2. Elizabeth Glaser Pediatric AIDS Foundation, Maseru, Lesotho
3. Ministry of Health, Maseru, Lesotho
4. Milken Institute School of Public Health, The George Washington University, Washington D.C., United States of America
5. Centre for International Health, University of Bergen, Bergen, Norway

* Corresponding author

Email: atiam@pedaids.org

Abstract

Introduction

Population-based HIV-free survival at 18-24 months of age among HIV-exposed infants in high prevalence settings in the era of treatment for all is largely unknown. We conducted a community-based survey to determine outcomes of HIV-exposed infants at 18-24 months in Lesotho.

Methods

Between November 2015 and December 2016, we conducted a survey among households with a child born 18-24 months prior to data collection. Catchment areas from 25 health facilities in Butha-Buthe, Maseru, Mohale's Hoek and Thaba-Tseka districts were randomly selected using probability proportional to size sampling. Consecutive households were visited and eligible consenting caregivers and children were enrolled.

Rapid HIV antibody testing was performed on mothers of unknown HIV status (never tested or tested HIV-negative >3 months prior) and their children, and to children born to known HIV-positive mothers. Information on demographics, health-seeking behavior, HIV, and mortality were captured for mothers and children, including those who died. The difference in survival between subgroups was determined using the log-rank test.

Results

Of the 1,852 mothers/caregivers enrolled, 570 mothers were HIV-positive. The mother-to-child HIV transmission rate was 5.7% [95% CI: 4.0-8.0]. The mortality rate was 2.6% [95% CI: 1.6-4.2] among HIV-exposed children compared to 1.4% (95% CI: 0.9 – 2.3)

among HIV-unexposed children. HIV-free survival was 91.8% [95% CI: 89.2-93.8] among HIV-exposed infants. Disclosure of mother's HIV status (aOR = 4.9, 95% CI: 1.3-18.2) and initiation of cotrimoxazole prophylaxis in the child (aOR = 3.9, 95% CI: 1.2-12.6) were independently associated with increased HIV-free survival while child growth problems (aOR = 0.2, 95% CI: 0.09 – 0.5) were independently associated with reduced HIV-free survival.

Conclusion

Even in the context of lifelong antiretroviral therapy among pregnant and breastfeeding women, HIV has a significant effect on survival among HIV-exposed children compared to unexposed children. Lesotho has not reached elimination of HIV transmission from mother to child.

Introduction

HIV-free survival is the gold standard in measuring the effectiveness of prevention of mother-to-child HIV transmission (PMTCT) programs especially among breastfeeding populations [1,2].

Since 2010, new HIV infections among children have declined by 35%, from 270,000 [170,000–400,000] in 2010 to 160,000 [110,000–260,000] in 2018, demonstrating a nearly 64% decline since 2001when almost half a million of children were newly infected with HIV [3]. Most of the infections among children remain attributable to mother-to-child transmission (MTCT) [4,5]. Program data are now starting to emerge, demonstrating the effectiveness of these approaches in preventing MTCT

using real world data which strengthen global reports that use modelling data [2,6-8]. Most of these studies report HIV-free survival before lifelong antiretroviral therapy (ART) was rolled out.

With an estimated HIV prevalence of 25.6%, Lesotho has the second-highest national HIV prevalence worldwide [9,10]. Among antenatal care (ANC) attendees, HIV prevalence ranges from 5.4% among adolescents aged 15-19 years, to 21.5% among women aged 20-24 years, 37.5% among those aged 25-29 years, and 40% among those aged 30-39 years [11].

Lesotho's national guidelines recommend that opt-out HIV testing be offered to all pregnant women presenting to ANC clinic or in labor with unknown HIV status. If HIV-negative, women should be retested for HIV at 36 weeks gestation if the prior test was performed ≥ 6 weeks earlier, or if not done prior to delivery, then HIV testing should be done during labor and delivery [12]. HIV deoxyribonucleic acid polymerase chain reaction (DNA-PCR) or total nucleic acid (TNA) testing is recommended for infants younger than nine months of age in Lesotho. Infants with a positive HIV DNA-PCR or TNA test result are initiated on ART immediately while awaiting results of the repeat confirmatory DNA-PCR test. Final documentation of the child's HIV status is conducted six weeks following cessation of breastfeeding using HIV DNA-PCR testing among infants younger than nine months of age, or HIV rapid testing, with DNA-PCR if the rapid test is positive, among infants and children older than nine months of age [12].

Traditionally, MTCT rates are assessed at health facility level and focused on six-week MTCT, though in breastfeeding populations, children are exposed to HIV through breastmilk for a prolonged period of time [13]. Facility-based HIV-free survival rate

estimates are potentially biased because they exclude women and children who do not utilize health facilities especially in populations where formal health care utilization rates are low. Community-based studies to estimate HIV-free survival among HIV-exposed children overcome some bias concerns in facility-based studies. However, since 2013, when the Lesotho Ministry of Health (MOH) adopted the World Health Organization PMTCT guidelines on universal treatment [8], no community-based assessment of HIV-free survival has been undertaken in Lesotho. The Lesotho population-based impact assessment survey found an overall MTCT rate of 2.8% [14,15]. We carried out a community household survey to estimate the HIV-free survival of HIV-exposed children 18-24 months of age after the introduction of lifelong ART.

Materials and methods

Study design

Between November 2015 and December 2016, we conducted a cross-sectional, community-based survey among households with children born in the previous 18-24 months to estimate MTCT rates, mortality, HIV prevalence, and HIV-free survival among children.

Sampling procedures

Four districts were purposively selected for the study: Butha-Buthe, Maseru, Mohale's Hoek, and Thaba Tseka. The first three districts represent the three national ecological zones: highlands, foothills and lowlands. The ecological zones account for variation in overall PMTCT service delivery coverage and heterogeneity in health-seeking behavior due to changing terrain. Maseru was included as the most populous

district in the country. The selection of these districts was expected to make the sampling nationally representative. Of the 78 facilities across the four districts, 16 were excluded from the sampling frame, including facilities that were determined to be difficult to reach (e.g., typically only accessible by helicopter). Within each district, we used a multi-stage sampling approach to obtain a representative sample of HIV-positive and HIV-negative mothers and children. First, health facilities from each study district were randomly selected using probability proportional to size sampling based on the annual number of live births. Ten facilities from Maseru District and five facilities each from the other three districts were randomly selected, for a total of 25 facilities. Communities were defined as the villages within the catchment area of a health facility. In each catchment area, all villages were assigned a number and then random selection was used to indicate the village from which recruitment would begin. Recruitment continued according to a pre-determined direction in the next closest village until the approximated sample size target was obtained. Study teams recruited participants from all households meeting the inclusion criteria within a village. If the study team reached approximately half of the target for that catchment area in one village, the team then stopped recruitment and moved to the next village to ensure at least two villages were captured for each catchment area.

To determine the sample size per facility catchment area, we estimated the expected number of HIV-infected women who had given birth in each area proportionate to the catchment population size. To identify eligible households within the catchment area, we went door-to-door or engaged community health workers to identify households in which a mother had delivered a child 18-24 months prior to data

collection. If the mother or a primary caregiver was not available to complete the survey, a return appointment was made.

Recruitment and enrollment of study population

The study population included mothers and children who were born 18-24 months prior to the data collection time. Household visits were made by trained research assistants responsible for consenting and data collection, and HIV counselors responsible for HIV testing. All eligible households were included in the study if the primary caregiver was willing to provide verbal informed consent. Households were eligible for study participation, regardless of the vital status of the child (alive/deceased). Households were excluded if members of household were not willing to participate, or if the primary caregiver was less than 18 years and was not the father or mother of the child. Households were visited consecutively, informed about the study, and caregivers were invited to participate in the study. If the mother was deceased or was not currently living in the household, consent for study participation was obtained from the child's primary caregiver.

Data collection

Caregivers or parents were interviewed in Sesotho or English by research assistants using a structured data collection tool with responses captured electronically. The questionnaire was developed specifically for the purpose of this study and collected data needed to answer study questions. Research assistants first determined the child's HIV exposure status and then administered the questionnaire to the mother or caregiver. Information collected from the questionnaire included demographics, use of

health facilities for HIV and maternal and child health services, maternal HIV status, maternal and infant receipt and use of antiretroviral drugs during pregnancy and after delivery (if known HIV-positive), infant feeding practices, and general well-being, including growth and development. The child health card and/or maternal health card were used to confirm caregiver information. Mortality information for mothers and children who died was also captured, and attempts were made to determine HIV status prior to death.

Biologic measurements

During household visits, blood was collected through finger stick from study participants by a trained community counselor for HIV rapid antibody testing according to the national HIV program testing algorithm. The first test performed was the Determine™ HIV-1/2 (manufactured by Alere). Participants with a negative Determine™ assay were told their HIV negative status. All participants who tested positive underwent a second test (with a new finger prick sample) using Uni-Gold™ Recombigen® HIV-1/2 and were told their HIV positive status if the second test was also positive. All maternal participants had a DBS sample taken to be stored in the laboratory for quality control. In addition, DNA-PCR testing was performed on the DBS sample for all maternal participants to confirm the status recorded from the field. Further, DBS specimens were collected from all enrolled children for HIV DNA-PCR confirmatory testing regardless of rapid test results.

All tests conducted for this study were recorded in a log at the testing laboratory using the unique study identification number. Study laboratory test results were

obtained directly from the laboratory and entered into the study database to link questionnaire responses with laboratory results. Study data collectors and investigators reviewed laboratory results and identified discordant or confirmatory results that required follow-up by the HIV counselors.

Sample size calculation and Data analysis

Based on previous PMTCT evaluation studies in Rwanda and Malawi, we expected HIV-free survival among 18-24-month-old HIV-exposed infants would be lower than the estimate from Rwanda (91.1%) due to less extensive PMTCT coverage in Lesotho in comparison to Rwanda [16]. Assuming HIV-free survival in Lesotho was about 85%, we needed 545 HIV-exposed infants to estimate HIV-free survival with ±3% precision. Adjusting the sample size by 10% for miscellaneous events (e.g., missing data, consent to some but not all study activities) we targeted to enroll 600 HIV-exposed infants. Given the HIV prevalence of approximately 25.4% among pregnant women in ANC, we would need to visit or approach 2,363 households with children in the 18-24-month age range to identify 600 HIV-exposed infants. To account for potentially 15% refusal rate to participate in the study and interviews with non-maternal caregivers of HIV-exposed children (who may not be able to adequately answer questions needed to address the research aims) we needed to visit approximately 2,700 households to reach the required sample of HIV-exposed infants and their mothers.

We summarized categorical variables using frequencies and percentages. Continuous variables were summarized using means and standard deviations, or medians and interquartile ranges as appropriate. We estimated HIV-free survival as the

proportion of children alive and HIV negative among all HIV-exposed children. The precision around survival estimates was summarized using 95% confidence intervals. We used the log-rank test to determine whether there were differences in HIV-free survival between subgroups. Comparisons included HIV-infected versus HIV-exposed but uninfected children; and HIV-exposed versus HIV-unexposed children. Type I error rate (α) for statistical tests was set at 0.05. We performed complete case analysis, and missing data were not imputed.

We used multivariate logistic regression models to test the independent association of ANC attendance, mode and place of delivery, gestational age at birth, birth weight, maternal vital status, adherence to and timing of initiation of antiretroviral drugs, infant feeding method and nutritional status with HIV-free survival among HIV-exposed children. All statistical data analysis was performed using STATA version 14.2 (Stata Corp).

Ethical considerations

The study was approved by the Lesotho MOH Research and Ethics Committee and the George Washington University's Office of Human Research. All participants were informed of the study objectives, were given the opportunity to ask questions and provided verbal informed consent to participate in the study.

Once a household was determined to meet the eligibility criteria, the mother/caregiver of the eligible child was identified and informed about the evaluation. The verbal informed consent text included statements that described the purpose of the evaluation, the activities to be conducted and confidentiality protections, in addition to a specific

statement for mothers/caregivers to provide consent for themselves and their children (if applicable). The interviewer read each of these statements in the participants' language of choice (English or Sesotho) exactly as written and documented the response on an electronic device used for data collection with a password verification process. The mother/caregiver had the opportunity to ask questions regarding enrollment into the evaluation. The consent process was structured so that a participant could agree to participate in some activities, but not others. Biological mothers were the preferred participants, but if the mother was deceased or not currently living in the household, consent was obtained from the child's primary caregiver, who was asked to respond to questions to the extent possible.

Results

Between November 2015 and December 2016, we screened 11,169 households for the study eligibility (Fig 1). Of the 2,190 eligible households, 1,852 mothers/caregivers consented and were enrolled in the study. Of the 338 households that declined to participate, the following reasons were provided: caregiver, child or both were unavailable (n=178), (157 of whom were at work); lack of time to participate (n=71); no incentive provided (n=25); not comfortable to participate (n=16); caregiver did not feel they had enough information to answer questions (n=15); mother needed husband's permission (n=9); caregiver was below 18 years of age (n=6); and other reasons (n=18).From the enrolled households, there were 1,884 deliveries (including 66 twin births) that occurred 18-24 months prior to data collection. Most of the children (77.1%, n=1,428) were being cared for by their biological mothers. Nearly a third

(30.7%) of biological mothers reported and knew their HIV-positive status. Thirty-nine mothers were newly diagnosed HIV-positive during the study, of whom 38 had previously tested HIV-negative during the index pregnancy. A total of 1,199 (64.7%) biological mothers were HIV-negative and 83 mothers were of unknown HIV status and unavailable for testing

Fig 1. Participants Screening, Enrollment, and Outcomes

Characteristics of caregivers

The mean age (±SD) of mothers was 27.8 ± 6.1 years (Table 1). Overall, 95.8% of mothers attended ANC at least once with over 80% of women delivering in health facilities. One-fifth (20.8%) of mothers presented with known HIV status at ANC. Most HIV-positive mothers (94.7%) disclosed their HIV status with 59.9% disclosing it to their spouse or partner.

Table 1. Antenatal, Delivery and HIV Service Utilization by Study Participants

	HIV-negative Mothers (N= 1199) n (%)	HIV-positive Mothers (N=570) n (%)	Mothers with Unknown HIV Status (N=83) n (%)	Total (N=1852) n (%)
Mean Age (SD)	26.7 (5.9)	30.1 (6.0)	27.7 (5.2)	27.8 (6.1)
Attended ANC during pregnancy	1152 (96.4)	530 (94.8)	56 (93.3)	1738 (95.8)
Mean gestational age in weeks at first ANC (SD)	16.7 (7.5)	16.6 (7.9)	14.7 (8.1)	16.7 (7.6)
Attended 4+ ANC visits	710 (70.6)	302 (68.8)	7 (53.8)	1019 (70.0)
Child delivered at a health facility	934 (81.1)	416 (78.5)	45 (83.3)	1395 (80.4)
Mother known HIV-positive during pregnancy	-	114 (20.8)	-	114 (6.6)
Mother tested for HIV during pregnancy	1161 (98.1)	418 (73.3)	8 (80.0)	1587 (85.7)
Mother disclosed HIV status to anyone	918 (79.7)	514 (94.7)	-	1432 (84.3)
Mother disclosed HIV status to spouse/partner	706 (76.9)	308 (59.9)	-	1014 (70.8)
Mother disclosed HIV status to other family members	362 (39.4)	373 (72.6)	-	735 (51.3)
Mother disclosed HIV status to others	4 (0.4)	26 (5.1)	-	49 (3.4)

Characteristics of children

The child participants included 64 twins (3.4%) and 52.5% of the children were male (Table 2). Preterm births occurred in 9.1% of children, with HIV-exposed children slightly more likely to have a preterm birth. Overall, 95.2% of infants were ever breastfed, with a breastfeeding rate of 90.7% among HIV-exposed infants. The mean (SD) birth weight was 3.0 (0.5) with HIV exposed infants having slightly lower mean birth weight compared to HIV unexposed infants. HIV-unexposed infants were breastfed longer with a mean of 15 months compared to HIV-exposed infants, who were breastfed for a mean of 11 months.

Table 2. Characteristics of Children by HIV Exposure Status

	HIV-unexposed children (n=1207) n (%)	HIV-exposed children (n=582) n (%)	Children with unknown HIV exposure (n=95) n (%)	Total (N=1884)
Twin	34 (2.8)	30 (5.1)	0 (0)	64 (3.4)
Male	609 (50.5)	324 (55.7)	56 (59.0)	989 (52.5)
Stillbirth	4 (0.3)	2 (0.3)	2 (2.1)	8 (0.4)
Preterm birth	106 (9.0)	54 (9.9)	3 (3.6)	163 (9.1)
Mean birth weight (SD)	3.05 (0.5)	2.9 (0.6)	3.06 (0.4)	3.02 (0.5)
Child ever breastfed	1169 (97.6)	519 (90.7)	81 (91.0)	1769 (95.2)
Child still breastfeeding after 18 months	200 (17.1)	47 (9.1)	3 (3.7)	250 (14.1)
Median time child was breastfed (IQR) (months)	15 (10 – 18)	11 (6 – 14)	12 (6 – 16)	12 (7 – 18)

There were 37 reported child deaths, 16 of whom were from HIV-positive mothers. Thirty-three children were identified as HIV-positive within the participating households among whom 10 were newly diagnosed during the study. Of the ten children who were newly diagnosed with HIV, five had previously tested HIV-negative, three were from known HIV-positive mothers but had never been tested, and two were from mothers who were newly diagnosed during the study.

Maternal use of antiretroviral therapy

Overall, 86.8% of HIV-positive women used ART during pregnancy. Based on child HIV status, 87.4% and 71.4% of mothers of HIV-uninfected children and HIV-infected children were enrolled on ART respectively (Table 3). Most mothers (89.1%) initiated ART either before or during pregnancy. Among mothers of HIV-infected children, 37.6% started ART either during or after breastfeeding period compared to 9.6% of mothers of HIV-uninfected children. At the time of the study, 88.0% of HIV-positive mothers reported currently taking ART. Among HIV-exposed children, 89.3% received nevirapine prophylaxis within three days of delivery. Of the 13 HIV-infected children who had records about their treatment initiation, 12 were initiated on ART.

Table 3: Maternal Use of Antiretroviral Drugs During Pregnancy by Child's HIV Status, Excluding Caregivers

	Uninfected, HIV-exposed children (N=367) n (%)	Infected, HIV-exposed children (N=19) n (%)	Total HIV-exposed children (N=396)
Mother had a CD4 test done when pregnant	282 (83.7)	12 (75.0)	294 (85.3)
Missing	30	3	33
Mother received ART during this pregnancy	285 (87.4)	10 (71.4)	295 (86.8)
Missing	41	5	46
Medicine received by mothers who took antiretroviral drugs during pregnancy*			
AZT	16 (5.7)	0	16 (5.5)
ART for life	261 (92.9)	10 (100.0)	271 (93.1)
Other	4 (1.4)	0	4 (1.4)
Missing	4	0	4
Time mothers started taking ART*			
Before pregnancy with this child	131 (39.5)	4 (25.0)	135 (38.8)
During pregnancy with this child	169 (50.9)	6 (37.5)	175 (50.3)
During breastfeeding of this child	20 (6.0)	5 (31.3)	25 (7.2)
After breastfeeding of this child	12 (3.6)	1 (6.3)	13 (3.7)
Missing	35	3	38

Regimen taken by the mother during pregnancy†			
TDF+3TC+EFV	204 (80.3)	8 (80.0)	212 (80.3)
TDF+3TC+NVP	16 (6.3)	2 (20.0)	18 (6.8)
AZT+3TC+EFV	10 (3.9)	0	10 (3.8)
AZT+3TC+NVP	21 (8.3)	0	21 (8.0)
Other	3 (1.2)	0	3 (1.1)
Missing	7	0	7
Mother currently taking ART	313 (88.2)	16 (84.2)	329 (88.0)

300* Total N is among those who received antiretroviral drugs during pregnancy
301†Total N is among those who received ART for life
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304 Mortality, HIV transmission, and HIV-free survival among

305 children

306 Tables 4a and 4b summarize child outcomes by HIV exposure status. The
307 estimated child mortality was 2.6% (95% CI: 1.6- 4.2) among HIV-exposed children
308 compared to 1.4% (95% CI: 0.9 – 2.3) among HIV-unexposed children. The estimated
309 HIV transmission was 5.7% (95% CI: 4.0-8.0). HIV-free survival was 91.8% (95% CI:
310 89.2-93.8). The majority of child deaths occurred after the neonatal period (Table 4b).

311 **Table 4a: Child Mortality and HIV-Free Survival by HIV Status**

Child Outcomes	HIV-unexposed Children % [95% CI]	HIV-exposed Children % [95% CI]	Total Children % [95% CI]
Child mortality	1.4 [0.9 – 2.3]	2.6 [1.6 – 4.2]	1.8 [1.3 – 2.5]
HIV infection	-	5.7 [4.0 – 8.0]	-
HIV-free survival	-	91.8 [89.2 – 93.8]	-

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Table 4b. Timing of Children's Death by HIV Exposure (N=28)

Timing of death	HIV-unexposed children n (%)	HIV-exposed children n (%)	Children with unknown status n (%)	Total Children (N=28)
Day 0	4 (26.7)	0	2 (100)	6 (21.4)
Within 24 hours	1 (6.7)	1 (9.1)	0	2 (7.2)
Within 28 days	3 (20.0)	3 (27.3)	0	6 (21.4)
More than 28 days	7 (46.6)	7 (63.6)	0	14 (50.0)

Factors associated with HIV-free survival among children

Table 5 summarizes factors associated with mortality, HIV transmission and HIV-free survival among children. Disclosure of mother's HIV status (aOR = 4.9, 95% CI: 1.3-18.2) and initiation of cotrimoxazole prophylaxis in child (aOR = 3.9, 95% CI: 1.2-12.6) were independently associated with increased HIV-free survival while child growth problems (aOR = 0.2, 95% CI: 0.09 – 0.5) was independently associated with reduced HIV-free survival.

Table 5. Factors Associated with child mortality, HIV transmission and HIV-free survival

Variable	Unadjusted OR [95% CI]	p-value	Adjusted OR [95% CI]	p-value
Factors Associated with Infant Mortality				
Infant was a twin (ref=No)	7.1 [3.0-16.8]	0.00	1.4 [0.2-8.7]	0.70
Mother HIV-positive (ref=Negative)	1.9 [0.9-3.7]	0.09	2.0 [0.6-6.8]	0.25
Child delivered in health facility (ref=No)	0.3 [0.1-0.5]	0.00	0.3 [0.1-1.0]	0.06
Timing of child birth (ref=before time)	0.3 [0.1-0.9]	0.02	0.5 [0.1-2.0]	0.32
Mother told child had a growth problem (ref=No)	4.0 [1.5-10.6]	0.01	2.8 [0.8-9.8]	0.11
Child ever breastfed (ref=No)	0.1 [0.0-0.2]	0.00	0.2 [0.0-0.9]	0.04
Infant breastfed for more than 6 months (ref=No)	0.2 [0.1-0.5]	0.00	0.7 [0.2-2.9]	0.60
Factors Associated with HIV Infection				
Disclosed HIV test result (ref = no)	0.3 [0.1-0.9]	0.04	0.3 [0.1-1.7]	0.18
Mother told child had a growth problem (ref=no)	2.6 [1.1-6.4]	0.03	4.6 [1.5-13.5]	0.01
Breastfeeding for 6+ months (ref=no)	2.3 [0.9-5.6]	0.06	2.3 [0.8-6.5]	0.12
Started ART (ref=before BF)				
During pregnancy	1.5 [0.5-4.4]	0.49	1.2 [0.4-3.9]	0.75
During breastfeeding	9.3 [2.6-32.9]	0.00	3.2 [0.6-15.9]	0.16
Not on ART/After breastfeeding	6.3 [1.8-22.2]	0.00	1.9 [0.2-17.0]	0.57
Received ART throughout breast feeding (ref=yes)	0.3 [0.1-0.8]	0.01	2.2 [0.4-12.8]	0.38
Child was given first dose of NVP syrup within 3 days after delivery (ref = no)	0.3 [0.1-0.6]	0.00	0.3 [0.1-1.1]	0.08
Child was given CTX to take daily beginning at 6 weeks (ref = no)	0.2 [0.1-0.6]	0.00	0.4 [0.1-1.5]	0.18
Factors Associated with HIV-free Survival				
Disclosed HIV test result (ref = no)	2.9 [1.0-8.1]	0.04	4.9 [1.3-18.2]	0.017
Delivered in a facility (ref = no)	2.3 [1.4-4.7]	0.00	1.7 [0.7-4.2]	0.22
Mother told child had a growth problem (ref=no)	0.2 [0.1-0.5]	0.00	0.2 [0.1-0.5]	<0.01
When started ART (ref=before BF)				
During pregnancy	0.7 [0.3-1.6]	0.39	0.7 [0.3-1.8]	0.44
During breastfeeding	0.1 [0.0-0.4]	0.00	0.3 [0.1-1.3]	0.11
Not on ART/After breastfeeding	0.2 [0.1-0.6]	0.01	0.7 [0.1-4.2]	0.69
Received ART throughout breast-feeding (ref=yes)	2.8 [1.3-5.9]	0.01	0.4 [0.1-1.8]	0.26
Child was given first dose of NVP syrup within 3 days after delivery (ref = no)	4.0 [2.2-7.5]	0.00	2.1 [0.6-7.0]	0.22
Child was given CTX to take daily beginning at 6 weeks (ref = no)	5.4 [2.7-10.8]	0.00	3.9 [1.2-12.6]	0.02

Discussion

We found that HIV-free survival in Lesotho was 91.8%. Our results demonstrate the effectiveness of the Lesotho PMTCT program in averting HIV infection and deaths in children within the first 18-24 months of life after introduction of lifelong ART for all HIV-positive pregnant women in 2013. Our finding corroborates with a systematic review of 18 studies published between 2005 and 2015, that estimated HIV-free survival in a breastfed population found that 18-month HIV-free survival estimates were 89.0% (95% CI 83.9%, 94.2%) with maternal ART for six months (five studies) and 96.1% (95% CI: 92.8%-99.0%) with maternal lifelong ART (three studies) [17]. HIV-free survival at 24 months was 89.2% (95% CI: 79.9%-98.5%) for children whose mothers were on ART for six months (two studies) [17]. Among breastfed infants, HIV-free survival ranged from 87% (95% CI: 78%-92%) to 96% (95% CI: 91%-98%).

Similarly, in a study in Eswatini, that reported HIV-free survival prior to the era of lifelong ART for pregnant and breastfeeding women in that country, HIV-free survival was 95.9% (95% CI: 94.1-97.2) though the authors reported a low death rate due to cultural sensitivities in collecting death data which may have impacted overall HIV-free survival [8].

Although approaching elimination, the MTCT rate estimated in our study was 5.7% [95%CI 4.0 – 8.0]. In the context of Lesotho, this shows much progress compared to the UNAIDS MTCT rates reported to be 12.7% (9.9-14.4) in 2019, and 30% in 2004 [18]. As UNAIDS estimates are based on models, which make assumptions that may not be accurate, estimates from surveys like ours are more reliable. The current

UNAIDS estimates were based on PMTCT coverage of 77% (59-89) and early infant diagnosis coverage of 70% (60-90) (15). Further, assuming all children who died were HIV-infected, the percentage of children who died or were HIV infected was 8.2% [95%CI 6.2-10.8] versus 12.7% (9.9-14.4) for UNAIDS transmission. Although mortality among HIV-infected children is high, at most only 50% of HIV-infected children will die by their second birthday making it unlikely that all children who died were HIV-infected [4-7]. However, much needs to be done to ensure women living with HIV are diagnosed even ahead of their index pregnancy or ideally ahead of their first pregnancy because women who start ART before pregnant and are virally suppressed are less likely to transmit HIV to their babies in utero, perinatally or through breastfeeding. The MTCT rate in Lesotho remains unacceptably high, and over three years after the rollout of lifelong ART for HIV-positive pregnant women, the program has not yet achieved elimination (an MTCT rate of < 5% and 2% in breastfeeding and non-breastfeeding population respectively) [19]. In addition, our study shows differences between MTCT rates reported from clinical trials and real world rates. Former studies have consistently reported MTCT rates lower than 5% [18,20, 21].

One-third of HIV-infected children were diagnosed during the study. Eight of the ten children have been in contact with health system for various services. However, contrary to the recommendation that all HIV-exposed children should have the final HIV test at 18-24 months or six weeks after cessation of breastfeeding, this was not the case for the eight children [8]. Five out of ten had tested HIV-negative initially but had not had subsequent test, while three children whose mothers were on ART had never

been tested. This finding shows a need to look for HIV-infected children beyond PMTCT setting. In a systematic review of 26 papers, Cohn et. al found that HIV prevalence was highest in pediatric inpatient settings (21.1%, 95% CI: 14.9-27.3), nutrition centers (13.1%, 95% CI: 3.4-22.7), immunization centers (3.3%, 95% CI: 0-6.9), then pediatric outpatient (2.7%, 95% CI: 0.3-5.2). Symptom-triggered universal testing in pediatric outpatient settings had a diagnostic yield similar to that found in the inpatient ward (21.3%, 95% CI: 11.6-31.0 in triggered testing vs 20.9%, 95% CI: 13.5-28.3 in universal testing) [22]. In a recent paper, data from Cameroon showed nontraditional PMTCT entry points [adjusted odds ratio (aOR): 1.95; 95% confidence interval (CI): 1.36 to 2.80] was independently associated with HIV positivity among HIV-exposed children [23]. In a high prevalence country like Lesotho, optimizing testing with screening of all HIV-exposed children at 18-24 months who do not have final HIV status will assist in diagnosing more children.

Our study also explored factors associated with MTCT and/or death. Factors associated with MTCT are well elucidated in the literature [22,24, 25]. Factors associated with HIV-free survival in Lesotho included HIV-positive mothers disclosing their HIV status and HIV-exposed infants taking cotrimoxazole prophylaxis. It can be hypothesized that women who disclosed their HIV status got more support to be adherent to their treatment [26,27]. HIV-exposed infants who had growth difficulties were more likely to be HIV-infected and/or to die.

The limitations of the study include the fact that information for more than a quarter of children was received from caregivers who were not biological mothers.

Although the team took all measures to verify the information in the handheld health records, in some instances such information was not available. However, our results remain valid considering the large sample size, which took these challenges into consideration.

In addition, for several deceased children, we did not know their HIV status and the cause of death was not recorded in any home-kept document. This limitation did not have impact on our results because the study was measuring HIV-free survival and was not set to describe HIV-related deaths.

Although the data collection was completed almost five years ago, the results of this study remain relevant especially because it is the first and so far the only community-based survey carried out in Lesotho to measure HIV-free survival.

Conclusion

Even in the context of lifelong ART among pregnant and breastfeeding women, HIV has a significant effect on survival among exposed children. HIV transmission to mothers and children continue to occur during breastfeeding period, therefore it is important to test mother-child pairs during the breastfeeding period while they attend routine clinics such as postnatal care, immunization clinics, outpatient and inpatient services. This study has shown that Lesotho has not reach elimination MTCT despite introduction lifelong ART for all HIV-positive women. Since this study measures the effectiveness of PMTCT program when the guidelines were just changed, it is expected that the performance of program will continue to improve to reach the MTCT elimination level.

We recommend that the MOH develop a national registry that links HIV-exposed infants with their mother until final HIV status is ascertained at 18-24 months as per national guidelines. In addition, there is a need to repeat this study and triangulate data to measure impact of increased coverage of PMTCT services across Lesotho.

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