[The effectiveness of PMTCT in the Free State-*An anonymously linked cord blood survey*]

BY

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DECLARATION

I Marian Ama Amoo hereby declare that the work of this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any other part of it has been, is being, or is to be submitted for any other degree in this or any other university.

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DEDICATION

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ACRONYMS AND ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Care
ART	Antiretroviral Treatment
ARV	Antiretroviral
AZT	Zidovudine
CDC	Centers for Disease Control and Prevention
DBS	Dried Blood Specimens
DOH	Department of Health
EBF	Exclusive Breast Feeding
EPI	Expanded Program for Immunisation
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
MCHW	Maternal and Child Health Welfare
MTCT	Mother to Child Transmission
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PMTCT	Prevention of Mother to Child Transmission
RCT	Randomised Controlled Trial
sdNVP	Single Dose Nevirapine
UNAIDS	Joint United Nations Program on HIV/AIDS
UNGASS	United Nations General Assembly Special Session
VCT	Voluntary Counseling and Testing
WHO	World Health Organisation

ABSTRACT

Background: PMTCT has become freely available in many African countries however the impact of these interventions at the population level has not been widely estimated.

Aim: The aim of this study was to estimate the proportion of HIV infected/exposed mother and infant pairs who received the appropriate prophylaxis.

Methods: Cord blood specimens were collected anonymously from women delivering in 10 facilities in the Free State from November 2007 to April 2008. Collected specimens were tested for antibodies to HIV. Specimens found to be seropositive were tested for the presence of nevirapine using chromatography. All PMTCT sites used single dose nevirapine as the minimum prophylaxis, a few used dual therapy including zidovudine and nevirapine and some included nevirapine-based HAART for eligible women. Information was also collected from the clinical records. Maternal PMTCT coverage was determined through cord blood chromatography and infant coverage was determined from documentation of receipt on the clinical records.

Results: 1619 specimens were collected from women who gave birth to live infants were collected and tested (3.6% collection rate). 472 specimens tested positive for HIV antibodies on cord blood testing giving an HIV prevalence of 29.2% (95% CI 26.9-31.4%). Only 45.8% (95% CI 41.2-50.4%) of the 472 live infants born to HIV-infected mothers received both the maternal and infant doses of ARV prophylaxis. Reasons for failed dosing included, pre-test counseling not offered, refused testing, positive test result not received, prophylaxis was not dispensed, mother did not adhere and infant did not receive the prophylaxis dose.

Conclusion: This study showed that coverage in the Free State Province is poor despite the national expansion of PMTCT services to all antenatal sites. Failures occurred at each step of the PMTCT cascade and resulted in low coverage. Interventions should be introduced at each step of the PMTCT cascade to increase coverage.

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INTRODUCTION

Problem Statement

Vertical transmission of HIV is the main source of HIV infection in children under 15 years. In 2008 sub-Saharan Africa accounted for 90% of all new infections in children worldwide (UNAIDS/WHO. 2009c). In an effort to decrease vertical transmissions, PMTCT services have been integrated with antenatal care and are freely available in many parts of Africa. Many of these programs offer short course prophylaxis which has demonstrated efficacy in randomised controlled trials. Despite PMTCT being widely available, not all HIV-infected pregnant women access the intervention. In 2008, only 45% of HIV infected pregnant women from middle and low income countries received antiretroviral treatment (ART) prophylaxis (UNAIDS/WHO. 2009d). Women need to follow a sequence of events in order to access a PMTCT intervention. They need to first access antenatal care, be offered HIV testing, accept testing, obtain the result, if HIV-infected be offered and accept the intervention, receive the intervention, adhere to the dosage and their infants should be given the prophylaxis. There are many barriers to access the intervention including fear, denial and stigma associated with HIV and poor health service infrastructure. It is therefore crucial to establish at which steps failed coverage occurs in order to target interventions appropriately and strengthen program performance.

The Global HIV/AIDS Epidemic

HIV/AIDS is an important public health problem worldwide. In 2007 there were 33.2 million people living with the virus and 7000 incident cases daily. The number of people living with HIV has increased rapidly since the beginning of the epidemic. In 1990 there were approximately 7 million people living with HIV globally, and a decade later in 2001 this increased to 29 million. The number of new infections has declined from 3 million in 2001 to 2.7 million in 2007. The decline in incidence has contributed to the stabilisation of prevalence; however the prevalence of HIV has increased as more people

have access to ART and the accumulation of on-going new infections has outweighed the deaths (UNAIDS/WHO. 2008b). At the end of 2008 there were more than 4 million people receiving ART in low and middle income countries. The ART coverage in these countries increased from 33% in 2007 to 42% in 2008, showing a 36% increase in one year. Sub-Saharan Africa had the greatest increase in coverage; from 33% in 2007 to 44% in 2008 (ibid).

Sub-Saharan Africa bares the bulk of the burden of this disease with 22 million people living with HIV/AIDS. Although current data has shown that the prevalence has either declined or stabilized (UNAIDS/WHO. 2007a), this region still remains the worst affected with 68% of all adults and almost 90% of all children living with HIV/AIDS (UNAIDS/WHO. 2007a). Within the region there is great variation in the rates, with southern Africa accounting for most of the infections. In 2007 it was estimated that southern African alone accounted for a third of global deaths and new infections (ibid). South Africa has one of the highest prevalences globally. In 2008 the prevalence among adults aged 15-49 years of age was 16.9% (95% CI 15.5-18.4%) (Shishana et al. 2008) and amongst pregnant women it was 29.3% (95% CI 28.5-30.1%) (DOH. 2009c).

In many developing countries the high rates of poverty and an already overburdened health care system has made the epidemic difficult to control. HIV/AIDS further burdens the health care system because of the need to expand resource intensive ART programs and the need to retain large numbers of HIV-infected persons in care (Van Damme, Kober & Laga 2006). The link between HIV and poverty is complex and wealth and unequal income distribution are associated with HIV transmission (Veenstra, Whiteside 2005). Poor people are often less educated and may also engage in risky sexual behaviour for favours or gifts because of need (ibid). The epidemic has significant multi-level economic implications globally.

The long term macro-economic impacts of HIV are important to consider in high prevalence countries. There is likely to be a decline in economic growth resulting from reduced skilled human capital and productivity, with increased morbidity and early mortality from HIV (Whiteside 2002, Beck, Miners & Tolley 2001). The health care sector particularly is bearing the burden of the disease. There has been increased government expenditure for the provision of extra care, staff, treatment and support services for ART delivery (Veenstra, Whiteside 2005). The capacity to meet these demands are limited as resources (both human and financial) are inadequate, feeding into the poverty cycle. In Africa, many HIV infected people do not seek health care and thus the actual economic burden on health services may be masked, as embedded cultural beliefs in traditional healers, fears of stigma and denial result in low health care utilization. The most devastating impacts of HIV/AIDS however, are faced at the household level, due to loss of income and out-of-pocket payments for health care, increasing the likelihood of or deepening poverty (Veenstra, Whiteside 2005, Whiteside 2002).

HIV/AIDS among Women of Child Bearing Age

Women are particularly vulnerable to the disease because of gender inequalities and economic dependency on men (Hayes 2007, De Vogli, Birbeck 2005). These socio-economic circumstances may result in riskier sexual behaviour, sexual abuse and exploitation with increasing risk of exposure (De Vogli, Birbeck 2005). In 2007 there were 15.4 million women living with HIV globally, representing half of all HIV infections, with 60% living in Africa (UNAIDS/WHO. 2007a). Similarly in sub-Saharan Africa, 60% of the total numbers of adults infected were women. In many parts of Africa and the Caribbean young women (15-24 years of age) are six times more likely to be infected (United Nations. 2006). Gender differences are also present in South Africa. In 2007 it was estimated that 3.2 million women (aged 15+) were infected (UNAIDS/WHO. 2008b). In the 15-19 year old category the female prevalence was 2.7

times higher than males and among the 20-24 year olds, the prevalence was 21.1% and 5.1% in women and men respectively (Shishana et al. 2008).

HIV/AIDS among Infants

The infection rate among women of childbearing age has a direct bearing on the epidemiology of HIV in children. In 2007 the WHO estimated that over 400 000 children less than 15 years of age were newly infected with the virus, mostly through mother–to-child transmission (MTCT) (UNAIDS/WHO. 2007a, WHO. 2004c). The number of children living with HIV has increased globally. In 2001 1.5 million children were infected and by 2007 this increased to 2.5 million worldwide. HIV/AIDS is the leading global cause of death in children and has increased infant and child mortality rates (UNAIDS/WHO. 2007a, WHO. 2004c). In 2001 the disease caused 40.3% of all deaths in children under five (Bradshaw, Bourne & Nannan 2003). In five sub Saharan high prevalence countries (South Africa, Zimbabwe, Botswana, Namibia and Swaziland), the mortality in children under five attributed to HIV was in excess of 30/1000 live infants (Volmink et al. 2007). In South Africa demographic projections for 2010 show the infant mortality rate with AIDS will be 67.4/1000 live births, and without AIDS, would have been 31.6/1000 live births, thus the excess mortality due to HIV was 35.7 (Essex et al. 2002).

Breastfeeding of infants by HIV-infected mothers is one of the reasons for the large discrepancy in transmission rates in developed and developing countries. Prior to the introduction of PMTCT interventions in developed countries the rate of transmission among non-breastfeeding populations ranged between 14% and 32%, while in predominately breastfeeding populations rates were between 25% and 48% (De Cock et al. 2000). In South Africa the rate ranges between 12% to 39% depending on breastfeeding and the duration of feeding and this is similar to other African countries (Bobat et al. 1996, Coutsoudis et al. 2001b). Member states at the United Nations General Assembly Special Session (UNGASS) made a commitment to reduce HIV

infant infections by 50% by 2010. In order to reach this target the number of pregnant HIV-infected women with access to PMTCT services will have to increase. Progress has been made in this scale-up. In 2005 less than 10% of pregnant women requiring PMTCT had access to the service globally (United Nations. 2006), however by the end of 2008 this increased to this increased to 45% in low and middle-income countries (UNAIDS/WHO. 2009d). The number of new infections in children globally has also decreased from 460 000 in 2001 to 420 000 in 2007 and the deaths due to HIV in children declined from 360 000 in 2005 to 330 000 in 2007(UNAIDS/WHO. 2009d).

Causes of HIV/AIDS among Infants

HIV transmission from mother to infant occurs during the intrauterine, intrapartum and breastfeeding periods. In non-breastfeeding populations it is estimated that 30% are intrauterine infections and the remaining 70% occur during labour and delivery. However among breastfeeding populations, 20% are intrauterine, 45-50% intrapartum and 30-35% postpartum infections (De Cock et al. 2000). The risk factors for transmission include maternal factors (low CD4+ cell count, high viral load, AIDS diagnosis/advanced HIV disease, high HIV viral genital secretions), obstetrical factors (vaginal delivery, prolonged rupture of membranes), infant factors (pre-term delivery) and breastfeeding (maternal advanced disease and longer duration of exposure). Maternal viral load has been noted to be the strongest independent risk factor (Volmink et al. 2007).

Prevention of Mother-To-Child Transmission (PMTCT)

MTCT can be reduced by improving access to and uptake of HIV testing and counselling by pregnant women followed by the use of ART as prophylaxis in those that are HIV-infected. ART primarily acts by reducing the viral load and thus provides prophylaxis to infant when HIV exposed in utero and after delivery. Other interventions include: the provision of safe delivery, increasing the availability and accessibility of

elective caesarean section, and particularly in Africa, the promotion of safe feeding practices. In Africa breastfeeding avoidance may not be feasible, affordable, sustainable or safe. Extended prophylaxis to the infant (Bedri et al. 2008, Kumwenda et al. 2008) and the use of highly active antiretroviral therapy (HAART) by mothers during breastfeeding has been shown to reduce postnatal transmissions (Kilewo et al. 2009, Palombi et al. 2007). Exclusive breastfeeding (EBF) for the first six months and early weaning may improve child survival by providing the infant with the nutrients of breast milk and also reduces the risk of transmission. However this has been controversial as some studies have shown no overall benefit in HIV-free survival compared with longer breastfeeding (Kuhn et al. 2008) while others found that early weaning reduces HIV transmission without increasing mortality (Leroy et al. 2002).

Numerous clinical trials have been conducted to show the clinical efficacy of ARV therapy to prevent mother-to-child transmission of HIV (Connor et al. 1994), and the implementation of these programs in developing countries has decreased transmission rates. However coverage remains low and MTCT rates are considerably higher in developing countries. Some high HIV prevalence settings like Zimbabwe, South Africa and Cameroon have managed to reduce transmission to between 6% and 15% (Coetzee et al. 2005, Dube et al. 2008, Ayouba et al. 2003). However a South African study (Rollins et al. 2007a) in KwaZulu-Natal reported a vertical transmission rate of 20.2%, suggesting that rates vary within countries. Other reasons for high rates include; fear and stigma associated with HIV, poor knowledge about HIV and PMTCT, inadequate maternal and child health facilities, low uptake and implementation of voluntary counselling and testing (VCT) services, health system failures such as drug and rapid test kit stock-outs and limited resources for widespread implementation (Mofenson, McIntyre 2000, Paintsil, Andiman 2009). Many industrialized countries have managed to achieve low rates through the implementation of effective strategies. For example, the United States has managed to reduce transmission to less than 1% (Mofenson 2004) through high PMTCT coverage, using interventions including HAART, caesarean

sections and replacement feeding (Mofenson, McIntyre 2000). As a result field effectiveness, which refers to whether an intervention works among a broadly defined population, and takes into account the logistics and challenges of implementing the program in routine settings (Glasgow, Lichtenstein & Marcus 2003), closely mimics clinical efficacy trials conducted under ideal, experimental conditions (Stringer et al. 2008).

PMTCT Roll out in South Africa

In 1990 the prevalence of HIV among antenatal attendees in South Africa was less than 1% and by 2008 this increased to 29.0% (DOH. 2009c). It appears that the prevalence is stabilising as it has not increased since 2006. Provinces with the highest prevalence among antenatal attendees include KwaZulu-Natal, Mpumalanga and the Free State (ibid).

In 2007 the UNAIDS estimated that less than 60% of HIV-infected pregnant women received prophylaxis. By the end of 2008 this had increased to over 70%; however coverage in South Africa still remains below the UNGASS target of 80%. Reasons for failing to achieve wide coverage include a lack of political support for HIV treatment and prevention programs. The former President of the country, Thabo Mbeki questioned the scientific evidence relating to HIV and the former Minister of Health raised concerns about the safety and costs of PMTCT. This delayed the introduction of PMTCT programs. In 1999 the only province that had started a pilot program was the Western Cape Province which was launched in Khayelitsha (Schneider, Fassin 2002). The initial regimen was zidovudine twice daily from 36 weeks until labour. During labour 300mg of zidovudine was given every three hours until delivery, based on evidence from the Thai study (Shaffer et al. 1999). Mothers who chose not to breastfeed were provided with commercial formula milk for nine months (Draper, Abdullah 2008).

In 2002 the Treatment Action Campaign (TAC) brought a court action against the national government and the Constitutional Court ordered the universal provision of prophylaxis to all identified seropositive pregnant women. The National Department of Health implemented a pilot program in 18 sites (two in each province) (DOH. 2008a). Single dose nevirapine given to the mother at the onset of labour and a dose to the baby within 72 hours of delivery was included in the package based on evidence from the HIVNET 012 trial (Guay et al. 1999). Rapid HIV antibody tests were performed at 12 months (Doherty, McCoy & Donohue 2005).

The current WHO guidelines recommend that HIV-infected pregnant women with a CD4 lymphocyte count greater than 350 μ/l are provided with a combination of short course zidovudine and single dose nevirapine, short course zidovudine and nevirapine to their infants and again post delivery to the mother, a „tail' of zidovudine and lamivudine to decrease nevirapine resistance (Maheswaran, Bland 2009, WHO. 2001a). If the mother has advanced disease (WHO stage 4 or the CD4 count is less than 350 μ/l) the WHO recommends HAART be initiated as early as possible in pregnancy. In South Africa only women with a CD4 lymphocyte count less than 200 μ/l are initiated on HAART. The first line HAART regimen in South Africa for pregnant women comprises zidovudine, lamivudine and nevirapine. Women not requiring HAART receive zidovudine from 28 weeks of gestation until labour together with single dose nevirapine at the onset of labour. On delivery the infant is given single dose nevirapine plus zidovudine for 7 days. In the event that the mother received less than the required one month of zidovudine then the infant is given zidovudine for 28 days.

The mother is counselled during antenatal care and after delivery on safe feeding options. She is either encouraged to practice exclusive breastfeeding for the first six months or formula feed if this is affordable, acceptable, sustainable, feasible and safe. Mothers who choose to formula feed are provided with formula for six months.

At six weeks irrespective of feeding option chosen, all infants exposed to HIV are tested using the HIV DNA polymerase chain reaction (PCR) test. This is an antigen and not an antibody test, and identifies the virus in children younger than 18 months when maternal HIV antibodies may be present in the child. This test is conducted at 6 weeks of age as infants receive their first scheduled immunisation at 6 weeks in accordance with the WHO initiated Expanded Program on Immunisation (EPI). In routine settings the test has a sensitivity of 98.8% (95% CI 98.0-99.5%) at six weeks (Sherman et al. 2005). Testing is repeated for infants who are HIV negative at 6 weeks and who are breastfed, after the cessation of breastfeeding (DOH. 2008a).

Rates of mother-to-child transmission in South Africa

Studies in South Africa show that the rates of MTCT range from 8.6% to 22.3% (Coetzee et al. 2005, Rollins et al. 2007, Colvin et al. 2007). The Johannesburg Coronation Women and Children's study which was conducted in an urban setting found a transmission rate of 8.7% at 6 weeks and 8.9% at 3 months (Sherman et al. 2004). The relatively low rate of transmission at 3 months was attributable to the small proportion of the women who breastfed. In another high HIV-prevalence urban setting in Cape-Town with a high rate of replacement feeding a similar rate (8.8%) was reported (Coetzee et al. 2005). Replacement feeding, high rates of caesarean sections and the use of zidovudine in addition to single dose nevirapine contributed to the low rate. On the other hand in KwaZulu-Natal transmission rates at six weeks were 20.2% (95% CI 17.8-23.1%) overall and 15% in those who reported to have taken nevirapine only (Rollins et al. 2007). In this study 14% of infected mothers reported that they had not taken nevirapine and almost 7% of seropositive mothers were either late seroconverters or received false negative results during antenatal care and thus were not initiated on the PMTCT program (Rollins et al. 2007).

Free State and PMTCT Services

The Free State has one of the highest HIV prevalences in South Africa. In 2008 the prevalence among antenatal attendees in the Free State was 33.5% CI (95% 28.3-39.1%) (DOH. 2009c). The provincial PMTCT program is co-ordinated by the Maternal Child and Women's health (MCWH) directorate. In 2001 the initial pilot sites were 16 clinics that referred pregnant women to hospitals in Virginia and Frankfort for delivery. These sites were chosen to reflect the different socio-economic conditions in the province. During the pilot 58% of pregnant women in Virginia and 43% in Frankfort received pre-test counselling and accepted HIV testing. There were stock outs of rapid HIV test kits for a period of three months in 2002 and specimens had to be sent away to a central laboratory for testing. This resulted in missed opportunities for PMTCT initiation since many mothers did not return for their results. There was also a period when nevirapine was not available (Doherty et al. 2003a, Doherty et al. 2003b).

Reasons for low coverage included that the nurses forgot to administer the infant dosing, mothers did not receive nevirapine because of nevirapine stock-outs, mothers delivered outside the facility, and omissions on the part of the health care worker to supply the mother with nevirapine. At the time HIV testing was done by antibody testing at nine months. Forty three percent of mothers did not bring their infant for rapid testing between 9 and 12 months of age or, were lost to follow up. Of the 129 live infants born between August 2001 and March 2002 only 56 infants (43%) were tested and 21 identified as HIV infected. In the Free State 64% of mothers' breastfed, the province with the highest rate in South Africa (Doherty et al. 2003a).

In 2003 the PMTCT program was expanded to all antenatal services. This expansion resulted in the training of professional nurses on the PMTCT protocol, operational issues and safe infant feeding options. Lay counsellors were trained to provide VCT. In 2007 the PMTCT program was evaluated in three sub-districts in the Free State as part of a wider study of PMTCT in four African countries.

CHAPTER II

LITERATURE REVIEW

Prevention of Mother-to -Child Transmission of HIV

The prevention of vertical transmission of HIV is a priority in countries with a high prevalence of HIV among women of child bearing age. The WHO provides a four stage framework for interventions to successfully reduce infections in infants. The comprehensive guidelines includes; primary prevention of HIV in women of child bearing age, prevention of unintended pregnancies in HIV infected women, prevention of HIV transmission to infants and the provision of care, treatment and support of mothers, children and families living with HIV.

Strategies need to be targeted at women not infected with HIV to achieve the first and second goals. In many settings interventions targeted at HIV negative women during pregnancy are not conducted yet it is well established that acquisition of HIV infection during pregnancy or lactation increases the risk of transmission considerably as viral loads are very high during seroconversion (Semba, Neville 1999). Interventions should empower women with regards to their reproductive rights and choices, increase access to contraceptives, free condoms, VCT services and provide support systems for women facing gender based violence (DOH. 2008a, WHO. 2001b).

Drug Therapy and Efficacy Trials

Numerous clinical trials have shown the efficacy of different ARV regimens administered during the intrauterine, intrapartum and postnatal period to reduce mother-to-child transmissions. In the United States, the ACTG076 was the first trial conducted to show that a long course of zidovudine starting from 14-34 weeks of gestation, intravenously during labour and an oral dose to the infant for 6 weeks reduced the risk of transmission by two-thirds (Connor et al. 1994). Trials have since been conducted to

demonstrate the efficacy of simpler, less expensive short course regimens that are feasible for implementation in developing countries.

A randomised placebo controlled trial (RCT) in Thailand showed that short course zidovudine for mothers and infants halved the risk of transmission in formula fed infants (Shaffer et al. 1999). The RETRO study in Ivory Coast applied the same regimen to breastfeeding populations (Wiktor et al. 1999) and showed a 37% reduction in transmission at 3 months; however efficacy diminished with time due to postpartum infections occurring through breast milk (Wiktor et al. 1999).

Although these trials demonstrated efficacy the costs of this regimen at that time limited its implementation in many resource constrained settings. The HIVNET 012 trial in Uganda therefore developed a more affordable and feasible short course protocol based on nevirapine. The long half life obtained in both maternal and infant plasma concentrations made it suitable for single dosing and subsequently would improve adherence. The study compared single dose nevirapine (administered to the mother at the onset of labour and to the infant within 72 hours of birth) with zidovudine (administered to the mother during labour and to the infant for 1 week) among predominantly breastfeeding women. The results showed that single dose nevirapine was both efficacious and safe to prevent vertical transmissions decreasing the risk of transmission by 47% at 14 to 16 weeks (Guay et al. 1999). Controversies however developed in relation to the conduct of the HIVNET 012 study and claims were made involving irregularities in record keeping and failure to adhere to procedures during the reporting of safety data that may have compromised the validity (Cohen 2004). However several studies conducted subsequently demonstrated the low toxicity and efficacy of single dose nevirapine (Moodley et al. 2003).

The findings from HIVNET 012 and the first zidovudine trials prompted the development of protocols that combined both regimens. The PHPT-2 in Thailand was among the many to demonstrate the added benefit of nevirapine and zidovudine for prophylaxis. The investigators conducted a double blinded three arm randomised trial among a predominately non-breastfeeding population. The study compared the Thai zidovudine regimen, given from 28 weeks of gestation, intrapartum and for one week to the new born infant with two other regimens. The second arm compared the addition of single dose nevirapine to the mother and infant and the third to the mother only. In the interim analysis the zidovudine only arm had a higher transmission rate than the nevirapine arms and the trial arm was stopped early (6.3% in zidovudine alone and 1.1% in the other two arms). The results of the other two arms showed that nevirapine-based regimens were highly efficacious with a 2.0% transmission rate in the zidovudine plus both maternal and infant nevirapine arm compared to 2.8% in the maternal nevirapine only arm (Lallemant et al. 2004). The study showed that maternal zidovudine at 28 weeks of gestation and single dose nevirapine at the onset of labour with or without the infants dosing were more efficacious in reducing transmission. In contrast, the PACTG 316 International study in developed countries showed that the addition of maternal and infant nevirapine to standard antiretroviral therapy had no benefit among non-breastfeeding populations. In this study elective caesarean sections were performed for all women and this may have lowered the intrapartum risk considerably (Dorenbaum et al. 2002).

The intrapartum and postpartum dose of nevirapine is essential for women presenting late in pregnancy and who may not have received sufficient zidovudine antepartum (Dao et al. 2007). The PETRA study assessed the efficacy of different combinations of zidovudine and lamivudine in three African countries among breastfeeding populations. Women randomised to the first arm received zidovudine and lamivudine given from 36 weeks of pregnancy, intrapartum and for one week to their infants. Those in the second received zidovudine and lamivudine given intrapartum and to the infant for one week,

the third received intrapartum zidovudine and lamivudine to the mother only and those in the fourth received the placebo. The transmission rates were; 5.7%, 8.9%, 14.2 and 15.3% respectively at six weeks. Intrapartum zidovudine and lamivudine alone regimens were less efficacious in reducing the rate of HIV transmission (PETRA study group. 2002). The SAINT trial in South Africa compared the efficacy of maternal and infant single dose nevirapine to zidovudine and lamivudine given intrapartum and to the infant for one week. At eight weeks there were more infections in the nevirapine arm than in the zidovudine and lamivudine arm (12.3% vs. 9.3%) but this was not statically significant (p=0.11). The overall efficacy in preventing transmissions was 50.6% for the nevirapine group and 58.8% for the zidovudine and lamivudine group (Moodley et al. 2003).

Some HIV-infected mothers do not attend antenatal care and present too late in labour to initiate intrapartum dosing. Trials have determined the efficacy of infant dosing alone in the absence of maternal intrapartum and antepartum dosing. A RCT in Malawi compared the efficacy of nevirapine alone and nevirapine added to zidovudine among infants exposed to HIV whose mothers had not received the intrapartum dose. Heel-stick dried blood specimens from the infants were taken and tested for HIV infection at birth, 6-8 weeks and at 3 months using nucleic acid sequence based amplification assays (Taha et al. 2003). This test detects HIV-1 RNA even in the primary infection stage when HIV antibodies have not developed for serological testing (Oehlenschläger, Schwille & Eigen 1996). At birth the transmission rate was 12.1% in the nevirapine alone arm and 7.7% in the nevirapine and zidovudine arm (p=0.03), while the overall rate of transmission was 20.9% and 15.3% in the two arms respectively at 6 to 8 weeks (Taha et al. 2003). Combination therapy is therefore essential in the absence of maternal dosing. Another RCT in Malawi among breastfeeding populations compared the efficacy of adding a weeks' supply of zidovudine to the nevirapine given to infants when the maternal nevirapine was received. The results showed no difference in the rate of transmission at 6 to 8 weeks for infants not infected at birth between the two

regimens (6.5% for nevirapine alone versus 6.9% nevirapine and zidovudine p=0.88) (Taha et al. 2004).

Short Course Nevirapine and Resistance

Although single dose nevirapine regimens have proved to be efficacious and well tolerated, there are concerns over the development of resistance in both women and infants. The long half life of nevirapine increases the risk of resistance. High viral loads and low CD4 lymphocyte cell counts have been associated with the development of resistance (Eshleman, Jackson 2002). This may affect the efficacy of nevirapine in later pregnancies and after the initiation of nevirapine as part of a HAART regimen in both mother and infant at a later stage (McIntyre 2005). A follow up study found that resistance appeared to wane with time (Eshleman, Jackson 2002, Eshleman et al. 2001), suggesting that efficacy is maintained for subsequent pregnancies following single dose nevirapine. Observational studies conducted in South Africa, Ivory Coast and Uganda showed that previous exposure to single dose nevirapine did not decrease efficacy for subsequent pregnancies (Martinson et al. 2007, McConnell et al. 2007).

Eshleman et al 2001 analysed blood samples from both the women and infants from the HIVNET 012 study and found the rate of resistance was 18% in women and 46% in infants at 6 to 8 weeks of age. Possible explanations for higher rates in infants may be due to the increased exposure levels, since infants receive both the maternal dose and the dose administered within 72 hours (Eshleman et al. 2001).

Nevirapine resistance not only occurs in single dose regimens but also in combination with other ARV drugs, however at a lower rate when drugs with shorter half lives protect the “tail” after nevirapine is stopped. It has been observed that the addition of either zidovudine or lamivudine reduces the time during which the mother is exposed to

nevirapine only and thereby reduces the likelihood for the development of resistance (Chaix et al. 2006). The Ditrame Plus trial evaluated the resistance rate of single dose nevirapine in combination with zidovudine and lamivudine together with a 3 day postpartum course of the latter two drugs. The resistance rate among women at 4 weeks postpartum was 1.14% (Chaix et al. 2006).

While there may be resistance problems associated with the use of single dose nevirapine alone this needs to be balanced against the cost-effectiveness, efficacy and simplicity in resource constrained settings (Eshleman, Jackson 2002, Eshleman et al. 2001).

Treatment Guidelines and WHO Recommendations

Single dose nevirapine is a simple and practical option to reduce MTCT in resource constrained settings. This regimen has several advantages. It is less expensive than zidovudine based regimens (Moodley et al. 2003), easy to administer and women presenting late during pregnancy or at delivery can be HIV tested and treated. There is likely to be better adherence since single dose therapy can be administered in clinic settings and the half life (61-66 hours) in women and in neonates (45-54 hours) is long. Finally, the regimen is well tolerated with very few reports of adverse events. Hence the WHO recommends nevirapine to both mother and infant as the minimum prophylaxis to be given to HIV infected women where resources may not permit the implementation of combination therapy (WHO. 2001b).

The WHO recommends dual therapy for women in WHO stage 1-3 or who have a CD4 lymphocyte count greater than 350µ/l, starting with zidovudine from 28 weeks of gestation, lamivudine and zidovudine plus single dose nevirapine during labour, followed by lamivudine and zidovudine for one week for the mother, and single dose nevirapine and zidovudine for one week for the infant (WHO. 2001b). For women with

WHO stage 4 or WHO stage 3 and CD4 count less than 350 µ/l or WHO stage 1 or 2 and CD4 count of between 200 and 350 µ/l the WHO recommends HAART. First line regimen is zidovudine, lamivudine and nevirapine (Dao et al. 2007).

Breast Feeding Avoidance

Breast milk remains one of the most important routes of MTCT in many resource limited settings. It has been estimated that 50% of all HIV paediatric infections occur through breastfeeding (WHO. 2004c). Clinical trials such as the RETRO-CI study in Ivory Coast found the efficacy of the PMTCT regimen decreased with time, due to the effects of breastfeeding. At 3 months of age efficacy declined from 44% to 37% (Wiktor et al. 1999) and by 24 months, had decreased to 23% in populations that continued to breastfeed (Leroy et al. 2002). Likewise in the PETRA study efficacy decreased by 18 months primarily as a result of postpartum infections (PETRA study group. 2002). The major risk factors associated with transmission during this period include long duration of feeding, mastitis and cracked nipples and sores in the mouth of the child. High maternal viral load due to recent seroconversion or late stage of disease results in elevated breast milk viral load and increased rates of transmission (Semba, Neville 1999, UNICEF 2009, Rousseau et al. 2003). In Kenya, a ten-fold increase in breast milk viral load was associated with a 2 fold increase in the risk of transmission (Rousseau et al. 2003). It has not been fully established whether infection occurs through cell free virus or through HIV-infected cells (Rousseau et al. 2003, Newell 2006).

Studies have demonstrated that the avoidance of breast milk eliminates these postpartum infections. An early randomised controlled study in Kenya found a higher probability of HIV infection in breastfed infants than in formula fed infants (Nduati et al. 2000). Similarly, the MASHI trial in Botswana showed decreased HIV transmission among infants not breastfed (Thior et al. 2006).

Formula or replacement feeding may not be feasible, affordable, acceptable, sustainable or safe in many developing countries (WHO. 2004c). Breastfeeding is beneficial because of the immunological and nutritional properties that are necessary for growth and development. In areas where the availability of clean water is limited, the excess morbidities and mortalities associated with formula feeding is of concern. For example, in many of the trials higher mortality rates were reported among infants who were formula fed. A cohort study in KwaZulu-Natal found that mortality rates more than doubled in replacement fed infants as compared to exclusively breastfed infants (15% vs. 6%) (Coovadia et al. 2007). The MASHI trial, also found mortality rates were higher in formula fed infants by seven months (9.3% vs. 4.9% p=0.003), with the most common causes of mortality related to diarrhoea and pneumonia (Thior et al. 2006).

In resource constrained settings, EBF and early weaning have thus been proposed as a strategy to reduce postnatal transmissions and still offer the benefits of breast-milk. EBF refers to the consumption of only breast milk until six months of age, and excludes other milk products and any liquids or solid foods (Iliff et al. 2005). It is thought that the EBF method preserves the intestinal mucosa and thus presents a barrier against HIV. It further protects against breast complications such as mastitis where higher rates have been noted to occur among mothers that practice mixed feeding (Coovadia et al. 2007).

The extent of the benefit of EBF is controversial since postpartum infections still occur through breastfeeding. A RCT conducted in Malawi found that most postpartum infections occurred by 6 months of age and thus the benefits of early weaning did not appear to be significant (Nduati et al. 2000). Similarly a longitudinal analysis in Kenya showed that HIV viral loads were highest in early milk as compared to mature milk, corresponding to a higher risk of transmissi**o**n (Rousseau et al. 2003). This was also demonstrated in the PETRA study where most infections occurred through breastfeeding in the first six weeks (PETRA study group. 2002). Latest evidence from the ZEBS study in Zambia confirmed no benefit to early weaning and abrupt cessation at four months.

The HIV free survival at 24 months in children that were weaned at four months was 68.4% while children that were breastfed for longer than 4 months was 64.0% p=0.13 (Kuhn et al. 2008). Early weaning has also been associated with an increased risk of malnutrition and increased mortality and morbidity from infectious diseases giving no overall advantage (Kuhn, Reitz & Abrams 2009, Mofenson 2008).

The administration of postpartum doses of zidovudine, lamivudine and nevirapine may reduce early postpartum transmissions due to breast milk (Kourtis et al. 2007). The MASHI trial showed no difference in the rates of HIV transmission between formula and breastfed infants at 1 month during which zidovudine was administered (Thior et al. 2006). On the other hand the PEPI trial conducted in Malawi showed that prophylaxis with either nevirapine alone or nevirapine and zidovudine given from birth till 14 weeks of age reduced postnatal transmissions. In the control group (single dose nevirapine plus 1 week zidovudine) HIV transmission at 9 months was 10.6% (95% CI 8.7- 12.8%), in the extended nevirapine group (control regimen plus nevirapine daily for 14 weeks) the rate was 5.2% (95% CI 3.9-7.0%) p<0.001 and in the extended nevirapine and zidovudine (control regimen plus nevirapine and zidovudine for 14weeks) 6.4 (95% CI 4.9-8.3%) p=0.002 (Kumwenda et al. 2008).

Reducing postpartum infections in developing countries still remains a challenge. There seems to be consensus that HIV-infected women should be given ART (Shapiro et al. 2005) and continue to breastfeed or their infants should given prophylaxis during breastfeeding (Bedri et al. 2008, Kumwenda et al. 2008).

Role of Elective Caesarean Sections

Although maternal viral load has been noted to be the strongest risk factor for transmission for interuterine and postpartum infections, direct contact with the virus by the infant in the genital tract, leads to intrapartum infections (Newell 2006). Prolonged

duration (over 4 hours) of rupture of membranes and vaginal delivery are obstetrical factors associated with increased transmission. Caesarean section in combination with ARV therapy has been implemented in many developed countries as part of PMTCT. Evidence shows that caesarean section alone may reduce HIV transmission to infants by 50% (Brocklehurst 2002) and when used in combination with zidovudine it decreases the risk to less than 1% (Kourtis et al. 2001). A randomised trial compared elective caesarean section with vaginal delivery and found an 83% decreased risk (Brocklehurst 2002). In developing countries the introduction of this option on a wide scale is not available due to expense and lack of accessible facilities. In addition the procedure is not without risks. Studies conducted in developed countries found that maternal mortality was 5 to 25 times higher (Fiore, Newell & Thorne 2004) and morbidity was six times higher (Lapaire et al. 2006) after caesarean section compared to vaginal delivery. Other studies reported higher post operative risks among HIV positive women such as severe anaemia requiring blood transfusions, minor complications and infections such as urinary tract infections, post-caesarean endometritis and postpartum fever compared to HIV negative women (Fiore, Newell & Thorne 2004, Lapaire et al. 2006, Marcollet et al. 2002, Björklund et al. 2005). Another method to decrease HIV transmission is the avoidance of artificial rupture of membranes.

Measuring Operational Effectiveness

Interventions to prevent mother-to-child transmission of HIV have been based on evidence from clinical trials showing efficacy conducted under ideal conditions. Measures of the effectiveness of the implementation of these interventions in resource constrained settings are lacking and the effectiveness of PMTCT programs in many countries is largely unknown (Stringer et al. 2003). Weak information systems remain a major challenge faced in estimating effectiveness as resources to collect reliable and accurate information may not be available (Reithinger et al. 2007).

Effectiveness is defined as the extent to which a health intervention results in desired outcomes in response to the needs of the population. It comprises a wide range of variables including efficacy, inputs, quality assurance mechanisms, patient compliance and health behaviour factors (WHO 2001). Coverage on the other hand is a proxy used to measure effectiveness and refers to the proportion of the population in need of an intervention who actually receive the intervention. The numerator indicates the number of units that are receiving the health intervention and the denominator comprises the population that would need the intervention indicated in the numerator (ibid). Utilisation and access (which includes accessibility, availability, affordability and acceptability) are other closely related terms linked to coverage and may be considered determinants of effective coverage (ibid).

Using the assumption that the clinical benefits of ARV for the prevention of mother-to-child transmissions will rebound to a mother and infant population that access the intervention appropriately, PMTCT program coverage can be evaluated (Stringer et al. 2008). The PMTCT cascade consists of a sequence of events that must occur for prophylaxis to be administered and serves as a mechanism in which to measure coverage (ibid).

PMTCT Cascade

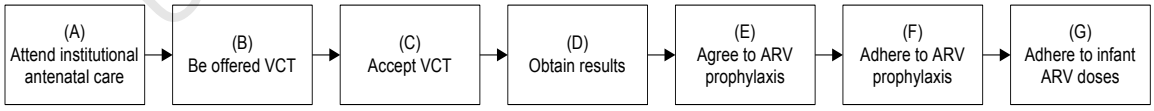


Figure 1 PMTCT coverage cascade. Source (Stringer et al. 2008)

Figure 1 shows the events which must occur in order for PMTCT to be delivered. A pregnant woman must attend an antenatal care facility, be offered VCT, accept testing, receive the test result, if infected be offered prophylaxis, agree to ARV prophylaxis, comply with prophylaxis and the prophylaxis must be provided to the infant (Stringer et

al. 2008). The degree to which these events occur gives an indication of program performance and hence coverage. Attrition may occur at each step resulting in reduced coverage and program effectiveness. A study in rural Malawi showed that 55% of HIV positive mothers were lost to follow up by 36 weeks of pregnancy, when nevirapine was dispensed, and by delivery this increased to 68%. Thus, only 32% of HIV infected/exposed mother-infant pairs received nevirapine prophylaxis (Manzi et al. 2005). In this study the high attrition was attributed to the long distances women had to travel to access clinics and multiple visits required. Home deliveries and traditional birth attendees' sites were common in the district and this contributed to the low coverage (ibid).

Studies conducted in routine settings suggest that demographic factors are associated with coverage. An increase in mothers' age, a higher level of education, an increase in the number of antenatal visits, living with a partner and delivering via caesarean section as compared to vaginal deliveries are some factors found to be positively associated with coverage (Peltzer et al. 2008, Albrecht et al. 2006, Bii et al. 2007, Kiarie et al. 2003).

Women need to access antenatal care to access PMTCT interventions. In most places in Africa services are widely available and freely accessible. The majority of women in Africa therefore access these services (Maheswaran, Bland 2009, Myer, Harrison 2003, Luo et al. 2007, Moses et al. 2008). In 2008 92% of pregnant women in South Africa accessed antenatal care (UNICEF, 2008). However some women may not attend antenatal care or attend few visits late in pregnancy and this may not be adequate to complete the steps required to receive full prophylaxis (Maheswaran, Bland 2009, Myer, Harrison 2003). Lack of physical access is regarded as a major barrier to access antenatal care especially in remote rural settings (Myer, Harrison 2003, Tanser, Gijsbertsen & Herbst 2006, Chapman 2003).

VCT is a crucial entry point to the PMTCT program. VCT uptake varies across settings. The Ditrame Plus study found 66% of women accepted HIV testing when HIV testing was on site (Ekouevi et al. 2004). In the Gulu District of Uganda, an area that faces civil unrest, the VCT acceptance rate was less than 60%. Another study in Burkina-Faso found acceptability to be very low (18%) (Pignatelli et al. 2006) .

"Opt out" as opposed to the traditional „opt in' has improved testing coverage. With "opt in" women are given counselling and asked if they wish to be tested. With "opt out" women are informed about testing and are routinely tested unless a women specifically refuses to be tested (Doherty, McCoy & Donohue 2005, Manzi et al. 2005). In Zimbabwe, a country with high HIV prevalence there was 79% acceptance with "opt out" (Perez et al. 2006). Similarly in Botswana, in a before and after study, the percentage of women tested increased from 76% to 95% and the percentage of those who received their test result increased from 72% to 82%. After five years of on-site testing the percentage of women delivering in regional hospitals who knew their status increased from 47% to 78% following the implementation of „opt- out' VCT (Creek et al. 2007). Although this method has achieved successful results, there are concerns that HIV infected women may become victims of stigmatisation and gender based violence upon disclosure of their status to their partners (Pignatelli et al. 2006).

Giving PMTCT to all women irrespective of HIV status is another strategy that has been proposed to overcome the low uptake of VCT. The universal access approach would increase uptake since pregnant women would not need to learn of their HIV status, in addition this approach would be suitable for settings where VCT is not available such as areas experiencing wars and conflict (WHO 2001b, Stringer et al. 2003). Using an RCT cross over design in Zambia, the investigators compared the universal strategy to the targeted strategy of giving prophylaxis to HIV positive women identified through VCT. The study found uptake, defined as the proportion of women accepting the intervention

without testing, was higher compared to infected women who accepted testing and received the intervention (70% vs. 59%; p <0.001).

However a potential disadvantage to giving nevirapine to women who do not know their status is that adherence may be compromised since these women may not fully perceive the actual benefits of the prophylaxis as compared to those who know that they are HIV infected (Sint et al. 2005, Stringer et al. 2003). In this study non-adherence was higher in the universal arm compared to the targeted arm (39% versus. 26%) (Stringer et al. 2003). Resistance in the mother and the risk of toxicity especially in HIV uninfected women are also concerns. VCT services should be integrated with antenatal care not only for the prevention of infant infections but also to educate, support and refer those mothers identified as HIV-infected and to reinforce HIV prevention in those who are not infected. Failing to offer VCT to pregnant women would therefore not achieve this (WHO 2001b, Stringer et al. 2003).

Notwithstanding these disadvantages and the fact that the universal approach would only be effective for intrapartum transmissions, this may be the only suitable, feasible and safe approach in some settings. In places where counselling and testing is of high quality and accessible, the targeted approach is the method of choice. In other settings the combined approach may be a more realistic to reduce MTCT as it incorporates both strategies. In this case, women accessing antenatal care are offered VCT in the conventional targeted way, and those who do not wish to be tested are provided with treatment through mass administration (WHO 2001b, Stringer et al. 2003).

In many settings women who participate in VCT do not return and obtain their test results. In a recent cohort study in rural KwaZulu-Natal, only 57% of women who accepted to be tested returned for their results (Mkwanazi et al. 2008). Similarly, in Kenya almost 31% of the women counselled did not return and these women were more

likely to be HIV positive, reflecting a crucial missed opportunity for prevention (Kiarie et al. 2000). Rapid HIV testing with same day result has been associated with increased uptake and enrolment into the program (Pignatelli et al. 2006, Pai et al. 2007). However even with rapid testing women may be given the option to return at a later date for the results (Maheswaran, Bland 2009). In KwaZulu-Natal a cohort study showed that women were more likely to return at a later date for their results than receive them the same day (65% versus 50% $p<0.001$) (Mkwanazi et al. 2008). Women may need time to assess and determine the implications of obtaining the result and the quality of counselling may have been poor (Mkwanazi et al. 2008). Another study in an urban setting in Ivory Coast conducted over a two year period to determine the acceptability of rapid testing, found that despite a high acceptance rate (89.4%), a quarter of women did not return for their results (Ekouevi et al. 2004). Similar findings were reported in Buhera, a rural in District in Zimbabwe, where the acceptance rate for testing was very high (92.9%) but return rate for those who decided to test was 74.3% (Perez et al. 2004).

In order for an identified pregnant HIV-infected woman to receive the PMTCT intervention she needs to be given the prophylaxis from the antenatal facility. Less than 60% of HIV infected women were provided with nevirapine in an Eastern Cape cohort that was followed (Peltzer et al. 2008). In Mombasa Kenya almost 50% of women who received a positive test result did not return to the clinic to collect the prophylaxis (Temmerman et al. 2003).

Further attrition occurs when HIV-infected women do not adhere to the prophylaxis. Maternal adherence is defined as the proportion that actually take the medication (Stringer et al. 2005). Factors associated with non-adherence include low education levels, home deliveries and referral to a tertiary hospital (Albrecht et al. 2006, Bii et al. 2007). The length of time between taking the medication and the birth of the infant is also critical to ensure that optimal therapeutic plasma levels are achieved to reduce the probability of transmission. Maternal single dose nevirapine should be taken at least 1

hour before delivery (Stringer et al. 2005, Albrecht et al. 2006). The SAINT study found that intrapartum transmissions were three times higher among women that had ingested the tablet less than 2 hours prior to delivery. Similarly the HIVNET 012 trial found that women who took nevirapine less than 1 hour before delivery had significantly lower cord blood concentrations than those who took it more than hour before delivery. Mothers of HIV uninfected infants reported a longer time interval between ingestion and delivery compared to mothers of HIV infected infants diagnosed between birth and 6 to 8 weeks (Jackson et al. 2006).

Infant adherence rates are higher than maternal because in most settings the first dose is given in the facility by the nursing staff. The evaluation of pilot sites in South Africa showed that almost all the infants (99%) identified received nevirapine syrup (Doherty, McCoy & Donohue 2005). A cohort study in Durban South Africa, similarly found high infant adherence with almost 90% reported having been given nevirapine (Coutsoudis et al. 2001a). In rural Uganda although home deliveries were common 96% of the infants were given the nevirapine dosing within the appropriate time limits. The nevirapine syrup was wrapped in aluminium foil and packaged in an opaque polythene bag similar to the bags containing multi-vitamin syrup given to HIV negative mothers. This increased acceptability as this did not divulge the woman's HIV status to the traditional birth attendees and family members (Kagaayi et al. 2005).

Even where participants are provided with support as in clinical trials non-adherence rates are high. In the Zambian Exclusive Breast Feeding Study (ZEBS) adherence rates were very high (94%), but only 78% received the dose within the correct time limit. Adherence was measured by self reports and from clinical records and this may have compromised validity (Albrecht et al. 2006). In Kenya 60% of rural women who delivered at home reported not to have taken the required dose (Songok et al. 2003). Women reported that they were ashamed to take medication in the presence of traditional birth attendees. Different findings were noted in a follow up study in Eastern Cape where both maternal and infant adherences were high. In this study self reported

maternal adherence (86%), was associated with disclosure of status to partner and a high HIV knowledge score (Peltzer et al. 2008). Similar findings were reported in Rakai Uganda, where 85% of HIV positive women reported taking nevirapine (Kagaayi et al. 2005).

Giving nevirapine during delivery and not to mothers prior to labour to take at the onset of labour, may increase adherence as it could be directly observed. In a study conducted in Zambia, Stringer et al achieved almost perfect adherence (99.4%) among HIV positive women. This approach has its disadvantages. Women who do not deliver at a facility do not receive the prophylaxis. Women may present late in labour and there is the risk of sub-optimal therapeutic concentrations (Stringer et al. 2004). This method could be used to improve coverage in women who were not given nevirapine or who forget to take the dose. Double dosing does not seem to be associated with severe adverse events or risks (ibid).

Study Designs Used to Measure Effectiveness

Prospective Follow-up Study Designs

Prospective cohort studies have been used to measure the effectiveness of PMTCT programmes (Ayouba et al. 2003, Songok et al. 2003, Tonwe-Gold et al. 2007, Palombi et al. 2007, Leroy et al. 2008). In these studies PMTCT exposed and unexposed are compared and HIV free survival estimates and transmission rates are obtained as the measure of effectiveness (Stringer et al. 2008). HIV free survival has been proposed as the ideal outcome indicator for the effectiveness of PMTCT interventions (Stringer et al. 2008, Stringer et al. 2005). This measure gives an assessment of the number of children who are alive and negative.

A cohort study in rural Kenya determined the effectiveness of short course prophylaxis among a breastfeeding population. The study followed a cohort of volunteer antenatal attendees for two years. 216 HIV infected women were included. The criteria for inclusion was being married, gestational period of 18-22 weeks, no life threatening illness and haemoglobin levels greater than 7g/dl. The HIV free survival among the women who reported to have taken the prophylaxis was 59.2% compared to 29.6% in those who had not taken prophylaxis ($p<0.0002$). This study may not be generalisable because recruitment was voluntary and women with life threatening illness and single women were excluded from the study. Almost 30% of HIV exposed live infants were lost to follow up. Validity may have been compromised if those lost to follow up were more likely to have been HIV-infected and died from HIV infection (Songok et al. 2003).

A cohort study in Cameroon determined the field effectiveness of nevirapine. Women attending antenatal care were informed of the study purpose and those who agreed to participate were included. At six weeks using PCR testing, the transmission rate was 10% (95% CI 5-16%) and by 5-6 months follow up this was 13% (95% CI 7-19%) (ibid). 23% (n=133) of HIV positive mothers were lost between testing and receiving

the results, almost 30% (n=166) had not delivered at the time of analysis (Ayouba et al. 2003).

Follow up non-randomised observational studies that compare HIV free survival and transmission rates of different interventions have also been conducted (Tonwe-Gold et al. 2007, Palombi et al. 2007, Leroy et al. 2008). In Ivory Coast a two-tiered effectiveness cohort study was carried out. All HIV-infected women attending antenatal care were enrolled. Women either received HAART or short course ARV therapy. Over 70% of mothers breastfed for a median duration of 5.4 months. At 1 month the rate of transmission was 1.0% (95% CI 0.0%- 3.1%) in the HAART group and 3.1% (95% CI 0.1-6.1%) in the short course ARV group. At 12 months this was 3.3% (95% CI 0.0-6.9%) for the HAART group and 7.5% (95% CI 2.8%-12.3%) in the short course ARV group. Although loss to follow up was minimal with 86% of the live infants available for testing, it is likely that more of these infants died from HIV infection (Tonwe-Gold et al. 2007).

While it is ideal to obtain HIV free survival for measuring effectiveness of PMTCT programs in routine settings, cohort studies have limitations which are important to consider. Cohort studies are expensive and complex to conduct as large numbers of infants and mothers need to be followed for over 6 months after cessation of breast feeding. In Ivory-Coast infants were followed for 12 months (Tonwe-Gold et al. 2007) and in rural Kenya for 24 months (Songok et al. 2003). Investigators are ethically bound to provide standard of care and therefore should provide the appropriate prophylaxis or ensure that mothers are referred for care (Ayouba et al. 2003). Hence findings may not be inferred to other settings as the results of these studies may be biased towards better outcomes. Selection bias resulting from loss to follow-up is a threat to validity as infants who are not retained in the cohort may die as a result of HIV-infection. In Kenya and Cameroon loss to follow up was over 20% (Ayouba et al. 2003, Songok et al. 2003).

Survey/Cross-Sectional Surveys

Facility based cross-sectional studies can also be used to estimate program effectiveness. This study design is simpler and less expensive than prospective designs as data is collected at one point in time or retrospectively (Stringer et al. 2008). They describe the burden of disease and provide population based outcomes since the entire population is included in the study (Stringer et al. 2008). Trends over time can also be established to determine the effectiveness of program implementation (Stringer et al. 2008). Missing or incomplete data may compromise validity.

A clinic based survey in among infants aged between 6 and 10 weeks was conducted in Khayalitsha, Cape Town to assess the operational effectiveness of the PMTCT programme. The study used clinic registers to identify all HIV-infected mothers and their exposed infants were tested. Initially only zidovudine was provided but during the study there was a change to dual therapy of zidovudine and nevirapine. This improved PMTCT coverage as women who missed or did not receive sufficient zidovudine had the benefit of nevirapine during labour and delivery. The transmission rate was less than 10%. In this study 24% of mothers who had not received sufficient zidovudine dosing before delivery received nevirapine and only 3% had not received any PMTCT intervention. Reasons for this low rate included a high uptake of VCT and over a quarter of the infants were delivered by elective caesarean. The authors commented that as replacement feeding was common postpartum infections would have been low. However the study had a number of limitations; almost 20% of mother infant pairs enrolled in the PMTCT program were lost to follow up at the six week follow up visit when infant HIV testing was conducted. This could have biased the result, particularly if the infants who were lost to follow up were more likely to have been HIV-infected or to have died from HIV infection (Coetzee et al. 2005). Therefore a strategy that will pick up all HIV exposed infants and minimises loss to follow up is necessary.

Using Cord Blood Specimens

Anonymous cord blood specimens can be collected and tested for HIV antibodies from all women delivering in facilities in order to measure the effectiveness of PMTCT interventions. Specimens found to be positive are then tested using chromatography to detect nevirapine. Information from clinical records can be collected to determine if the infant received nevirapine. Coverage is the proportion of mother-infant pairs with HIV antibody positive cord blood with confirmed receipt of maternal and infant prophylaxis. This method yields a valid coverage estimate because all women who deliver in facilities are included in the study as opposed to only those who consent (Ayouba et al. 2003, Songok et al. 2003). This method has other advantages. The use of cord blood specimens allows for verification of maternal adherence instead of relying on self reports. In Zambia a RCT found that nevirapine was not detected in the cord blood specimens of 28% of women who reported they ingested the dose (Stringer et al. 2003b). Nevirapine is rapidly absorbed and crosses the placenta easily and is thus easily detected through chromatography analysis (Mirochnick et al. 1998). Antibody serological testing is also cheaper and easier to conduct than infant PCR testing.

In Zambia a cross-sectional PMTCT effectiveness study was conducted among 10194 women delivering in 10 public facilities using anonymous cord blood specimens to detect HIV antibodies and nevirapine. From the population of HIV exposed infants in the surveillance program, only 30% received the PMTCT package (both maternal and infant dose). Failed coverage occurred along the entire cascade, HIV positive mothers were not offered VCT (18%) or declined testing (27%). At later stages, significant attrition occurred due to non-compliance as more than 32% of women who were given nevirapine did not actually ingest the tablet at the onset of labour (Stringer et al. 2005). 6% of mothers who were identified as negative during pregnancy were positive at delivery, possibly due to recent seroconversion or false negative results (ibid).

Cord Blood Testing to Estimate Prevalence

Anonymous cord blood HIV antibody testing also facilitates the accurate estimation of HIV prevalence because all women who deliver in facilities are tested. There is no need to obtain consent and thus those who might not have consented will be included. In most settings, the HIV prevalence is estimated from women who accept testing during antenatal care (Reithinger et al. 2007, Mpairwe et al. 2005). Women who consent to HIV testing may have a different HIV prevalence than those that reject but are anonymously tested. For example in Uganda, women who underwent VCT had higher HIV prevalence to those who refused but were anonymously tested (20% versus 11%), since women that considered themselves to be at risk for infection were more likely to accept VCT (Mpairwe et al. 2005). In Lusaka, women who refused testing during VCT were more likely to be positive than those who accepted. Programs which estimate HIV prevalence from women who accept testing during VCT may underestimate the true prevalence (Reithinger et al. 2007).

Objectives of the Study

Primary objective

- To determine PMTCT coverage in women delivering at public sector facilities in the Free State.

Secondary objective

- To determine the prevalence of HIV at the time of delivery in Free State health facilities
- To determine if the prevalence of HIV differed amongst women who refused and those who accepted HIV testing during antenatal care in the Free State
- To determine factors associated with maternal adherence (nevirapine detected in the cord blood) and successful coverage (infant receipt of prophylaxis and maternal adherence)

CHAPTER III

SUBJECTS AND METHODS

Description and Background of Study Community

The Free State covers an area of about 129,480 km^2 and has a population density of 23 people per km^2. This large and sparsely populated area is the third largest province in South Africa and lies between the Vaal River in the North and the Orange River in the South. About 6.8% of the total South African population lives in this province. The province is divided into five districts namely; Fezile Dabi, Thabo Mofutsanyane, Motheo, Xhariep and Lejweleputswa. The provincial capital, Bloemfontein is located in the Motheo District. Thabo Mofutsanyane is the largest district, with almost 30% of the population in the province living in this district, followed by Lejweleputswa and Motheo with just over 25% of the inhabitants. The majority of the population is African (89.6%), 9.9% white, 3% coloured and only 0.1% Asian. Most people (72.8%) live in urban areas, with almost 20% living in Metropolitan areas and 50% in small towns (Jacobs& Punt, 2009, DOH, 2009b, Davis& Tavasci, 2006).

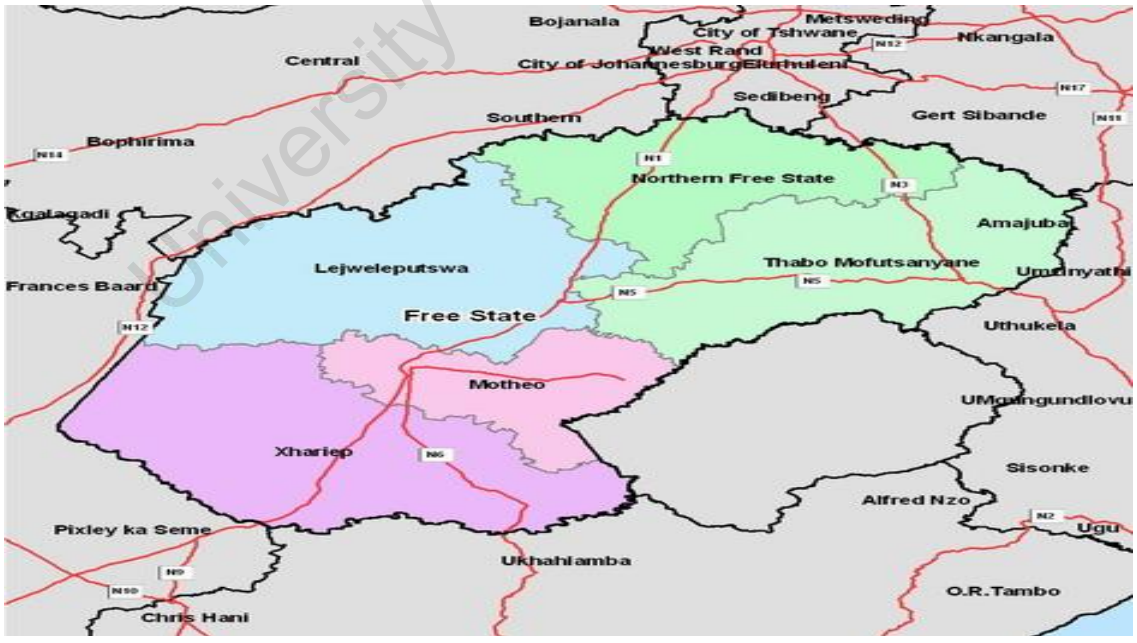


Figure 2 Map of the Free State

Nationally this province contributes about 5.5% to the GDP. Agriculture and mining are the major economic activities. Mining provides most employment opportunities, and produces about 30% of South Africa's total gold output. The rich fertile lands allow for agricultural activity. Many African households are involved in small scale subsistence farming. For commercial purposes, whites are generally the farm owners and the wealthiest while the Africans who generally provide labour, are the poorest (Jacobs, Punt 2009, Davis, Tavasci 2006).

The official unemployment rate for the first quarter of 2009 in the Free State was 25.4% (Statistics South Africa. 2009b). This rate was higher than the national average of 23.1% during the same period (Statistics South Africa. 2009b). The African population in the Free State, like the rest of South Africa, has the largest unemployment rate, at 30.8%, which is slightly higher than the countries national unemployment rate (Jacobs, Punt 2009). The Free State is not considered to be the poorest province. The districts lie within the third and fifth socio economic quintile (1=poor; 5=rich). Thabo Mofutsanyane district lies in the third quintile and has the highest deprivation index of 2.92 (Health Systems Trust. 2008). The levels of inequalities between the rich and poor are high as in the rest of South Africa (0.75) (Jacobs, Punt 2009). The Gini coefficient is an indicator used to show the income inequalities, and ranges from 0 or perfect equality to 1 or complete inequality (World Bank. 2009). In the province this value is 0.66 and is highest within the African population 0.60 (Jacobs, Punt 2009).

Most households (97.3%) in the province have access to safe drinking piped water, either inside the dwelling or outside in a communal area. Almost 20% of the households in the Free State are informal settlements, higher than the national average of 14.4% and the province is ranked third highest. 61.4% of the households have flush toilets, 22.6% use pit latrines (7.9 with ventilation, 14.7 without ventilation), 12.7% use the bucket system and 3.2 have no toilet facility. The province has the highest percentage of

households still using the bucket system as compared to the national average (2.2%) (Statistics South Africa. 2007a) .

Health Service Provision

Within the province four health complexes are responsible for providing public sector health services. These health complexes are compromised of 24 district hospitals, 5 regional hospitals, 1 tertiary and 1 psychiatric hospital. There are 258 fixed clinics, 121 mobile clinics 14 community centres responsible for primary health care (Table 1).

Table 1 Free State health facility distribution

	Xhariep	Motheo	Lejweleputswa	Thabo Mofutsanyane	Fezile Dabi	Province
Population	133 668	790 955	758 097	767 862	518 024	2 968 604
District Hosp	4	4	5	8	4	25
Regional Hosp	0	1	1	2	1	5
Tertiary Hosp	0	1	0	0	0	1
Central Hosp	0	1	0	0	0	1
Specialist Hosp	0	3	0	0	0	3
Fixed Clinics	17	69	45	67	33	231
Mobile Clinics	21	20	25	21	25	112
*CHCs	1	5	1	1	27	35
Total Clinics	38	93	71	89	59	350

Table adapted from Health Systems Trust 2007/2008 * Community Health Center

In the province, 15.8% of the population have health insurance and 84.2% depend on the public sector. Motheo district has the highest medical aid coverage (24.7%), while Thabo Mofutsanyane (5.3%) and Xhariep (8.4%) have less than 10%. The nurse clinic work load in Free State is 36.9 patients per nurse per day, and is higher than the 23.7 patients per nurse per day in South Africa. Fezile Dabi, Lejweleputswa and Thabo Mofutsanyane have patient workloads over 40.0 patients per nurse per day, while Motheo has the lowest nurse work load of 28.9 patients per nurse per day. The utilisation rate of primary health services in the province is 2.0 visits per person per year and is similar to the national average of 2.2 visits per person per year. This rate is below the Department of Health target of 3.5 visits per person per year. Lejweleputswa has the lowest utilisation rate in the province (1.6) (Health Systems Trust 2008) (Table 2).

Most women attending antenatal services in the province are tested for HIV (80.1%). In 2007 over 90% of the pregnant women in Fezile Dabi, and Xhariep were tested, whilst it was lowest in Lejweleputswa (74.7) and Motheo (75.6). Most women (84.8%) deliver in heath facilities, higher than the national average of 80.6%. Xhariep has the lowest percentage (57.9%) while over 90% of women in Lejweleputswa and Motheo deliver in health facilities. 11.8% of pregnant women deliver via caesarean section, this is lower than the national average (15.6%), and Fezile Dabi (16.6%) and Motheo (17.3%) have higher percentages compared to Lejweleputswa (9.6%) and Thabo Mofutsanyane (9.7%) (Table 2).

Table 2 Provincial health indicators

	Fezile Dabi	Lejweleputswa	Motheo	Thabo Mofutsanyane	Xhariep	Free State	National
Population total	518 024	758 097	790 955	767 896	133 666	2 968 604	47 844 347
Medical aid coverage	18.9%	14.6	24.7	5.3%	8.4%	15.8%	14.3
Access to piped water	97.1%	97.4	98.8	96.4%	92.5%	97.3%	85.8%
Deprivation Index *	1.59	2.04	2.12	2.92	2.27	-	-
Social Economic quintile **	5	4	3	3	3	-	-
Nurse clinical workload	44.2	40.5	28.9	40.3	35.9	36.9	23.7
Caesarean section rate	16.6%	9.6%	17.3%	9.7%	0.0	11.8%	15.6%
Proportion ANC clients tested for HIV	93.9%	74.7%	75.9%	79.9%	90.9%	80.1%	79.6%
HIV prevalence among ANC clients	33.0%	37.0%	27.4%	30.7%	28.3%	33.6%	28.3%
Utilisation rate	2.0	1.6	1.9	2.4	2.8	2.0	2.2
Diarrhoea incidence under 5	170.2	171.7	161.4	158.7	260.1	168.9	254.0
Percentage of woman delivering in public facilities	78.7%	91.7%	92.4%	80.5%	57.9	84.8%	80.6%

Table adapted from the Health Systems Trust Health Barometer 2007/008 * High value most deprived ** Social economic quintile 1=poor 5=rich

HIV/AIDS Disease Burden in the Free State

In 1991 HIV prevalence amongst pregnant women attending antenatal services in the public sector in the Free State was 1.5% and by 1996 this increased to 17.5% (DOH. 2009b, DOH 2009c). Since 2001 there has been no statistically significant increase in HIV prevalence (DOH. 2009b, DOH 2009c). In 2001 the prevalence was 30.1% and 33.5% in 2007 (DOH. 2009b, DOH 2009c). In 2007 Lejweleputswa (37.0%) had the highest prevalence while Xhariep (24.0%) had the lowest (Table 3).

Table 3 HIV prevalence among antenatal attendees in the Free State 2007

	Prevalence %	95% Confidence Interval
Free State	33.5	28.3-39.1
Fezile Dabi	33.0	28.2-38.1
Lejweleputswa	37.0	33.1-41.1
Motheo	27.4	23.8–31.3
Thabo Mofutsanyane	30.7	26.9-34.8
Xhariep	24.0	16.7–32.6

Table adapted from the National HIV and Prevalence Survey 2008

In 2006 the ASSA model estimated that HIV/AIDS accounted for almost 51% of deaths (Dorrington et al. 2006). In 2006 it was estimated that 3.6% of infants were infected perinatally while a further 2.5% were infected through breast milk in the province (Dorrington et al. 2006). Provincially infant and child mortality rates have declined since the peak in 2001. In 2001 the infant mortality rate was 72/1000 live births and the under five mortality was 106/1000 (Dorrington et al. 2006). By 2006 the infant mortality rate decreased to 57/1000 live births and the under five mortality to 87/1000 (Dorrington et al. 2006). The decrease in mortality may be related to an increase in the number of women accessing PMTCT in the province. In 2006/2007 it was estimated that about 60% of HIV infected mothers and their exposed infants received prophylaxis in the province (Health Systems Trust 2008).

Definition of Terms and Measurements for Surveillance

Population NVP Coverage: the proportion of HIV-exposed infants in the population in whom both maternal and infant doses were ingested (Stringer et al. 2005). Infant HIV exposure was determined by cord blood testing, maternal nevirapine ingestion by the presence of nevirapine in the cord blood and infant nevirapine ingestion from the clinical records at the delivery facility.

Calculation of maternal adherence: the number of women in whom nevirapine was detected in the cord blood divided by the total number of HIV infected women who were dispensed with the intervention.

Study Design

This study used a cross-sectional design.

Study Population and Sampling

The study population included women who delivered within three randomly selected sub-districts in the Free State. These women attended maternity sites in one metropolitan area (Botshabelo) and in four small towns (Ladybrand, Ficksburg, Clocolan and Senekal) in Motheo and Thabo Mofutsanyane districts. From Thabo Mofutsanyane district, Itemoheng District Hospital in Senekal, JD Newbury District Hospital in Clocolan and Phutuhola District Hospital in Ficksburg were selected. From Motheo district, Mantsopa district hospital in Ladybrand and Botshabelo district hospital in Botshabelo and five supporting 24 hour clinics were selected.

From November 2007 to April 2008 cord blood specimens were collected from all live deliveries. Infants born before arrival at the maternity site and those still born were excluded from the study.

Sample Size

The required sample size was calculated using the EPICALC 2000 software. Sample size estimates were based on 50% PMTCT coverage, (based on earlier unpublished studies), and a margin of error around the estimate of 5% (i.e. 95% confidence interval between 0.45 and 0.55). The sample size required was 384 HIV-infected specimens. As the average prevalence in the areas where the study was conducted was 30%, an overall sample size of 1078 was required. The sample size for each site was based on the HIV prevalence at that site. The study was commenced at each site and continued until over 95% of specimens from the required sample size was collected.

Measurement

After delivery cord blood specimens from discarded placentas were collected by the health care worker performing the delivery. Five cm^3 of blood was collected and placed in an anticoagulated (EDTA) tube and assigned a number. In instances where multiple births were recorded, only one cord blood specimen was obtained. Information was collected from the mother's chart relating to the pregnancy. No patient identifiers were included. The blood specimens were sent to a central location where a rapid assay for HIV antibodies was conducted, in an effort to preserve anonymity. For every positive result, a small amount of blood was placed on a filter paper, placed in a plastic bag and refrigerated. These specimens were tested for nevirapine using chromatography at the University of Cape Town.

Surveillance Chart

This following information was collected from the mother's clinical chart: the mother's age, gravidity, the month and year of delivery, the delivery site, the mode of delivery, if the mother was offered pre-test counselling, if she accepted HIV testing, if the results of the test were given back to the mother, if ART was dispensed to the mother, whether the infant was administered prophylaxis and if formula milk was given on discharge. This information was collected by nurses. All forms were checked by study staff for quality assurance to see if information was complete. In instances where women delivered at sites different to where they attended antenatal care, information was retrieved from the health passport or otherwise recorded as unknown.

Validity and Reliability

The cord blood specimens were tested for HIV antibodies with a rapid test algorithm (Determine HIV 1/2 test, Abbott Laboratories, Chicago, Illinois). In the case of discrepancies, a second Determine test was done. A second Determine test was conducted by a different person on 10% of specimens for quality control. The Determine test kit has a sensitivity of close to 100% (95% CI 99.8-100.0%) and specificity of 99.9% (95% CI 99.8-99.4%) (Wright, Stringer 2004).

High performance liquid chromatography was used to detect nevirapine. Nevirapine was extracted from the dried blood spots (DBS) with 80% methanol, 20% 0.2M zinc sulphate containing neostigmine as internal standard. The chromatography analysis was carried out on the Phenomonex Fusion RP column (5x2x4um) using a methanol/10 mM ammonium acetate gradient to effect elution. Detection was achieved using an Applied Biosystems API 3200 tandem mass spectrometer in the MRM detection mode. Detection of nevirapine in dried blood spots (DBS) was carried out by a validated method using minor modifications (Koal et al. 2005).

For qualitative assessment blank and quality control cut off samples were included with each run. The limit of detection for nevirapine was set at 0.1μg/ml. Values detected above this limit were reflected as positive and those below as negative. For quantitative assessment standard curves were run in the range 0.1– 10μg/ml and appropriate quality control samples run with each batch. The limit of quantification was 0.1 μg/ml.

Data Management and Analysis

Data was collected, checked and double entered at a central location by trained data capturers. The data was entered into an encrypted MS Access database for security. For quality assurance purposes, data entry errors were identified by comparing every 10^{th} entry into the database.

Data was exported from MS-Access to STATA version 10 for analysis. T- tests were used to compare continuous variables between different groups and Chi squared tests for categorical variables. Both univariate and multivariate logistic analysis were used to determine the factors associated with maternal and infant adherence. Results were analysed as prevalence odds ratios with their corresponding p-values and confidence intervals.

Ethical Considerations

Ethics approval was granted by the University of Cape-Town Ethics Committee (approval number REC REF 038/2007), the Centre for Disease Control (CDC) and the University of Alabama.

Confidentiality and anonymity were assured as there were no identifiers on cord blood specimens and the surveillance form. In this way no information could be traced back to

the women in the study. Confidentiality was maintained as only the study personnel had access to the database which was securely protected by a password. Women were informed of the study through talks given by service providers at the antenatal care services.

Individual informed consent was not obtained from mothers for the following reasons:

- There was no risk to the patient as blood was taken from the discarded placenta.
- The rights of the patient were not harmed. Patients still had access to the facilities and the usual care that the facilities provided. Anonymity was assured since HIV assay results could not be linked to a woman.
- Obtaining informed consent from women participating would have led to selection bias. A study in Lusaka showed that women who refused testing were more likely to be HIV infected. (Stringer et al. 2005) .

The study had no direct benefits for the participants. Information gathered by this study could be used to strengthen the PMTCT program.

CHAPTER IV

RESULTS

During the surveillance period 1679 women delivered in facilities in the Free State and 1650 (98.27%) specimens were collected and tested for maternal HIV antibodies. Specimens were not collected from 11 women (0.66%) and 18 (1.07%) of those collected were not tested[1]. Mothers in whom specimens were not collected or tested had a shorter median interval between the last antenatal visit and delivery; their infants also had a lower median birth weight as compared to those in whom specimens were obtained and tested. The gravidity, the number of antenatal visits and the age of the mother did not differ between mothers in whom a specimen was collected or tested and those in whom specimens were not collected or tested (Table 4). A further 31 HIV positive women were excluded from the analysis because their babies died or were stillborn (n=8) and because no nevirapine cord result was obtained (n=23). The remaining 1619 (96.4%) women therefore make up the surveillance population of which 29.2% were HIV positive (Figure 3).

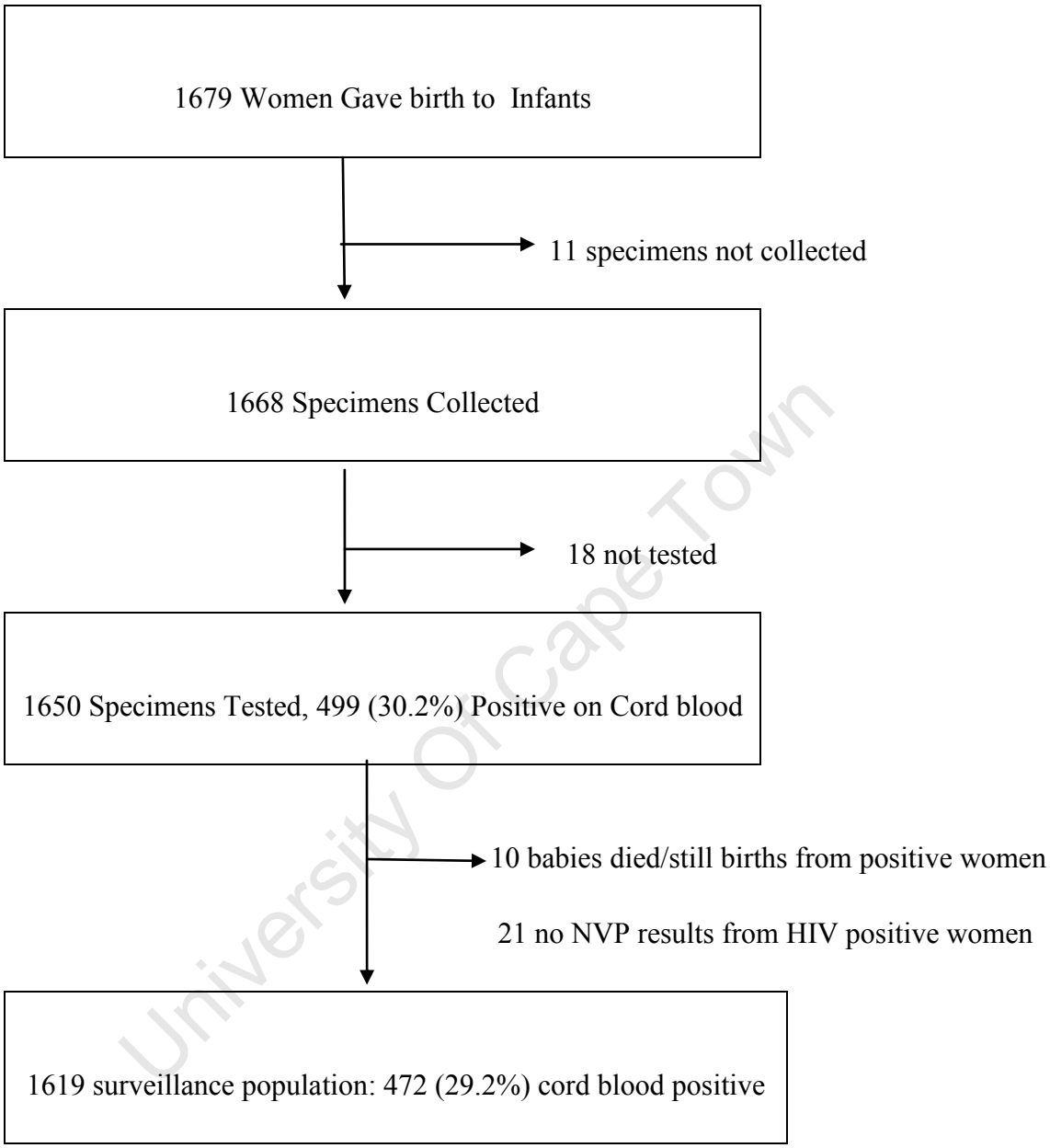
Table 4 Characteristics of mothers in whom cord blood testing was performed compared to those in whom no test or specimen was obtained

Variable	No specimen Result n=29	Specimen Results Obtained n=1650	P-value
Median Infant Birth weight (g)	2700 [IQR 1900-3100]	3017.5 [IQR 2780-3300]	0.001
Median Mothers Age (years)	26 [IQR 22-30]	24 [IQR20-29]	0.322
Median Gravidity	2 [IQR 1-3]	2 [IQR 1-3]	0.496
Interval in months between last ANC visit and delivery	1 [IQR 0-1]	0 [IQR 0-1]	0.012
Interval between testing and delivery (in months)	3 [IQR 2-4]	3 [IQR 2-4]	0.329
Mode of Delivery			
Vaginal	93.1% (n=27)	90.4% (n=1448)	0.617
Caesarean	6.9% (n=2)	9.7% (n=159)	

[1] 11 Specimens not collected (1 born before arrival, 1 cord snapped, 2 forgot to take sample, 1 fresh stillbirth, 4 macerated stillbirth, 1 Other-Unspecified, 1 resuscitating patient)

18 Specimens not tested (11 severely haemolysed, 3 blood clotted, 4 Other-Unspecified)

Figure 3 Free State cord blood study profile



Characteristics of Surveillance Population

Almost 80% of the women that made up the surveillance population were from the Hospital facilities (Botshabelo Hospital, Phuthuloha Hospital, Mantsopa Hospital, JD Newbury Hospital, Itemoheng Hospital and Dr Pedro Hospital) the remaining 20% were from the clinic facilities (Winnie Mandela, Pule Sefatsa, Maletsatse Mabaso, Itumelang).

The median age of mothers was 24 years [IQR 20-29yrs]. The median number of pregnancies was 2 [IQR 1-3]. Mothers had a median of 4 [IQR 3-6] antenatal visits. 93.8% of the mothers who delivered in the facilities attended at least one antenatal visit. There was no significant difference in number of antenatal visits, the mode of delivery, the interval in months between testing and delivery and the interval between the last antenatal visit and delivery between HIV-infected and HIV-negative women. The median age for HIV-infected women was 27 years and 23 years for HIV-negative women. A minority of the mothers 10.2% reported that they had previously been tested for HIV. All women who reported that they were HIV-infected were positive on cord blood HIV antibody testing. Twenty three point five percent of women who reported that they were negative were found to be positive on cord blood testing. The majority of the mothers (90.5%) delivered vaginally and 9.5% delivered via caesarean section (Table 5).

Table 5 Profile of surveillance population by cord blood HIV testing status

Characteristics	Overall (n=1619)	HIV-infected (n=472)	HIV-Negative (n=1147)
Median Baby Birth weight (grams)	3025 [IQR 2800-3340]	3000 [IQR 2700-3300]	3053 [IQR 2800-3340]
Median Mothers Age (years)	24 [IQR 20-29]	27 [IQR 24-31]	23 [IQR 20-28]
Median Gravidity	2 [IQR 1-3]	2 [IQR 2-3]	2 [IQR 1-3]
Median Interval between testing and delivery (months)	3 [IQR 2-4]	3 [IQR 2-4]	3 [IQR 2-4]
Median number of antenatal visits	4 [IQR 3-6]	4 [IQR 3-6]	4 [IQR 3-6]
Mode of Delivery			
Caesarean	9.5%	9.5%	9.5%
Vaginal	90.5%	90.5%	90.5%
Previous HIV test done			
Yes	10.2%	12.7%	9.2%
No	89.8%	87.3%	90.8%
Previous Positive result	16.4% (20)	100% (n=20)	0% (0)
Previous Negative result	83.6% (102)	23.5% (n=24)	76.5% (n=74)

HIV Prevalence of Women Delivering in Facilities in the Free State

The overall HIV prevalence in the surveillance population was 29.2% (n=472) (95% CI 26.9-31.4%). HIV prevalence did not differ significantly between facilities p=0.795 (Table 6). Mothers aged less than 19 years and those aged 20-24 years had the lowest HIV prevalence 9.8% (95% CI 6.7-13.7%) and 23.5% (95% CI 19.9-27.3%) respectively. Mothers aged 25-29 years and those aged 30-34 years had the highest HIV prevalence of 42.2% (95% CI 37.3-47.1%) and 41.6% (95% CI 35.1-48.3%) respectively (Table 6).

Mothers who had not received pre-test counselling had a lower prevalence than those who received pre-test counselling (23.0% n=52, 30.2% n=392) p=0.028. Similarly mothers who refused testing had a lower prevalence as compared to those who accepted testing (22.7% vs. 31.5%) p=0.016 (Figure 5). These results are based on the cord blood analysis results.

Table 6 HIV prevalence of mothers delivering in public facilities in the Free State

Facility	HIV Prevalence	95% Confidence Interval
Botshabelo Hospital	29.2% (72/247)	23.6-35.2
Dr. Pedro Hospital	34.5% (20/58)	22.4-48.1
Itemoheng Hospital	29.1% (70/241)	23.4-35.2
Itumelang M Clinic	31.3% (21/67)	20.5-43.8
JD Newbury Hospital	24.5% (52/212)	18.9-30.8
Maletsatse Mabaso B Clinic	24.1% (21/87)	15.6-34.5
Mantsopa Hospital	31.7% (109/344)	26.8-36.9
Phuthuloha Hospital	30.1% (64/213)	24.0-36.7
Pule Sefatsa U Clinic	27.8% (20/72)	17.8-39.6
Winnie Mandela J Clinic	29.5% (23/78)	19.6-40.8
Total	**29.2% (472/1619)**	**26.9-31.4**
Age Category		
Less than 19	9.8% (30/305)	6.7-13.7
20-24	23.5% (122/519)	19.9-27.3
25-29	42.2% (172/408)	37.3-47.1
30-34	41.6% (94/226)	35.1-48.3
35+	33.5% (54/161)	26.3-41.4

Free State Population PMTCT/Coverage Cascade

The PMTCT cascade describes the events that need to be followed for the intervention to be delivered and allows for coverage to be estimated. Coverage refers to maternal (nevirapine present in cord blood) and infant ingestion (documented in records) of the prophylaxis. Of the 1619 mothers in the surveillance population 94.1% (n=1523) had complete information in the clinical folder of whether they had been offered or accepted testing during antenatal care (Cascade Step1 Figure 4 & Figure 5). Of the mothers who had complete information in the folder, 85.2% received pre-test counselling (Cascade

Step2 Figure 4 & Figure 5). However 28.7% (n=65) of all the mothers who did not receive pre-test counselling according to the clinical records had not attended antenatal care. Of the mothers that were pre-test counselled, 85.7% agreed to testing (n=1112) (Cascade Step3 Figure 4 & Figure 5). All mothers who tested received either a positive or negative test result. 790 (71.0%) mothers received a negative test result and 322 (29.0%) received a positive result (Cascade Step4 Figure 4 & Figure 5.). Of those that received a negative test result during antenatal care, 752 were also negative on cord blood testing at delivery but 38 tested positive on cord blood antibody. Of the 322 women that received a positive test result during antenatal care, 312 were also found to be positive on cord blood testing, and 10 women who were given a positive test result during antenatal care were found to be negative on cord blood testing (Table 7).

Table 7 Comparison of HIV test results received during antenatal care and results obtained during cord blood testing.

	Cord blood Testing		
Antenatal Care Test Result	Positive	Negative	Total
Positive	312	10	322
Negative	38	752	790
Total	350	762	1112

312 mothers were identified as HIV-infected on cord blood testing (Cascade Step5 Fig.2 & Fig.3). According to the clinical records 93.7% (n=291) of HIV-infected women on cord blood testing were dispensed with PMTCT, the remaining 6.9% (n=21) did not receive any intervention. Of these women who received the intervention, 245 (Cascade Step6 Figure 4 & Figure 5) had nevirapine detected in the cord blood. Thus maternal adherence was 84.2%. 88.2% (n=216) of infants born to mothers who were adherent to the intrapartum nevirapine, received the required dose directly observed. All infants received the nevirapine dose within 72 hours of birth. 29 infants did not receive their PMTCT dosing. The population coverage was therefore (216/472) 45.8% (95% CI 41.2-

50.4%), and thus 256 (54.2%) of the 472 HIV exposed live infants missed the opportunity to receive both the maternal and infant PMTCT doses. Of the infants who received both maternal and infant doses 46.7% were discharged with replacement feeding and only 11.8% of those infants who failed to receive the recommended prophylaxis.

Figure 4 PMTCT attrition cascade among surveillance population across 10 delivery sites in the Free State

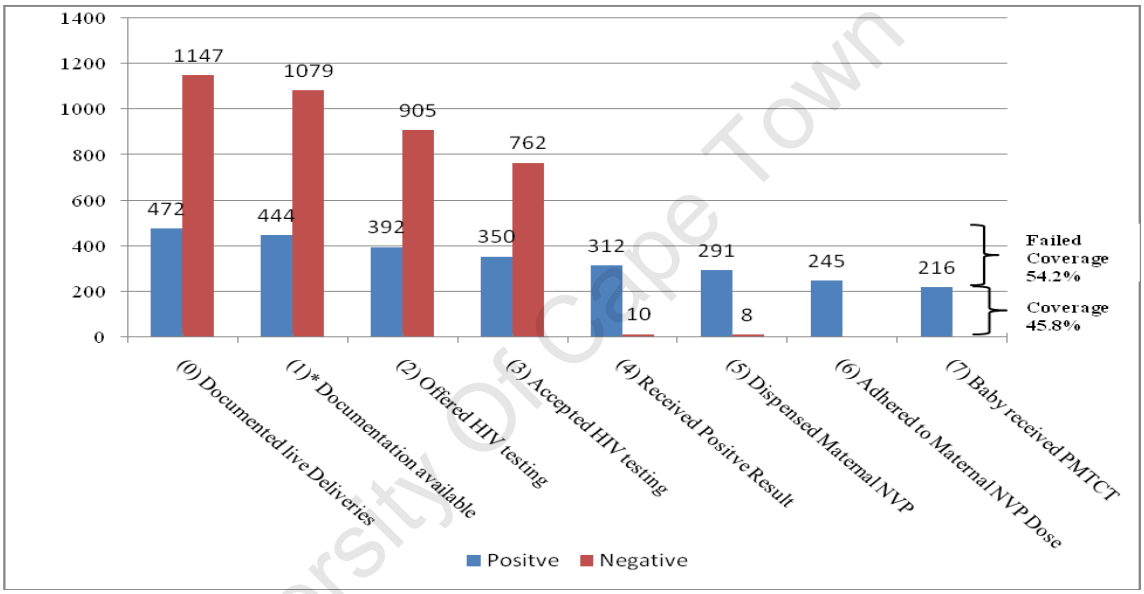
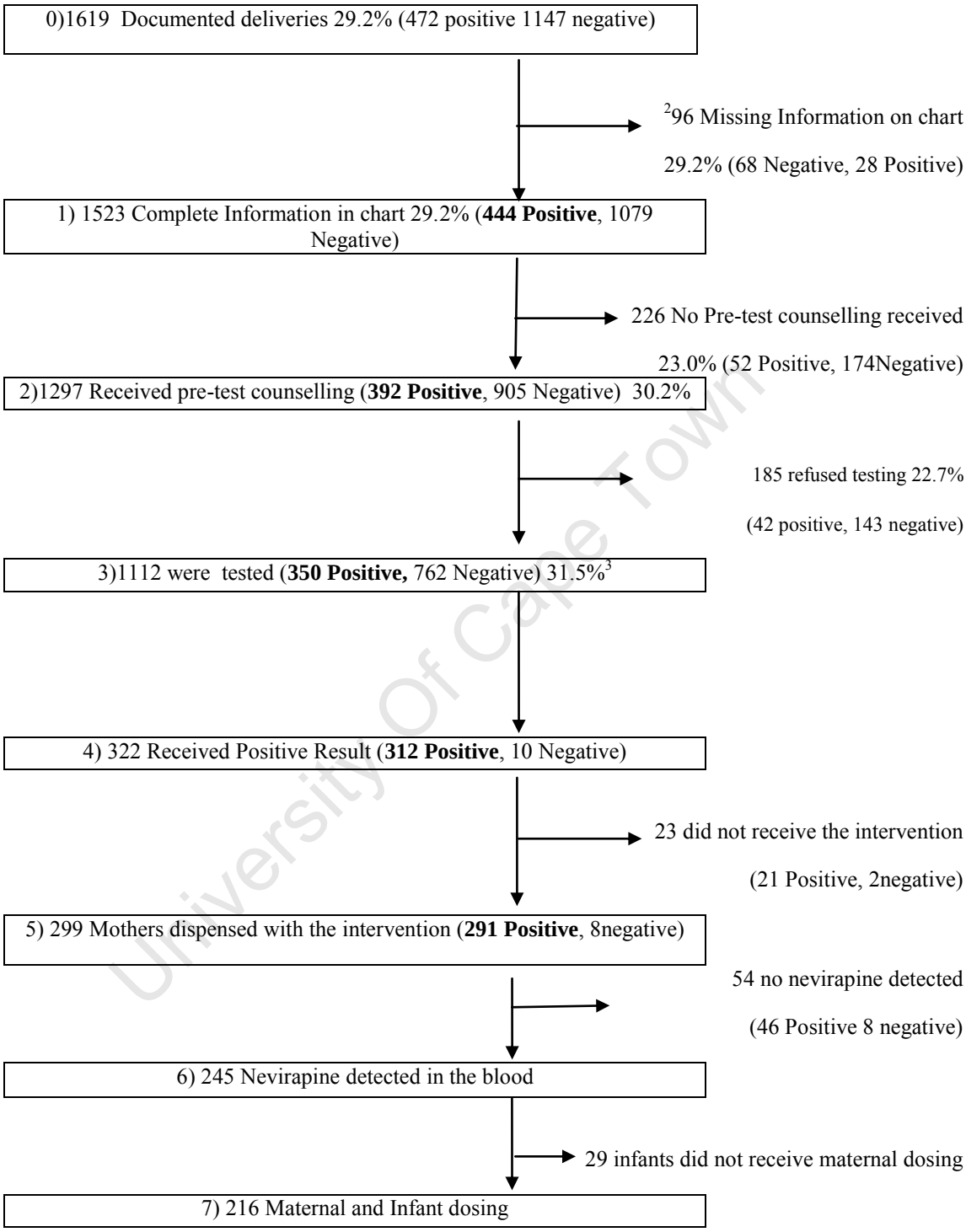


Figure 5 shows the sequence of events that had to be followed in order to achieve successful PMTCT coverage Step 0 (leftmost bar) represents all women in the surveillance population who stratified by cord blood HIV status at delivery. Coverage defined as the proportion of infected/exposed mother/infant pairs that received maternal and infant nevirapine doses was 45.8% (216/472). * There were 28 HIV infected/exposed cases who failed coverage (i.e. no nevirapine in the cord blood or infant did not receive dose but reason for failure could not be determined.

Figure 5 Flow diagram showing attrition from the PMTCT program in the Free State



[2] The 96 women who had missing chart information and those who did not for HIV testing-HIV results are based on the cord blood results.
[3] Results in brackets are based on cord blood results

Determining the factors related to maternal adherence

In a univariate logistic analysis, maternal adherence was not associated with the infants' birth weight, gravidity, the mothers age, the mode of delivery, the type of intervention received nor the interval between the last ANC visit and the interval between testing and delivery (Table 9). In a multivariate analysis, after adjusting for the mothers age and the type of intervention received, mothers who had attended more than 5 antenatal visits were 2.76 times more likely to be adherent than mothers who attended less than 4 visits (p=0.030). However there was no significant difference between mothers who attended 4 or 5 visits and those who attended 3 or less (p=0.645). The mothers' age and the type of intervention received were not significantly associated with adherence after adjusting for the other variables (Table 10).

Table 8 Characteristics of HIV-infected mothers in the Free State by adherence status

Variable	Adherent mothers n=245	Non-adherent mothers n=54
Median baby Birth weight(grams)	3000 IQR [2700-3300]	3016 IQR [2670-3400]
Median Mother age (years)	27 IQR[24-31]	27 IQR[25-30]
Gravidity		
1	20.8%	26.7%
2-3	63.7%	60.0%
4+	15.5%	13.3%
Interval Between Testing and delivery		
0-3 months	55.5%	56.5%
4 months or more	44.5%	43.5%
Interval between last ANC visit and delivery		
Less than 1 month	57.6%	54.4%
1 month or more	42.5%	45.7%
Number of Antenatal Visits		
3 or less	30.6%	37.0%
4-5	34.7%	43.5%
6 or more	34.7% (	19.6%
Mode of Delivery		
Vaginal	89.8%	91.3%
Caesarean	10.2%	8.7%
Type of Intervention Mother received		
NVP only	90.0%	95.7%
HAART or AZT+NVP[4]	10.1%	4.4%

[4] 10 started HAART 12 started NVP+AZT

Table 9 Univariate regression analysis for determining factors for maternal adherence in the Free State

Variable	Odds Ratio	95% CI	P Value
Birth weight	1.00	0.99-1.00	0.752
Gravidity			
1	1.00	-	-
2-3	1.47	0.71-3.07	0.301
4+	1.61	0.56-4.63	0.373
Mothers Age	1.01	0.96-1.08	0.633
No. of ANC Visits			
3 or less	1.00	-	-
4-5	0.96	0.47-1.97	0.919
6 or more	2.14	0.90-5.09	0.085
Mode of Delivery			
Caesarean Section	1.00	-	-
Vaginal	0.84	0.27-2.53	0.754
Type of intervention			
HAART/AZT+NVP	1		
NVP only	0.63	0.30-1.34	0.237
Interval between last ANC visit and delivery			
Less than 1 month	1	-	-
1 month or more	0.89	0.46-1.65	0.687
Interval between testing and delivery			
0-3months	1	-	-
4 months or more	1.04	0.52-1.97	0.889

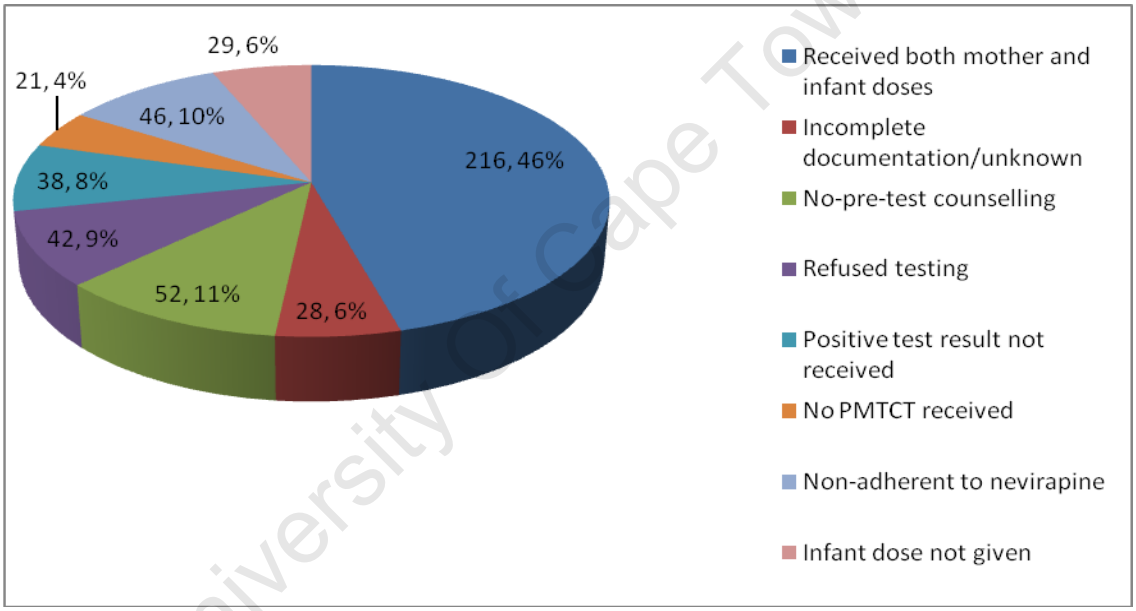
Table 10 Multivariate regression analysis for determining factors related to maternal adherence in the Free State

Variable	Adjusted Odds Ratio	95% CI	P Value
Mothers age	1.01	0.95-1.09	0.586
Type of Intervention Offered			
AZT+NVP/HAART	1.00	-	-
NVP only	0.67	0.31-1.41	0.289
No. of ANC visits			
1-3	1.00	-	-
4-5	1.19	0.57-2.50	0.645
6+	2.76	1.10-6.91	0.030

Reasons for failed coverage

Of the 472 live infants born to HIV positive mothers in whom cord blood testing was performed, only 45.8% (95% CI 41.2-50.4%) had evidence that they received both the maternal and infant doses of ARV prophylaxis. Reasons for failed dosing included, not offered pre-test counselling, refused testing, did not receive a positive test result prophylaxis was not issued, the mother did not adhere and the infant did not receive the prophylaxis dose.

Figure 6 Population PMTCT coverage and reasons for failed coverage



Factors associated with successful PMTCT coverage

In the multivariate regression HIV-infected mothers who attended 4 or 5 antenatal visits were 2.16 times more likely to have their infants successfully received the appropriate prophylaxis than those who attended less than 3 (p=0.001). Similarly HIV-infected mothers who attended 6 or more visits were 2.71 times more likely to have their infants receive the full prophylaxis as compared to those who attended 3 or less visits (p=0.002).

Table 11 Characteristics of HIV-infected and exposed infants in the Free State by coverage

Variable	Successful Coverage N=216	Failed Coverage N=256
Median Mothers age (years)	26 IQR[24-30]	27 IQR[24-32]
Gravidity		
1	20.8%	27.5%
2-3	62.5%	60.4%
4+	16.7%	12.2%
Interval Between Last ANC visit and delivery		
Less than 1 months	57.9%	49.6%
1 month or more	42.1%	50.4%
Number of Antenatal Visits		
3 or less	28.2%	45.2%
4-5	37.1%	31.2%
6 or more	34.7%	23.6%
Median Birth weight (grams)	3000 IQR[2715-3300]	3000 IQR[2600-3290]
Mode of delivery		
Caesarean	11.1%	8.2%
Vaginal	88.9%	91.8%

Having had more pregnancies was significantly associated with successful coverage. HIV-infected mothers who had four or more children were more likely to have their infants receive both the infant and maternal doses as compared to those who only had one child (p=0.011). However this association was not significant in having had 2 or 3 children as compared to having had only 1 child. There was no significant association between successful coverage and the birth weight of the infant (Table 13). In the univariate regression, an increase in mothers' age was significantly positively associated with successful coverage, however during the model building process this effect diminished, and the adjusted odds ratio was no longer significant (Table 12).

Table 12Univariate regression analysis for factors related to successful PMTCT coverage

Variable	Odds Ratio	P-value	95% CI
Birth weight	1.09	0.007	1.04-1.11
Gravidity			
1	1.00	-	
2-3	1.38	0.179	0.89-2.15
4+	1.83	0.051	0.99-3.37
No. of ANC visits			
<3	1.00	-	-
4-5	2.13	0.001	1.37-3.31
6+	2.64	<0.001	1.67-4.19
Mothers age	1.04	0.038	1.00-1.07
Mode of Delivery			
Caesarean Section	1.00	-	-
Vaginal Delivery	0.71	0.286	0.39-1.32
Interval between last ANC visit and delivery			
Less than 1 month	1.00	-	-
1 month or more	0.72	0.073	0.50-1.03

CI, Confidence Interval

Table 13 Multivariate analysis for factors associated with successful PMTCT coverage

Variable	Adjusted Odds Ratio	P-value	95% CI
Birth weight	1.00	0.137	0.99-1.00
No. of ANC visits			
<3	1	-	-
4-5	2.16	0.001	1.37-3.42
6+	2.71	<0.001	1.66-3.70
Gravidity			
1	1.00	-	-
2-3	1.42	0.30	0.90-2.24
4+	2.30	0.011	1.21-4.37

CI, Confidence Interval

CHAPTER V

DISCUSSION

Despite the wide-spread availability of PMTCT services in the Free State, less than half of the infants born to HIV-infected mothers received appropriate prophylaxis. The study showed that attrition occurred at all stages of the cascade resulting in reduced coverage. Studies conducted in Africa have shown that coverage was poor (Manzi et al. 2005, Perez et al. 2004, Temmerman et al. 2003, Stringer et al. 2005). In Kenya 20% of mother infant pairs received the appropriate prophylaxis (Temmerman et al. 2003) and 30% in Zambia (Stringer et al. 2005).

Studies in routine PMTCT services in Africa have reported low uptake of VCT services among antenatal attendees (Ekouevi et al. 2004, Pignatelli et al. 2006, Meda et al. 2002). In this study more than 80% of women were tested and this was encouraging. The use of rapid tests, the availability of same day results and confidential „one-on-one' counseling sessions with well trained staff may have been associated with higher uptake of VCT. Other studies conducted in South Africa (Coetzee et al. 2005, Jackson et al. 2007) have also noted high uptake of VCT. However about 20% of HIV-infected women in this study failed to enroll into the program because they were not offered or refused testing and this has important implications for the success of PMTCT programs. Similar proportions of women were either not offered or refused testing. VCT services therefore need to be strengthened and all women should be offered an HIV test and need to be encouraged to test.

Counsellors and health care workers need to be motivated and encouraged to provide women with high quality counselling. In Zimbabwe in service training sessions and validated teaching tools such as flip charts were developed to strengthen interview skills and provide counsellors and health care workers with up-to-date information on PMTCT strategies

(Chandisarewa et al. 2007). Monitoring was introduced to ensure adherence to PMTCT protocols and staff competency was assessed (Chandisarewa et al. 2007). Community engagement through effective communication strategies that improve HIV/AIDS awareness including primary prevention of HIV and PMTCT need to be strengthened. Women in particular need to be empowered on their reproductive rights and encouraged to practise safer sex. Efforts also need to be established to reduce discrimination and stigma towards people living and affected by HIV/AIDS.

In this study the majority of women who delivered in the facilities (94%) attended at least one antenatal visit. This finding is consistent with reports from other parts in Africa (Moses et al. 2008). However 6% of HIV-infected women failed to receive prophylaxis because they had not attended antenatal services according to clinical records. Offering HIV testing in the labour ward is a strategy that can be adopted for mothers who did not attend antenatal care but deliver in facilities (Temmerman et al. 2003, Homsy et al. 2006). This also gives women who are not offered or those who refuse testing another opportunity to know their HIV status and participate in the program. These mothers and their infants will then have access to intrapartum and postpartum prophylaxis. Implementation of labour ward testing has been found to be acceptable and feasible (Pai et al. 2008). In India 98% of women offered testing were tested in the labour ward (Pai et al. 2008). In Uganda, 88% of women who presented in the labour ward with undocumented HIV status were tested during delivery (Homsy et al. 2006). Health care workers should also be encouraged to ask HIV infected mothers whether they were given or remembered to take their prophylaxis when they present in labour, and in instances where prophylaxis this was not taken they should be administered.

In addition to the 6% of HIV-infected women in this study who did not attend antenatal services, a further 5% of HIV-infected women had no record of whether they had been offered VCT services or if they had refused testing. Nevirapine was not detected in the cord blood of these women and this contributed to failed coverage. Good medical record

keeping should be emphasised. Staff and health care workers should be well-trained on record keeping and capture data for monitoring and evaluation purposes. Simple, effective and preferably electronic record keeping systems that link patient data should be developed for each facility (Reithinger et al. 2007). Centralised databases that allow for the access to patient information from any facility within the province will help reduce attrition from the program in the event that women are referred or transferred to a different facility for delivery or antenatal care.

The overall HIV prevalence in the surveillance population was 29.2%. This is consistent with the Free States Provincial antenatal prevalence at that time (DOH. 2009). Contrary to a study in Lusaka, Zambia (Stringer et al. 2005), women who were not offered pre-test counselling and those who refused testing, had a lower HIV prevalence than those who were tested during VCT. This may be explained by the fact that women who perceive themselves to be at risk of infection are more likely to accept testing (Mpairwe et al. 2005). This finding is consistent with findings reported in Uganda where women who were tested in pregnancy had a higher HIV prevalence than those who refused testing but were anonymously tested (Mpairwe et al. 2005).

This surveillance showed that 8% of HIV-infected mothers at delivery were seronegative when tested at their first antenatal visit. This finding is similar to the 6% reported in Zambia (Stringer et al. 2005). It is also consistent with findings reported in KwaZulu-Natal, where 7% of women in the study had a negative HIV test result during pregnancy, but were found to have HIV-infected infants when their infants were tested postnatally (Rollins et al. 2007a). These women either seroconverted during pregnancy or were false negatives when they tested at their first antenatal visit or testing information was incorrectly recorded at the antenatal facilities. Women may seroconvert during pregnancy and repeat testing later in pregnancy may be required (Moodley et al.

2009). A South African study recommended repeat testing late in pregnancy (Moodley et al. 2009) .

HIV-infected women in the Free State were given the nevirapine at 28 weeks of gestation. In this study 93% of mothers who were identified as HIV-infected during pregnancy were dispensed with the intervention. In a study in Lusaka less than 1% of HIV positive women either failed to receive the intervention or did not collect the test results. In a study in the Eastern Cape over 40% of pregnant women who were identified as HIV-infected failed to receive nevirapine (Peltzer et al. 2008). Women may not return and collect their prophylaxis because of fear and stigma. In Kenya women are now given the prophylaxis during post-test counselling irrespective of their gestational stage (Temmerman et al. 2003).

A strength of this study was the ability to objectively determine the rate of maternal adherence. Unlike other studies that have relied on pill counts or self reports to measure maternal adherence (Albrecht et al. 2006, Bii et al. 2007, Kiarie et al. 2003), cord blood specimens were used to detect whether nevirapine had been taken. In this study almost 20% of women who were given the intervention according to the clinical records had no nevirapine detected in the cord blood. This finding is lower than the 32% non adherence rate reported in the study in Lusaka, Zambia which used similar methods to measure adherence. Identified HIV-infected women in the Free State were given nevirapine to take once they went into labour. This surveillance showed that women who attended more antenatal visits were more likely to have nevirapine in their cord blood. This association is consistent with the findings reported in Kenya (Bii et al. 2007). This suggests that there are more opportunities for health care workers to encourage, support, and re-emphasise the importance and benefits of adhering to therapy if they attend more antenatal clinic visits.

In this study 6% of infants born to seropositive mothers who had nevirapine in their cord blood, did not receive their nevirapine dosing. All the infants who received their dosing received it within 72 hours of delivery in the facility by the health care worker. Other studies report that nevirapine syrup is provided in blister packets for self administration for women delivering at home and this has resulted in infant adherence of almost 90% (Peltzer et al. 2008). In this study HIV-infected women who attended more antenatal visits were more likely to have their infants receive both maternal and infant doses. A woman who refused or was not offered HIV testing on her first antenatal visit may accept testing or be offered testing at subsequent visits. This finding was similar to a study conducted in KwaZulu-Natal where women who attended less antenatal visits were at increased risk of transmission (Rollins et al. 2007). The WHO has recommended that pregnant women in resource constrained settings should attend that at least four antenatal care visits to achieve positive maternal health outcomes (Villa et al. 2001). An increase in gravidity was positively associated with successful coverage. This finding may suggest that mothers who have more children may comprehend and understand the need to follow all the steps in the cascade as they are likely to be more experienced in mother hood as compared to new mothers. Furthermore these mothers may have been informed of PMTCT during earlier pregnancies at ANC sites through communication strategies and thus managed to successfully complete all the steps in the cascade.

Study limitations

This study had a number of limitations. The study excluded women who did not deliver in public sector antenatal facilities. In the Free State province it was estimated that 15% of women did not deliver in health care facilities (Health Systems Trust 2008). This study therefore missed women who delivered at home. In KwaZulu-Natal, it was found that home deliveries were associated with an increased risk of transmission (Rollins et al. 2007a); therefore the coverage estimate in this study may be over-estimated. Home deliveries are common in other parts of Africa (Manzi et al. 2005, Moses et al. 2008, Bii et al. 2007, Kagaayi et al. 2005).

Another weakness of this study was that it was insufficiently powered to detect factors related to maternal adherence. Other studies have found that disclosure of status to the partner, education level, maternal age and mode of delivery were associated with maternal adherence (Peltzer et al. 2008, Albrecht et al. 2006, Bii et al. 2007, Kiarie et al. 2003). This study also relied on the medical records to determine if the infant received the nevirapine however if the infant was dosed and this not documented then the infant was incorrectly classified as non-adherent thereby underestimating the coverage.

Finally, the main outcome measure in this study was coverage, but this only serves as a proxy to measure effectiveness (Stringer et al. 2008). The HIV free survival has been proposed to be the most important metric for estimating effectiveness in high prevalence and resource limited settings (Stringer et al. 2008). Because this was a cross-sectional study and HIV-exposed infants were not followed up and tested to determine HIV infection the HIV free survival could not be estimated. This study was able to point out which failures occur along the PMTCT cascade.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

HIV prevalence was high amongst women delivering in facilities in this Province in 2007/2008. Anonymous cord blood studies together with clinical record documentation of PMTCT coverage and the infant dosing can be used to determine the effectiveness of PMTCT programs.

PMTCT coverage in the Free State Province was poor despite the national expansion of PMTCT services to all antenatal sites. Failures occurred at each step of the PMTCT cascade and resulted in low coverage. Interventions should be introduced at each step of the PMTCT cascade to increase coverage. The number of antenatal care visits was associated with higher coverage and maternal adherence and women should be encouraged to attend antenatal care regularly.

Recommendations

- Further research is needed to determine the impact of implementing the new PMTCT protocol which consists of longer and more efficacious regimens on coverage.
- Train counsellors on how to deliver high quality confidential counselling to improve testing coverage
- Improve HIV and PMTCT awareness through effective communication strategies and community engagement to decrease stigma towards people living with HIV and empower women on their reproductive rights.
- Offer repeat testing during late pregnancy to avoid women who seroconvert and women who receive false negative results from failing to receive PMTCT interventions
- Offer HIV testing during delivery and in the labour ward in addition to VCT during antenatal care for women who may refuse to test and those who do not attend antenatal care

- Offer infant prophylaxis in blister packs together with adequate information on how to correctly store and administer the prophylaxis for women who wish to deliver at home.

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UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
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09 February 2007

REC REF: 038/2007

Dr D Coetzee
IIDMM
Public Health and Family Medicine
Falmouth Building, Level 1

Dear Dr Coetzee

PROJECT TITLE: PMTCT EFFECTIVENESS IN AFRICA: RESEARCH AND LINKAGES TO CARE PART1: CORD BLOOD SURVEILLANCE PROTOCOL VERSION 1.0

Thank you for submitting your study to the Research Ethics Committee for review.

I have pleasure in informing you that the Ethics Committee has **formally approved** the above mentioned study.
This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

p/s **PROF.M BLOCKMAN**
CHAIRPERSON, HSF HUMAN ETHICS

lemjedi

PE3 - SURVEILLANCE FORM

Study number SA / 120 / 1601 — 04 - 05 (MONTH - YEAR)

PART A *Complete form with a blue or black pen*

1. Date of delivery: 04 (MONTH) - 05 (YEAR)
2. Mother's age (years): 25
3. Gravidity*: 02 **Total number of pregnancies including this one and any past stillbirths/miscarriages/abortions*

4 Where did mother have her first prenatal visit? ○ No ANC visits ● This facility ○ Other_____

5. Total number of ANC visits: 01
6. Date of last ANC visit: 04 (MONTH) - 05 (YEAR)

7. HIV test before this pregnancy? ○ Yes, when? __ __ (MONTH) - __ __ (YEAR) ● No ○ Unknown

8. Previous result? ○ Positive ○ Negative ○ Indeterminate ● Not applicable — If No or Unknown Skip to question 9

DURING THIS PREGNANCY:

9. Was mother pretest counseled for HIV? ● Yes, when? 11 (MONTH) - 07 (YEAR) ○ No ○ Unknown

10. Was HIV test performed? ● Yes, when? 11 (MONTH) - 07 (YEAR) ○ No ○ Unknown

11. If test performed, HIV test result: ○ Positive ● Negative ○ Indeterminate ○ Unknown

12. Maternal NVP dispensed? ○ Yes ● No ○ Unknown

13. AZT dispensed? ○ Yes, month started: __ __ (MONTH) - __ __ (YEAR) ● No ○ Unknown

14. HAART therapy dispensed? ○ Yes, month started: __ __ (MONTH) - __ __ (YEAR) ● No ○ Unknown

Staff name [redacted] Signature [redacted]

PART B

15. Mode of delivery: ● Vaginal ○ Caesarean

16. Hours after delivery the mother was discharged: 012 17. Hours after delivery the baby was discharged: 12

18. Not yet discharged (tick in the box) ☐

19. Did the baby receive ARV prophylaxis? ● No 20. Reason:
- ○ Mother was not tested
- ● Mother tested HIV-negative
- ○ Mother was transferred
- ○ Baby died or was stillborn
- ○ Baby was transferred
- ○ Baby NVP given in ANC
- ○ Other_____

○ Yes 21. Number of hours after delivery: ☐

22. Which ARVs? ○ NVP only ○ AZT only ○ AZT + NVP ○ Other_____

23. Was mother discharged with replacement feeding? ○ Yes ● No ○ Unknown

24. Birth weight of baby (in grams): 2200g

25. In case of twins, birth weight of baby 2 (in grams): ☐

Staff name N C Mlungu Signature [redacted]

PE4 - LABORATORY TEST

Study number **SA / 120 / 1601**

Laboratory facility: _____

PART A *To be filled in by midwives and nurses from maternity ward*

☑ Sample collected. Date and time:

Date 1 2 - 0 4 - 0 8 (DAY - MONTH - YEAR)

Time 1 1 : 4 2 (H H : M M)

Write date and time sample collected on the blood tube

☐ It was not possible to collect a sample. Tick the primary reason:

- ◯ Macerated stillbirth (MSB)
- ◯ Fresh stillbirth (FSB)
- ◯ Cord snapped / broke
- ◯ Sample spilled / broke
- ◯ Placenta / cord unhealthy / too thin
- ◯ Born before arrival at clinic
- ◯ Mother transferred
- ◯ Forgot to take sample
- ◯ Other reason, specify: _____

Staff name [illegible] Signature _____ Date 12/04/08

PART B *To be filled in by laboratory*

Date sample tested 1 2 - 0 4 - 0 8 (DAY - MONTH - YEAR)

1. Determine HIV test result. *Perform this test on all samples.*

- ◯ Positive
- ◯ Negative
- ◯ Not done. Reason: ◯ Blood clotted ◯ insufficient sample ◯ Sample spilled / broke ◯ Other reason, specify: _____

2. Dried Blood Spot sample. *Prepare when Determine test is positive.*

- ◯ Prepared
- ◯ Not prepared. Reason: _____

3. QC Specimen Prepared

- ◯ Prepared
- ◯ Not prepared
- ◯ Not applicable

4. Specimen selected for 10% QC

- ◯ Yes
- ◯ No
- ◯ Not applicable

Laboratory staff name [illegible] Signature _____ Date _____