Acceptability and feasibility of heat-treated expressed breastmilk following exclusive breastfeeding by HIV-1 infected South African women

by

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I dedicate this Ph.D. thesis to my incredible daughter Gabriella, your unconditional support nourishes and sustains me, always, I love you. I thank my parents, David and Elizabeth Sibeko, whose spirits inspire me to follow in their footsteps and serve Africa. To my brothers Bongani, Themba and David, as always, Ngiyabonga.

ABSTRACT

Qualitative and quantitative research methods were employed to evaluate the acceptability and feasibility of HIV-1 infected, urban South African mothers being able to feed their infants heat-treated expressed breast milk (HTEBM). Nutritional status assessment of HIV-infected breastfeeding mothers (n=84) indicated that maternal status was not compromised; mean body mass index 26.8 (4.0) kg/m^2, triceps skinfold 14.8 (5.50) mm and hemoglobin 11.6 (1.49) g/dL. However, severely immunocompromised mothers ($CD4^+ < 200$ cells/mm^3) were more likely to be anemic. Breastmilk viral loads were also higher in mothers with lower $CD4^+$ cell counts. Community based inquiry on the acceptability of HTEBM was accomplished through in-depth interviews of participants (n=31), at the individual (mothers), family (partners, grandparents, mothers-in-law) and at the community level (traditional healers, daycare worker, health care counselors). Although an unfamiliar concept for all interviewed, overall, HTEBM was found to be an acceptable feeding choice regardless of respondents' gender, age, maternal status, family or community role. Further, data indicated mothers rarely received quality infant feeding counseling, consequently mixed feeding, a high risk for HIV transmission, was a common practice. In a pilot longitudinal study, using mixed-methods, the feasibility of mothers successfully implementing a modified breastfeeding intervention (6 months exclusive breastfeeding (EBF), cessation of breastfeeding, followed by use of HTEBM with complementary diet) was evaluated. The majority of mothers (36/66) practiced EBF for 6 months, 42% of whom also used HTEBM, expressing a range of approximately, 65 ml to 600 ml of breastmilk daily, for varying durations (2 weeks to 5 months). Mothers did not experience breast pathology. Home visits were highly enabling as was disclosure of HIV status to a partner. This is the first study to demonstrate that use of HTEBM is a feasible infant feeding option for HIV infected women. HTEBM may offer one solution to reduce vertical transmission of HIV and help maintain nutritional adequacy, as a component of complementary feeding.

RÉSUMÉ

Des méthodes qualitatives et quantitatives ont été utilisées afin d'étudier l'acceptabilité et la faisabilité de l'utilisation du lait maternel exprimé et chauffé (*Heat-Treated Expressed Breast Milk*, HTEBM) par des mères infectées par le VIH-1 en Afrique du Sud. L'évaluation de l'état nutritionnel de 84 mères allaitantes indiquait un état non compromis, avec un indice de masse corporel (IMC) moyen de 26,8 (4,0) kg/m^2, un pli cutané tricipital (PCT) de 14,8 (5,5) mm et un taux sanguin d'hémoglobine de 11,6 (1,49) g/dL. En revanche, les mères dont le système immunitaire était sévèrement compromis (CD4+ < 200 cellules/mm^3) étaient plus à risque d'être anémiques. En outre, la charge virale était plus élevée dans le lait maternel des mères présentant un faible compte de cellules CD4+. L'acceptabilité de la méthode HTEBM a été évaluée à l'aide d'entrevues en profondeur auprès des principaux acteurs (n=31), notamment auprès des mères, des membres de la famille (partenaires, grands-parents, belles-mères) et des membres de la communauté (cliniciens, travailleurs en santé communautaire, guérisseurs traditionnels, travailleurs en garderies). Même si le HTEBM représentait pour tous une nouvelle technique, dans l'ensemble, celle-ci fut considérée comme étant un choix d'alimentation acceptable par les répondants, indépendamment de l'âge, du sexe ou du rôle social dans la communauté. Dans le cadre d'une étude pilote, nous avons étudié la faisabilité de l'utilisation d'une approche modifiée à l'allaitement constituée de l'allaitement exclusif pendant une période de 6 mois par des mères infectées par le VIH-1, suivi de l'utilisation du lait maternel exprimé et chauffé (HTEBM) accompagnée d'une diète complémentaire. Une majorité des mères (36/66) ont pratiqué l'allaitement exclusif pour une durée de 6 mois. Parmi celles-ci, 42% ont utilisé le HTEBM. Durant cette période, les mères ont exprimé entre 65 ml et 600 ml de lait maternel par jour, pour une durée variant de 2 semaines à 5 mois. Aucune mère n'a souffert de pathologie au niveau des seins. Les visites à domicile ainsi que la divulgation du statut VIH au partenaire ont favorisé l'implantation de cette méthode. Cette étude est la première à démontrer la faisabilité de l'utilisation de la technique HTEBM. Elle représente une option faisable et acceptable de nutrition infantile pour les mères infectées par le VIH-1. Cette technique pourrait contribuer à réduire la transmission verticale de VIH tout en offrant une alimentation complémentaire favorisant une nutrition adéquate aux enfants.

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STATEMENT OF ORIGINALITY

Contribution to knowledge, policy and practice

This doctoral dissertation presents the first longitudinal study to investigate the feasibility of feeding infants born to HIV-1 infected mothers their expressed and heat-treated breastmilk.

Preliminary data from this feasibility study were included as part of the discussion at a WHO infant feeding and HIV guidelines meeting in October 2006, presented by our collaborator Dr Anna Coutsoudis.

This research is also the first community-based study to evaluate the acceptability of heat-treated breastmilk within a South African resource poor setting, highly affected by the country's HIV epidemic.

Data and experiences from this study were shared by the candidate with researchers in Tanzania who are in the process of conducting a large prospective study, examining the feasibility of using heat-treated breastmilk by HIV infected mothers, examining maternal and infant health outcomes.

Based partly on the study data and experiences from this collaborative research, different sites in Africa now offer heat-treated breastmilk as an option for mothers who are unable to afford replacement feedings and have no alternative safe options.

The candidate was solely responsible for writing the successful operational grant (Principal Investigator Dr Katherine Gray-Donald) to the Canadian Institute of Health Research (CIHR), which was the main source of funding for this study. The candidate was also successful in attaining funding from the International Development Research Centre (IDRC).

In summary, there is a desperate need for safe breastfeeding options for women living with HIV, in areas of the world where alternative choices are not possible or present significant risk for their infants. Findings of this study have already made an impact on the choices now available to HIV infected women living in areas where breastfeeding is universal and a pillar of infant survival.

CONTRIBUTION OF AUTHORS

This is a manuscript based Ph.D. dissertation, comprised of four manuscripts:

Chapter 5: Heat-Treated Expressed Breast Milk: An Acceptable Feeding Option For Use By Urban African Mothers Living With HIV.

Lindiwe Sibeko, Anna Coutsoudis, S'phindile Nzuza, Katherine Gray-Donald. *Under review by Social Science and Medicine.*

Chapter 6: Mothers' infant feeding experiences: constraints and supports for optimal feeding in a HIV impacted urban community in South Africa.

Lindiwe Sibeko, Anna Coutsoudis, S'phindile Nzuza, Katherine Gray-Donald. *Under review by Public Health Nutrition*

Chapter 7: Heat-Treated Expressed Breastmilk Is A Feasible Feeding Option For South African Mothers Living With HIV: a mixed methods approach.

Lindiwe Sibeko, Anna Coutsoudis, S'phindile Nzuza, Katherine Gray-Donald. *To be submitted to JAIDS*

Chapter 8: Nutritional status and immunological profile of lactating HIV-1 infected South African urban women.

Lindiwe Sibeko, Dima Ousta, Anna Coutsoudis, S'phindile Nzuza, Katherine Gray-Donald. *To be submitted to Maternal and Child Nutrition*

The Ph.D. candidate, Lindiwe Sibeko, was completely responsible for developing the concept of the studies comprising this thesis. The candidate also developed all study protocols, data collection forms, development and delivery of breastfeeding counseling training program for community research partner, recruitment of participants for all studies and overall implementation, monitoring and management of all studies. Data analysis, interpretation and preparation of the above four manuscripts were conducted by the candidate.

Dr Katherine Gray-Donald, the candidate's thesis supervisor, participated in the study development and design, consulted in the analysis and data interpretation and made

editorial revisions to the manuscripts. Dr. Anna Coutsoudis provided the study site, collaborated in the study conception and study design, and made editorial revisions to the manuscripts. S'phindile Nzuza, trained community research partner, conducted participant recruitments, participated in all aspects of study implementation including data collection and data interpretation. She reviewed and provided feedback on all manuscripts.

The candidate collaborated in two additional studies which were integral components of the study with a focus on examining the safety of heat-treated expressed breastmilk. Reprints of the published manuscripts (K. Israel-Ballard, A. Coutsoudis, CJ. Chantry, A.W. Sturm, **L. Sibeko,** B. Abrams,(2006) Bacterial safety of flash-heated and unheated expressed breastmilk during storage. *Journal of Tropical Pediatrics,* 52(6), 399; Kiersten Israel-Ballard, Richard Donovan, Caroline Chantry, Anna Coutsoudis, Haynes Sheppard,H., **Lindiwe Sibeko,** Barbra.Abrams.(2007). Flash-heat inactivation of HIV-1 in human milk: A potential method to reduce postnatal transmission in developing countries. *Journal of Acquired Immune Deficiency Syndromes,* 45(3), 318) detailing these studies are included in appendices I and II. The candidate's contribution to these papers include: recruitment of study participants, data collection, data interpretation, and manuscript edits and revisions.

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LIST OF ABBREVIATIONS

AFASS	Acceptable, feasible, affordable, sustainable and safe
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretrovirals
AZT	Zidovudine
BM	Breastmilk
BMI	Body mass index
CD4+	$CD4^+$ T- lymphocyte cell count
CI	Confidence interval
CTL	Cytotoxic T lymphocytes
ELISA	Enzyme-linked immunoarbsobent assay
EBF	Exclusive breastfeeding
EBM	Expressed breastmilk
EFF	Exclusive formula feeding
FH	Flash-heating
HAART	Highly active anti-retroviral therapy
Hb	Hemoglobin
HIV	Human immunodeficiency syndrome
HIV-1	Human immunodeficiency syndrome subtype 1
HR	Hazard ratio
HTEBM	Heat-treated expressed breastmilk
HTST	High temperature short time
Ig	Immunoglobulins
IMR	Infant mortality rate
KZN	KwaZulu-Natal
MF	Mixed feeding
MTCT	Mother-to-child transmission of HIV
NHANES	National Health and Nutrition Examination Surveys
NVP	Niverapin
OR	Odds ratio

PB	Predominant breastfeeding
PCR	Polymerase chain reaction
pMTCT	Prevention of mother-to-child transmission of HIV
RF	Replacement feeding
RT	Reverse transcriptase
TSF	Triceps skinfold
UNAIDS	joint United Nations Programme on HIV/AIDS
UNFPA	United Nations Population Fund
UNICEF	United Nations Children's Fund
WHO	World Health Organization

"You are fighting for the survival of women and infants... You're fundamentally fighting for the emancipation of women, and there is no fight in this world more worthwhile"

Stephen Lewis, UN special envoy for HIV/AIDS in Africa

CHAPTER 1

INTRODUCTION

Three decades into the global AIDS pandemic, 22.5 million sub-Saharan Africans are living with HIV/AIDS. Unlike any other region in the world, the HIV burden in Africa is mostly (61%) shouldered by women (UNAIDS, 2007). African women, as is the case for women in most developing areas of the world, experience the highest level of poverty, further fueling their vulnerability to HIV infections. Consequently, high infection rates of women in their reproductive years have resulted in elevated antenatal infection rates.

South Africa, a country experiencing the most severe HIV epidemic in the world, has an estimated 29.1% prevalence of HIV infection in its pregnant population (Department of Health, 2007b). These infections pose a substantial risk for mother-to-child transmission of HIV (MTCT), as a result, 90% of pediatric infections are due to MTCT, the majority of these infected children live on the African continent (UNAIDS/WHO, 2005). Hence, the prevention of mother-to child-transmission (pMTCT) has emerged as a high priority public health challenge in sub-Saharan Africa, particularly in the sub-region of Southern Africa, the epicenter of the epidemic.

Given that prolonged and sub-optimal breastfeeding, a common practice in Africa, can result in the infection of up to 45% of African children (De Cock et al., 2000), elucidating safe breastfeeding practices within African settings is urgently needed. In the developing world, exclusive breastfeeding (EBF) is a pillar of infant survival, protecting infant and young children from common childhood morbidities like diarrhea and respiratory infections. Estimates indicate EBF to be the most cost effective, highest impact infant

survival strategy, capable of saving the lives of 13-15% of children who die annually from preventable causes (Jones, Steketee, Black, Bhutta, & Morris, 2003). Additionally, based on a strong body of evidence illustrating the minimal infection risk associated with EBF (Coovadia et al., 2007; Coutsoudis, Pillay et al., 2001; Iliff et al., 2005; Leroy et al., 2004), WHO recommends mothers living with HIV breastfeed their infants exclusively for six months if these mothers do not have access to a replacement feeding (RF) that is acceptable, feasible, affordable, sustainable and safe (AFASS). Unless a RF complying to AFASS conditions becomes available, infected mothers are further advised to continue breastfeeding until their infant is being fed a nutritionally adequate diet without breastmilk (WHO, 2006). For this reason, availability of a safe and sustainable feeding option for use when the infant discontinues EBF at 6 months of age provides mothers with a choice to cease breastfeeding early, thereby further minimizing transmission risk.

Heat-treated expressed breastmilk (HTEBM) has the potential of being a powerful feeding option, when combined with the complementary diet, due to the energy and nutrients it would contribute to the growing infant's dietary intake. Program or operational experience using HTEBM, despite being a WHO feeding suggestion (WHO, UNICEF, UNAIDS, & UNFPA, 2004), is lacking. Before HTEBM can be promoted as an infant feeding choice, the safety, acceptability and feasibility of this feeding alternative needs to be fully assessed. This identified need for important data derived from the evaluation of the practical applicability of HTEBM use, within the context of a highly prevalent HIV resource poor community, formulated the main purpose of this study. The outcomes of such research has a direct practice implication by providing health care workers with practical information on an infant feeding strategy that can be offered with confidence, as a feeding option during the counseling of HIV infected mothers.

As roll out of antiretroviral therapy (ART) takes place in the developing world, women and children will benefit substantially from the availability of treatment, and predictably a large impact on pMTCT will take place, as has been the case in the developed world.

In the meantime, urgently needed are infant feeding strategies that minimize postnatal transmission of HIV and are relevant to people living under limited economic conditions, within communities where breastfeeding is universal and contributes substantially to child survival. Accordingly, the modified breastfeeding intervention investigated in this thesis was developed and implemented with the intention of providing a safe, affordable feeding alternative for infants born to HIV infected mothers, living in a settlement community in South Africa.

The 2002 United Nations general assembly Special Session for Children addressed the global pediatric HIV/AIDS burden, resulting in a declaration of strategic goals aimed at the reduction of the proportion of infants infected with HIV by 20% by 2005 and by 50% by 2010 (United Nations, 2000). These albeit ambitious, but important goals were endorsed by many governments, including Canada and South Africa. The call for action inspired by such declarations fosters formation of global partnerships that can respond collectively to challenges presented by the epidemic. This thesis is comprised of research that was undertaken as a result of collaborative work between Canada, the U.S.A. and South Africa.

CHAPTER 2

REVIEW OF THE LITERATURE

This literature review sets out to present a contextual background providing the underpinning for this doctoral thesis topic, which investigates an infant feeding strategy aimed at use by HIV infected women from a HIV prevalent, resource-poor setting in South Africa.

Beginning with an epidemiological overview of the HIV epidemic and its impact on sub-Saharan Africa as a whole, the review then focuses on South Africa, the study location. A brief historical background of the HIV/AIDS situation in South Africa is presented, providing an important framework highlighting some of the forces that have contributed to the accelerated growth of the epidemic that has gripped the country.

A review of mother-to-child transmission of HIV and the role breastfeeding plays in the vertical transmission of the virus is discussed. Additionally, the evidence substantiating international infant feeding guidelines, aimed at risk reduction of postnatal transmission of HIV is presented. In this context, exclusive breastfeeding is discussed and its significant association with low transmission risk and vital role in protecting child health. Accordingly, a review of the characteristics of Flash-heated expressed breastmilk, a component of the feeding intervention in this thesis, is also presented.

The literature review ends with a discussion of qualitative methods, focusing on the mixed methods approach, since both qualitative and quantitative methods are employed in conducting the studies of this thesis topic.

2.1 The Epidemiology of HIV infection in Africa: a sub-Saharan Africa burden

The HIV/AIDS epidemic, first appeared in Africa in the early 1980's, but is now sweeping through sub-Saharan Africa, having devastating ramifications on a region of the world that is home to some of the most marginalized and poorest people on the globe.

Although sub-Saharan Africa represents only 10% of the world's population, it nonetheless accounts for approximately two thirds (22.5 million) of the 33.2 million people, around the world living with HIV/AIDS (UNAIDS, 2007). The epidemic shows no signs of abating. In 2007, an estimated 1.7 million African adults and children became infected with HIV/AIDS, resulting in the highest rates of infections taking place in a year. Worldwide, the epidemic has resulted in overwhelming mortality rates as evidenced by 25 million people losing their lives to AIDS in the past quarter century, most in the prime of their lives. Yet again, the mortality burden is most heavily represented in sub-Saharan Africa, with an estimated 72% (2.1 million) of the 2.9 million adults and children who died from HIV/AIDS in 2006 being from this corner of the world (UNAIDS, 2006).

The HIV burden is not equally distributed within the sub-Saharan region with rates ranging from a high of 33.4% in Swaziland to less than 1% in Senegal (UNAIDS, 2006). These disparate rates between western and southern regions of Africa illustrate a common HIV infection pattern of the continent. The most current surveillance data identify the Southern African sub-region as the global epicenter of the HIV/AIDS burden. On a worldwide basis, estimates indicate that close to a third of those living with HIV, as well as 34% of AIDS related deaths are from Southern Africa (UNAIDS, 2006). The most recent estimated adult rates of HIV infections are highest (>15%) in the following Southern African countries: Botswana, Lesotho, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe (UNAIDS, 2007). Consequently, Southern Africa is losing a considerable proportion of its human resources to AIDS.

Despite these grim figures, the tide does seem to be turning with various pockets of Eastern (Kenya), Western (Burkina Faso) and even the hard hit Southern regions of Africa witnessing rates that are stabilizing or declining. A point in case being Zimbabwe, which seems to be experiencing a decline in its national HIV prevalence rate (UNAIDS, 2007).

2.1.1 HIV/AIDS in South Africa: prevalence and incidence estimates

In the past decade, South Africa experienced the fastest growing HIV epidemic in the world and currently is recognized as having one of the most severe HIV burdens on the planet. In general, estimates of HIV infection rates are usually provided as prevalence rates, which measure the cumulative infections over a specified time subtracting the cumulative HIV deaths over the same period. Using available data, UNAIDS/WHO estimated South Africa's HIV prevalence to be around 18.8% for 15 to 49 year olds (range of 16.8 to 20.7%). These estimates are based on data from several surveys and the country's two major studies: the Department of Health "National HIV and Syphilis Sero-prevalence Survey in South Africa 2006" (Department of Health, 2007b), and the Medical Research Council's "South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey, 2005" (MRC, 2006). Additionally, a demographic and AIDS model based on available data has been developed by the Actuarial Society of South Africa, to predict the course of the epidemic and impact on the demography of the country (Dorrington, Johnson, Bradshaw, & Daniel, 2006). Overall, based on all of these data, South Africa has an estimated 5.5 million people infected or 11% of the total population (UNAIDS, 2006). At the current rate, the country is predicted to have over 6 million people living with HIV/AIDS by 2015 (Dorrington et al., 2006).

Incidence rates provide an estimate of new infections within a specified time and serve as a good indicator of the rate of HIV transmission or progression of the epidemic. As such, an incidence rate is a better measurement of the impact of prevention methods than prevalence rates (McDougal et al., 2005). However, incidence estimates rarely exist because they require large cohort studies that are expensive to manage. In an attempt to generate incidence data, researchers used the 2005 South African national HIV household survey to create national incidence estimates stratified by age, sex, province, race and locality (Rehle et al., 2007). Their findings indicated a HIV incidence rate of 1.4% per year for South Africans two years of age and older, which resulted in an estimate of 571,000 new infections in 2005 for the identified population. The high incidence of infections in the 15 to 24 age group, particularly in the female population, accounts for

most new infections, while incidence of infection for all other age groups peaked between 1997 and 2000 (Dorrington et al., 2006). Figure 2.1 presents a summary of demographic facts and the HIV burden in both sub-Saharan Africa and in South Africa.

2.1.2 Contributing factors to South Africa's HIV burden: historical background and demographic variables

South Africa is considered a middle-income developing country, with a multiracial and multicultural population (Fig. 2.1). The country's diverse population has resulted in the recognition of 11 official languages. However, from an economic perspective, South Africa is characterized by extreme inequities where a visible contrast exists between a minority of affluent citizens co-existing side by side with their counterparts living in stark poverty, and in much larger numbers. These economic inequities are divided by racial lines. Although the black African population accounts for 79% of the South African population, 52 % live in poverty (95% of the nation's poor) as opposed to 17% of the Mixed race group, and less than 5% of the Indian and White population (Wolard, 2002).

Likewise, the HIV burden is unevenly distributed amongst South Africa's diverse racial groups, the highest levels being in the African population (prevalence of 13.3%) followed by the Mixed race, Indian and White populations, 1.9%, 1.6%, 0.6%, respectively (MRC, 2006). This unequal distribution of HIV infections suggests that socio-cultural, political and economic factors as well as the remnants of the country's apartheid history and its damaging consequences to the African people are significant drivers of the HIV epidemic in South Africa.

Since the arrival of democratic rule in 1994, South Africa has been in the process of undergoing transformation on many levels. As the country changed, the growing HIV burden seemed to be ignored. Furthermore, repositioning the country as a democratic state progressed slowly, resulting in development in some areas but also an emergence, or a worsening of pre-existing conditions for those living in poverty - all factors that fueled

and sustained the epidemic (AVERT, 2007). An additional underlying factor has been a history of poor government response to the country's increasing HIV burden, thereby creating an atmosphere of HIV denial, confusion and stigma that continues to plague South Africa. Collectively, these mitigating factors have had devastating implications for the country's future, resulting in considerable setbacks. Mortality from HIV/AIDS occurs at an alarming pace, with an estimated 320,000 South African lives lost to the epidemic in 2005, implying an average loss of more than 800 people daily (MRC, 2006). These deaths have contributed significantly to the dramatic decline in South Africa's population growth, which fell from a growth rate of 10.4% in 2001 to 6.4% in 2007 (Dorrington et al., 2006). Other declines associated with the epidemic have been in the area of life expectancy, which decreased by 13 years, from 64 in the absence of AIDS to 51 years (MRC, 2006). The epidemic has also affected development progress, contributing to a drop of 35 places on the Human Development Index (UNAIDS, 2006). Yet of all countries in sub-Saharan Africa, South Africa has both the infrastructure and resources to respond proactively to the national AIDS crisis, thereby preventing the considerable losses it has experienced because of the epidemic.

2.1.3 South Africa's strategy to address the AIDS epidemic

The lack of government leadership in responding to the South African HIV epidemic has lead to years of conflict between the government and advocacy groups over access to antiretroviral therapy (ART) for infected citizens, including pregnant women. Following various court actions against the government, ART is now available for South Africans living with HIV/AIDS, although estimates indicate only a third in need of ART have access to treatment (WHO, 2007b). Legal action against the government also resulted in access to ART prophylaxis for infected pregnant women, and made help available to prevent the transmission of HIV from a mother to her newborn. Initially, the economical and effective antiretroviral drug nevirapin (NVP) was the prescribed ART for mothers and their infants. More recently, the national policy has been revised to provide HIV-infected pregnant women with dual therapy, consisting of zidovudine (AZT) and NVP, a

modification that reflects a WHO recommendation for developing countries (WHO, 2007b).

Overall, under national and international pressure, South Africa has recently implemented dramatic changes to the country's AIDS policies. Through a restructured and strengthened South African National AIDS Council, a National Strategic Framework has been developed under the leadership of the Deputy President Phumzile Mlambo-Ngcuka, with the aim of universal access to prevention, treatment, care and support to its citizens living with HIV/AIDS (Department of Health, 2007a). Such a shift at the government level may herald a turning point for South Africa, leading to declining infection rates, similar to experiences of other developing countries with heavy HIV burdens.

2.2 In Africa HIV/AIDS is a women's epidemic: biological and social vulnerability of women to HIV infections

On the African continent, HIV infection occurs mostly through heterosexual transmission. Although AIDS is gender neutral, epidemiological data indicate that HIV is increasingly a women's epidemic. The trend of higher rates of women living with HIV, 15 years of age and older, have been reported in all regions of the globe (UNAIDS, 2006). By the end of 2007, an estimated 15.4 million women globally, were living with HIV. Predictably, 13.8 million (11.5-15.2 million) women in sub-Saharan Africa over the age of 15 are living with HIV. Africa is the only region in the world where the greatest proportion of those living with HIV/AIDS is women, with approximately 61% of those infected on the continent being women (UNAIDS, 2007).

This striking vulnerability to HIV infections is partly due to physiological factors that render women more susceptible than men to HIV infections, through heterosexual contact. During unprotected sex, women are twice as likely to be infected by the HIV virus as men (WHO, 2007a). Women's vaginal anatomy is such that there is a greater surface area of mucus membrane exposed during sexual contact than men, increasing the

risk of micro-tears during penetration. Additionally, during sex women receive a high quantity of fluid from men (sperm), which has a higher viral content than vaginal fluids (WHO, 2007a). Exposure to this infectious fluid continues for some time following sexual contact, increasing the risk of infection. Additionally, the sexually transmitted infections (STI) women often suffer from without their knowledge are also a significant risk factor, since presence of STI increases the risk of HIV infection fourfold (WHO, 2007a).

Biological susceptibility is one side of the dual vulnerability that women in developing countries face. Gender inequality, advanced by social, cultural and economic factors, frames the other side of the burden. Lack of economic and social power often means that women are powerless to negotiate condom use or monogamy. Economic insecurity may lead women to sell or trade sex in order to survive and look after the family. Young women are particularly vulnerable since a lack of social support networks and economic independence often means that these young girls enter relationships with older men (who may be infected) as a means of survival (AVERT, 2007). Sexual violence or rape has also been identified as a significant contributor to HIV infections of women in South Africa (CIAC, 2006).

There may be other female subgroups that are particularly vulnerable to infections. A recent South African based study found that older widowed women, median age of 43 years, are at risk of HIV infection, having a high incidence rate of 5.8 % per year. The researchers had no explanation for this surprising finding (Rehle et al., 2007). It is conceivable that as widows, some women may experience greater poverty due to loss of income from the death of a spouse and as a result may have to enter into relationships of trading sex for money, with very little power to negotiate safe sexual practices.

Furthermore, women identified as being HIV positive are in danger of experiencing more severe discrimination than infected men and may be at a increased risk for spousal or other forms of violent physical abuse (AVERT, 2007).

These are the gender inequalities that have contributed to the rapid increase of HIV infections of women throughout the world, with greater impact in the developing world. There is a critical need for HIV prevention programs to address the social, economic and cultural issues specific to women. The South African government has recognized that women in the country face a triple burden of race, class and gender. Efforts to address these issues are being made through education and skills development (AVERT, 2007).

The identified vulnerability of women to HIV infection is exemplified in the high numbers of South African women living with HIV. In 2005, South Africa had 3,100,000 women, between the ages of 15 to 49, living with HIV. Within the same year, the estimated incidence of HIV infection for women in this age group was 2.4% per year. Overall, HIV incidence was highest at 5.6% for females in the 20 to 29 age group, six times (0.9%), higher than for their male counterparts. Amongst youth, aged 15 to 24, 90% of recent HIV infections were in women (Rehle et al., 2007).

Researchers can provide an important contribution to the emancipation of women, by ensuring that interventions targeted at HIV infected women build in a support and empowerment component, as an integral component of any proposed initiative.

2.2.1 South Africa: antenatal HIV infections

Elevated HIV infection rates of women in their reproductive years translate to high antenatal infection rates. Pregnant women seem to be particularly vulnerable to HIV infection as evidenced by studies from South Africa (Rehle et al., 2007) and Uganda (Gray et al., 2005); both indicating increased risk of HIV infection during pregnancy. South African data provides an estimated HIV incidence of 3.8% for non-pregnant women 15 to 49 years of age, while for pregnant women in the same age category new infections were occurring at a rate of 5.2% (Rehle et al., 2007).

Antenatal prevalence estimates derived from the South African Department of Health Study indicate that nationally, 29.1% of pregnant women were living with HIV in 2006. These figures are based on data collected on 33,033 women attending 1,415 antenatal clinics located in all of South Africa's nine provinces. The province of KwaZulu-Natal at 39.1% had the highest antenatal infection rate while the Western Cape had the lowest at 15.2% (Department of Health, 2007b). In comparison, countries outside of Africa rarely experience rates of antenatal HIV infections that exceed 5% (US Bureau of the Census, 2001).

2.3. South Africa: pediatric HIV burden

Globally, in 2007 there were 2.5 million children under 15 years of age living with HIV/AIDS, the majority (2.2 million) being from sub-Saharan Africa (UNAIDS, 2007). The South African picture is equally bleak with approximately, 240,000 children under the age of 15 years living with HIV/AIDS by the end of 2005 (Dorrington et al., 2006).

Prognosis for HIV infected children in developing countries is poor, consequently up to a third of infected infants die by their first birthday, greater than 50 % by the second year of life and most before their fifth birthday (Newell et al., 2004). Moreover, progression of the disease is more rapid in children living in resource poor settings, since these children tend to be exposed to a myriad of risk factors including multiple non-HIV infections, increased rates of malnutrition and micronutrient deficiencies (UNICEF, 2003).

In many areas of the sub-Saharan region, the HIV/AIDS burden has become the underlying cause of increasing infant and child mortality rates, resulting in the reversal of child survival gains experienced by many developing regions of the world (Dabis, et al., 2004). Likewise, the AIDS epidemic in South Africa is associated with 57% of child deaths and a 13% increase in the under 5-child mortality rate since 1990. The current national infant mortality rate (IMR) is 48 infant deaths per thousand live births, while the under 5-mortality rate is 73 (Dorrington et al., 2006). It is noteworthy, that pockets of

South Africa's children are at risk of a double burden of disease. Hence, the pediatric HIV burden is taking place alongside underlying childhood diseases such as malnutrition, pneumonia and diarrhea, morbidities that remain significant contributors to the deaths of South African children (Bourne et al., 2007).

2.3.1. Mother-to-child transmission of HIV

Mother-to-child transmission (MTCT) of HIV, describes the vertical transmission of the virus from an infected mother to her infant. The term MTCT implies that blame rests solely on mothers for transmitting the virus to their children. However, reality dictates that the mother is not the only conduit of the virus so that the term should be modified to parent-to-child transmission. However, in keeping with current convention and terminology and for issues of consistency, this thesis will refer to vertical transmission as MTCT.

Three possible routes of HIV infection via MTCT exist: initially through conception; during pregnancy, labor and delivery; and in the postpartum period, through breastfeeding. A significant number of the world's children become infected through MTCT, accounting for approximately 90% of the approximately 700,000 HIV infections that occur annually among children, throughout the world. Epidemiologically, the pediatric HIV burden is suffered mostly (90%) by African children (UNAIDS/WHO, 2005). South African estimates for 2006 indicate that, in a single year, 36,000 infants were HIV infected at birth (Dorrington et al., 2006).

Transmission risk estimates based on a meta-analysis of several studies indicates that in the absence of ART prophylaxis and effective obstetrical interventions, MTCT of HIV through pregnancy, labor and delivery is responsible for an infection rate of approximately 15 to 30% for infants born to HIV infected mothers (Table 2.1) (De Cock et al., 2000). Postnatal breastfeeding up to six months contributes to the cumulative transmission risk of 25 to 35 % and for extended breastfeeding 18 to 24 months. The

cumulative risk of infection can be as high as 45% (De Cock et al., 2000; Kourtis et al., 2006).

However, when pregnant women receive obstetrical interventions, use replacement feeding and highly active antiretroviral therapy (HAART), as is the case in the developed world, transmission rates can be as low as less than 2% (Cooper et al., 2002). In the developing world, use of ART is estimated to decrease transmission rate of HIV to newborns to 5-14% from 10-20% (Leroy et al., 2004).

2.3.2 Transmission risk estimates attributable to breastfeeding

Breastfeeding has been found to pose a significant risk for HIV transmission. An estimate of transmission risk through breastfeeding was first reported by Dunn and colleagues (1992) to be 14% (95% CI 2-22%), based on a meta-analysis of studies on breastfed and formula fed infants, using ELISA at 18-24 months for determining infection (Dunn et al., 1992). This estimate was later identified as a cumulative risk over 24 months.

A later estimate found that during the postnatal period, 5 to 20 % of infants are infected because of breastfeeding (De Cock et al., 2000). The study also indicated that risk of transmission increased with duration of breastfeeding, such that extended breastfeeding, a common practice in developing countries, increased the risk of infection (Table 2.1).

These early estimates of transmission risk attributable to breastfeeding, although providing essential data, have significant limitations.

In addressing the limitations of the studies, the transmission risk estimates were based on diagnostic tests used to determine child infection, and stand out as a primary consideration. Initially, studies estimating transmission risk used enzyme-linked immuno-absorbent assay (ELISA) to diagnose HIV infections of children. The ELISA test measures HIV antibodies but does not confirm the presence of the virus. Therefore, an

infant born to an infected mother may have circulating antibodies without being infected. Since maternal antibodies disappear by 15 months, ELISA tests are performed after this period, usually when the child is 18 months of age (BHITS, 2004). Although diagnostic use of ELISA under these conditions is appropriate, the test is unable to estimate the transmission point and therefore cannot distinguish transmission during breastfeeding from that of pregnancy or labour and delivery. Dependency on the ELISA for diagnosis in the early studies also meant transmission estimates were based on comparisons between breastfed and formula fed infants, which would provide inaccuracies since the groups might not be comparable (Coutsoudis, 2003).

An additional limitation of a majority of studies investigating infant feeding and HIV transmission has been a lack of clearly defined breastfeeding patterns of study participants. Therefore, earlier estimates of HIV transmission rates (10-20%) were unclear whether participants were practicing exclusive breastfeeding (EBF), predominant breastfeeding (PB) or mixed feeding (MF).

Standard WHO definitions, outlined on Table 2.2, provide specific definitions of breastfeeding patterns. Rigorous studies specify the operational definitions of breastfeeding patterns and provide clarity of results and guidance for policy development.

Infant diagnosis is now possible at an earlier stage of life using the polymerase chain reaction (PCR) test. This test amplifies genetic material from the virus and detects the presence of HIV infection. As a result, a PCR test that is initially negative at 6 weeks of an infant's age but can become positive at a later age (>6 weeks), is the current international standard for determining postnatal transmission attributable to breastfeeding (BHITS, 2004).

2.3.3 Low transmission risk associated with exclusive breastfeeding

In the area of postnatal HIV transmission, two key studies, one from South Africa (Coutsoudis et al., 1999) and the other, Kenyan (Nduati et al., 2000), were critical in elucidating the association between infant feeding and transmission risk. Data from these two studies were presented in high impact journals, received a wide audience and fueled an often-passionate debate amongst researchers, clinicians and frontline workers.

The Coutsoudis and colleagues study (1999) based in Durban, South Africa, is widely recognized as the landmark study that provided important data suggesting low transmission risk was associated with EBF of infants born to HIV-infected mothers. Although the primary outcome of this randomized trial was to determine the association between vitamin A supplementation and MTCT risk, the secondary objective was to asses the relationship between feeding patterns of HIV infected mothers (n =551) and MTCT of their infants. Women chose to either breastfeed or formula feed, following counseling.

The study was well designed in that transmission risk factors did not differ in the three feeding groups, EBF (n=18), formula fed (n=157) and mixed breastfed (n=276). Results indicated that infection estimates at 3 months of age found 21.3% (95% CI 17.2-25.5) of breastfed infants (n=393) to be infected in comparison to 18.8% (12.6-24.9) of never-breastfed infants (n=156), (p=0.5). Mixed feeding emerged as a transmission risk, since there was a significantly lower proportion of HIV infection of infants who were EBF for 3 months (14.6% [7.7-21.4]), compared to those who were mixed fed (24.1% [19.0-29.2]), for the first 3 months, p=0.03. The researchers were careful to adjust for potential confounders like maternal CD4-cell/CD8-cell ratio, syphilis screening test results, and preterm delivery (Coutsoudis et al., 1999).

Through Kaplan-Meier analysis, the cumulative probabilities of HIV infection up to 6 months were estimated to be similar between infants who were formula fed (0.194 [95% CI 0.136-0.260] and those who were EBF (0.194 [95% CI 0.125-0.274]), but higher

among mixed fed infants (0.261 [95% CI 0.205-0.319]). Essentially, from these data it seemed that EBF decreased infant risk of postnatal HIV infection, and that infants who were EBF for a minimum of 3 months had no excess risk of HIV infection at six months of age compared to infants who were formula fed. Moreover, the EBF infants had significantly lower rates of HIV transmission at 6 and at 15 months compared to infants who were partially breastfed plus received other foods and liquids (mixed fed) in early infancy (Coutsoudis et al., 2001).

The findings of this study were critical in the area of public health policy since they provided the first indication that safe breastfeeding, in the form of EBF, may exist as a potential feeding option for infants born to HIV infected mothers in breastfeeding communities. When these results emerged, the practice and philosophy around infant feeding in the context of HIV was very much focused on avoiding HIV infections at all cost. As a result the avoidance of breastfeeding was strongly advocated, a policy that disregarded issues of child survival. In settings where breastfeeding was universal, like Africa, avoidance of breastfeeding was challenging to implement operationally, since health care workers had spent decades promoting breastfeeding as an effective infant survival strategy.

The Kenyan randomized clinical trial contradicted most of the Coutsoudis findings. In the Nduati and colleagues (2000) trial, 425 pregnant women were randomized into breastfeeding (n=212) and formula feeding (n=313) groups. Mothers were followed for 24 months postpartum, at which point the cumulative probability of HIV-1 infection was found to be 36.7% for breastfed infants and 20.5% for their formula fed counterparts (p=.001). The rate of breastmilk transmission was estimated to be 16.2%, with 44% of HIV-1 infections in the breastfeeding group attributable to breastmilk (Nduati et al., 2000). Clearly, these findings indicated that there was a significantly higher transmission rate associated with breastfeeding. However, at the two year mark, the mortality rates were found to be similar in the feeding groups, with breastfeeding estimated at 24.4% compared to 20.0% for formula, (p=.30), this relationship was maintained following adjustment for HIV status. Additionally, the rate of HIV-free survival at two years was

significantly lower for breastfed infants at 58.0% than it was for formula fed infants (70%), p=.02. There were no significant differences found in morbidity outcomes (including diarrhea and pneumonia) between the two feeding groups although breastfeeding infants had significantly better nutritional status during the first 6 months of life (Nduati et al., 2000).

An additional controversial finding of the Kenyan trial was the higher number of maternal deaths in the breastfeeding group (18 mothers) compared to the formula feeders (6), p=.009 (Nduati et al., 2001). In contrast, the Coutsoudis study found that maternal mortality was not significantly different in their study, with two deaths from the breastfeeding group and three in the formula group reported (Coutsoudis et al., 2001). Increased risk of maternal mortality associated with breastfeeding for HIV-infected mothers has generated some concern. However, similar to the Durban study, other studies have not confirmed negative health outcomes associated with breastfeeding for infected mothers (Papathakis et al., 2004).

The substantially different outcomes, and the differing messages arising from the above two key studies, added confusion to the already complex issue of infant feeding and HIV transmission risk. The Coutsoudis study indicated that EBF had the potential of being a safe breastfeeding option for HIV infected women in resource poor settings, but the Nduati research group findings showed the reverse, identifying breastfeeding as highly risky, with formula being more protective.

Limitations exist for both studies; however, the Kenyan study had several confounders that would affect study outcomes. The South African study was limited in that the data emerged from a trial not specifically designed to examine infant feeding practices as they relate to transmission risk. As well, the study was deemed too small to provide strong evidence.

The initial concern with the Kenyan study was that exclusivity in both the breastfeeding and formula group was an issue, with an indication that the breastfeeding group had more

mixed feeders assigned to the group than to its formula counterpart. Furthermore, the formula feeding mothers seemed to be non-representative of the population, since the women were well resourced (i.e. access to a safe water supply). Participants using formula received significantly more support from the research team to insure appropriate preparation and feeding methods, while support seemed absent for the breastfeeding group. Finally, maternal characteristics were not similar amongst feeding groups since the immune status of the mothers in the breastfeeding group were more compromised than in the formula group, a factor that should have been dealt with as a result of randomization.

Since findings of these two studies were in conflict with one another, there was a call for larger scale studies designed specifically to elucidate the infant feeding determinates of HIV transmission from a mother to her child.

In response to the identified need, a large Zimbabwe based Vitamin A and HIV transmission study (ZVITAMBO trial), included in their research a study examining breastfeeding associated HIV transmission as it relates to infant feeding practices (Iliff et al., 2005). The ZVITAMBO study essentially confirmed the findings of the Coutsoudis research, illustrating the low risk of HIV transmission for HIV exposed infants that were exclusively breastfed to three months of age, compared to the mixed fed babies. The data were on 2,060 infants, all initiated on breastfeeding, and none of the mothers fed formula due to lack of availability in the study area. Postnatal transmission was determined when infants tested positive following a negative test at six weeks, using the PCR test. The postnatal transmission was 12.1 % at 18 months, with early mixed feeding (before three months) being associated with a four-fold (4.03, 95% CI 0.98-16.61) risk of transmission at 6 months compared to EBF. At 12 months, mixed feeding was associated with a three-fold risk (3.79, 95% CI 1.40-10.29) and a doubling of the risk (2.60, 95% CI 1.21-5.55) at 18 months (Iliff et al., 2005). Over the 18 month period, transmission risk with predominant breastfeeding ranged between 1.6-fold to 2.7 fold risk compared to EBF, a relationship that was only significant at 12 months (Iliff et al., 2005). These findings confirmed that the high HIV transmission risk associated with mixed feeding is

associated with the highest risk for infection. As well, the data showed that strict adherence to EBF is important given that even small amounts of non-milk liquids increased transmission risk.

Data from this large trial also indicated that a mother's immune status is a significant risk factor for MTCT. Immuno-suppressed mothers with CD4 levels less than 200 cells/ml were five times more likely to infect their infants during breastfeeding compared to women with higher CD4 levels (> 500 cells/ml) (Iliff et al., 2005). Other studies have also reported a strong association between immune status and postnatal transmission of HIV (Kourtis et al., 2006; Newell, 2006; Rollins et al., 2004). Table 2.2 outlines some of the maternal and infant risk factors associated with MTCT of HIV.

The Breastfeeding and HIV International Transmission Study Group (BHITS) carried out a meta-analysis of individual patient data from trials in breastfeeding communities. They estimated the rate of transmission to be 8.9 transmissions per 100 child-years of breastfeeding or 0.74% per month of breastfeeding. Thus 6 months of breastfeeding was estimated to be associated with a 4-5% risk of infection. This risk of transmission was related to mixed feeding, with infection risk being constant over the breastfeeding period (BHITS, 2004).

The most recent and most robust evidence to date on infant feeding as it relates to transmission risk is from a large, prospective cohort study from KwaZulu-Natal, South Africa (Coovadia et al., 2007). This is an observational study that encompasses rural and urban populations in KwaZulu-Natal, a highly HIV prevalent province of South Africa.. The study was designed specifically to investigate HIV transmission risk in relation to infant feeding practices as a primary outcome. In the study, HIV infected (n=1372) and non-infected (n=1345) women were recruited. Regardless of feeding practices, mothers received infant feeding counseling and regular scheduled visits for a six-month period, with detailed infant feeding intake documented by the mothers. Data were available for 1,276 infants (Coovadia et al., 2007).

Results of the study estimate a transmission rate of 4.04% (CI 2.29-5.76%), following 6 months of EBF. The high risk of adding solids to infant intake were clearly illustrated, with an 11 fold risk of HIV transmission in the first 6 months of life for infants mixed fed breastmilk plus solids compared to EBF infants (HR 10.87, CI 1.51-78.0, p=0.018). In comparison, infants mixed fed formula plus breastmilk (at 14 weeks), were twice as likely to be infected compared to EBF babies (HR 1.82, CI 1.7-3.06, p=0.057). There was no significant greater risk of infection by 6 months for infants fed breastmilk plus other liquids (HR 1.56, p = 0.308). Mortality risk by 6 months was significantly less for EBF (6.1%) than for replacement fed infants (15.1%), (HR 2.06 CI 1.00-4.27, p=0.051), (Coovadia, 2007). Through multivariate analysis, it was found that risk of infection for EBF infants was associated with maternal immune status. Hence, infants born to mothers with CD4 counts between 200-500 ml/mm^3 were twice as likely to be infected or die as infants of mothers with CD4 levels above 500. Infants of mothers with CD4 cell count below 200 had close to a four-fold risk of infection or death (Coovadia et al., 2007).

This important study also provided evidence for targeting and initiating ART for mothers with low CD4 counts as soon as possible, as an effective means of decreasing the risk of MTCT. The findings also confirmed the continued need to promote, protect and support EBF for women in resource poor settings, regardless of HIV status. Those women who are HIV negative or unaware of their status, still benefit from practicing EBF by improving the survival of their children. Infected women who EBF their infants benefit from a low transmission risk while protecting their children's health.

2.3.4 Why EBF results in low transmission rates: potential mechanisms

Mechanisms of how EBF results in lower transmission rates have not yet been elucidated. However, the site of transmission of HIV-1 is hypothesized to be at the lympho-epithelial tissues of the tonsils; gastrointestinal epithelium; M cells which are a component of the Peyer's patches in the gut mucosa and other lymphoid gut associated tissue (Hanson, 2004). It seems the mucosal surface of the infant's gastrointestinal tract might be the

most vulnerable port of entry for HIV, where the virus may enter the sub-mucosa through lesions or breaches in the gut (John-Stewart et al., 2004). Unrestricted access to the bioactive components of breastmilk (Table 2.3), takes place because of breastfeeding exclusively which results in the maintenance of the integrity of the mucosal barrier and limits the inflammatory response at the gut mucosa (Smith & Kuhn, 2000). Overall, human milk with its antimicrobial, anti-inflammatory and immuno-modulating function may play an active role in protecting the exclusively breastfed infant from transmission of the virus (Lonnerdal, 2006).

Given these hypotheses, authors of the Durban study (Coutsoudis et al., 2001) hypothesize that EBF infants are exposed to fewer bacterial contaminants from foods and other fluids that may damage the gut lining and cause small lesions in the immature gut. These lesions can provide access for the virus and facilitate infection. It is also possible that women who practice EBF will follow practices that minimize breast inflammation, mastitis, and cracked, bleeding nipples, all known risk factors for breastfeeding-related HIV transmission (Piwoz et al., 2004). Finally, breastmilk promotes the establishment of beneficial intestinal micro flora that may contribute to an infant's immune response against HIV (Smith & Kuhn, 2000).

2.4 Nutritional and immunological constituents of breastmilk

Breastmilk contains all of the nutrients needed by the newborn during the first six months of life (WHO, 2006). These include the metabolic fuels provided by macronutrients (fat, protein, carbohydrate), and the basic components required for tissue growth and development, such as fatty acids, amino acids, minerals, vitamins, and trace elements. These nutrients in human milk are highly bioavailable due to nutrient-nutrient interactions, thus enabling enhanced nutrient utilization (Lonnerdal, 1985). Additionally, several nutrients play a significant role on host immune defenses. For example, vitamin A influences the integrity of the mucosal surface so a deficiency may be related to HIV progression (Semba et al., 1994).

Given that infants consume a considerable amount of breast milk (approximately 750 mL/d), it is surprising that HIV postnatal transmission rates are not higher in infants breastfed by HIV infected mothers. This phenomenon is probably due to a well-integrated innate and adaptive immune system at work. Human milk is replete with bioactive components (Table 2.3) which contribute to the immune and non-immune protection of breastfed infants. For instance, proteins in human milk have bioactive functions, in addition to providing amino acids for protein synthesis by the infant (Hanson et al., 1978). Whey proteins (i.e., lactoalbumin, lactoferrin, lysozyme, lactoperoxidase) which are predominant in breast milk work synergistically to provide immune and non-immune protection to the developing infant (Lonnerdal, 1985). Casein in breast milk has been shown to prevent the attachment of *Helicobacter pylori* to human gastric mucosa, thus protecting infants from gut-associated pathogens (Hamosh, 1997). Other proteins such as epidermal growth factor and insulin-like growth factor function on the infant's immature gut, essentially helping close up the leaky mucosal lining (Hanson, 2004). All five basic immunoglobulins (IgA, IgG, IgM, IgD, IgE) have been identified in human milk, with HIV–1 specific IgG and IgA being the most abundant and believed to play a role in protecting the newborn from transmission of the virus (Becquart et al., 2000).

Breast milk also contains leukocytes, most (90%) being neutrophils and macrophages and the remainder (10%), lymphocytes. Majority of lymphocytes in breast milk are T cells, with the proportions of CD4 (helper) to CD8 cytotoxic T lymphocytes (CTL) being similar to those in blood. In plasma, HIV-specific CTL plays a crucial role in decreasing HIV levels. A collaborative study between African and U.S. researchers identified HIV-specific CTL in the breast milk of infected women in Zambia and in the U.S.A. Based on their findings, the authors suggest that breastfed infants of infected mothers are protected directly by HIV-specific CTL found in breast milk. They further propose that CTL may act locally on the mammary gland to decrease viral load in breast milk, thus decreasing the risk of HIV transmission to the infant (Sabbaj, et al., 2002).

2.4.1 Optimal feeding for high-risk settings, the role of EBF

There is irrefutable evidence that breastfeeding in all populations (not just the infected) reduces infant morbidity and mortality associated with common childhood infectious diseases, in both industrialized and developing countries (Kramer & Kakuma, 2002; WHO, 2000a). The gains are much greater within resource poor settings. Consequently, in most developing countries, compared to infants who are breastfed, formula-fed infants have a substantially higher risk of mortality due to non-HIV infectious diseases, such as diarrhea, malnutrition and respiratory infections (WHO, 2000a). A landmark Brazilian study demonstrated that formula feeding was associated with a 14-fold increase in diarrhea-associated mortality for all infants and a 25-fold increased risk in infants less than two months of age (Victora et al., 1987).

In Ghana and Peru, breastfeeding avoidance was associated with a 10-fold risk of the infants dying when compared to predominantly breastfed infants (Bahl et al., 2005).
Over time, numerous other studies have provided robust evidence for the countless child and maternal benefits of breastfeeding, particularly EBF (WHO, 2000a).

A feature Lancet series presented evidence on the most effective strategies to prevent death of under-5 year old children from common childhood illnesses in developing countries, and which account for the highest mortality rates in this age group (Jones et al., 2003). Analysis of data gathered from 42 developing countries that experienced 90% of these childhood illnesses was conducted by a panel of global experts. The evidence from this study identified that universal breastfeeding in resource-limited settings as the number one, most cost-effective child survival intervention, since it could prevent 13 to 15% of deaths in children under five years old. Overall, as an intervention, breastfeeding was found to be more effective than other strategies, such as vaccination or replacement feeding plus an ARV (Jones et al., 2003).

Moreover, in contrast to the estimated 242,000 infant deaths that occur annually, associated to MTCT of HIV through breastfeeding and the majority being non-

exclusively breastfed (UNICEF, 2000), WHO/UNICEF estimates that in developing countries about 1.45 million lives of children under two years of age are lost each year as a result of not breastfeeding (WHO, 2005).

The protective characteristics of breastfeeding are largely attributed to the high nutrient quality and immunological constituents of breast milk (Hanson et al., 1978). Hence, in poor settings, avoidance of breastfeeding by HIV infected mothers who lack access to safe replacement feeding methods, would greatly compromise infant health and survival.

Infant feeding guidelines outlined by WHO/UNICEF/UNAIDS (2006) recommend that HIV-infected mothers practice EBF in situations where women cannot ensure that replacement feeding is acceptable, feasible, affordable, sustainable, and safe (AFASS). While in industrialized countries HIV infected women are advised to avoid breastfeeding and use formula to feed their newborns instead.

Although formula is the recommended infant feeding option for HIV positive mothers in developed countries, this is often not a feasible or preferred choice for women in resource-poor communities due to the prohibitive cost of formula, the lack of infrastructure to ensure formula availability during unstable conditions, and the socio-cultural stigma associated with formula feeding (Coutsoudis et al., 2002). Additionally, most women in developing countries (particularly in Africa and Asia) initiate breastfeeding of their infants, with many continuing to the second year of life (WHO, 2000a).

Some countries (Botswana, South Africa) have responded to the HIV epidemic by providing free formula, a policy that can have dire consequences within an African setting, even in relatively well-resourced countries. Such an event occurred in Botswana between January to March 2006, where there was a diarrheal outbreak with 23,998 cases reported. The outbreak was a result of heavy floods that had the effect of overwhelming the country's sanitation system. The consequences were 486 infant deaths, with children under 12 months being the most vulnerable. Analysis of the situation indicated that not

breastfeeding was a primary risk factor for diarrhea (Odds Ratio=50) and mortality, OR=8.5 (Creek et al., 2006). Follow up studies found that of the 154 patients with diarrhea, 23% of the infants who died were not breastfeeding, while none of the breastfed infants died. Of the infants who died, 51% received the free formula program. The primary predictor of death was severe malnutrition related to insufficient formula, not HIV infection (Creek et al., 2007).

2.5 Infant feeding guidelines for infected women in resource poor settings

Infant feeding guidelines outlined by WHO (WHO/UNICEF/UNAIDS/UNFPA., 2004) recommended that:

> When replacement-feeding options are acceptable, feasible, affordable, safe, and sustainable **(AFASS),** avoidance of all breastfeeding is recommended; otherwise exclusive breastfeeding is recommended for the first few months of life, followed by weaning, only if a nutritionally adequate and safe diet is maintained.

In October 2006, WHO amended their recommendations with a substantial change, advocating the continuation of breastfeeding until replacement feedings are AFASS (Table 2.3). The new guidelines reflect a modified philosophical approach to infant feeding in the context of HIV. Previous guidelines focused on the prevention of HIV-infection of the infant, while the new guidelines promote the prevention of postnatal transmissions while improving HIV-free child survival (WHO, 2006).

While South Africa's infant feeding recommendations reflect those of the United Nations by advocating EBF for the first six months of an infant's life (Department of Health, 2000), the actual practice of EBF within the country appears uncommon. In fact, South Africa's estimated rate of EBF (10.4%) for infants under 4 months of age is amongst the

lowest in sub-Saharan Africa (UNICEF, 2001). We previously reported that early mixed feeding was a common practice of mothers from a South African urban settlement community, with close to a third of mothers feeding their infants solids by the first month of life (Sibeko et al., 2005).

Concern over transmission of HIV during breastfeeding presents HIV positive mothers and health care workers in developing countries with a challenging predicament of having to balance the risk of passing on the virus through breastfeeding versus the risk of infant mortality.

2.5.1 Risk, benefit balance and the infant feeding dilemma of infected mothers

While the evidence accumulated on the benefits of EBF for women living with HIV, the risk and benefits of breastfeeding continue to be debated, with governments in developing countries seeking guidance. Years have been spent in some countries on promoting, protecting and supporting breastfeeding for child survival. In the era of HIV, infant feeding became a more complex issue requiring development of new government policies, national guidelines and resource allocations.

One attempt at providing some direction for policy development within resource poor settings came from Ross and colleagues (2005). This group employed mathematical models using WHO infant feeding recommendations combined with infant mortality rates (IMR) that describe different condition and use recent data on postnatal transmission risk for EBF and mixed feeding. Through their assessment they found that for settings where IMR > 25/1000 live births, EBF for 6 months followed by early breastfeeding cessation is recommended, while in situations where IMR < 25/1000 the evidence implies replacement feeding from birth may provide the best outcome (Ross et al., 2004).

2.5.2 Successful promotion and support of EBF

Support of infant feeding practices by mothers plays a crucial role in empowering women to EBF. Various programs have used trained peer counselors to support women to EBF with great success (Haider et al., 2000; Haider et al., 2002; Nankunda et al., 2006).

In South Africa, it was found that antenatal home visits, by trained counselors, were significantly associated with 75% of HIV-infected mothers being able to adhere to their prenatal intention to EBF their infants (Bland, et al., 2007).

The same researchers also investigated whether outside of support, access to clean water, adequate fuel, refrigeration and regular maternal income combine to influence infant feeding choices. Of 1,253 HIV-infected women, only 3% of women had access to all resources whereas 23% of these women chose replacement feeding. Overall, significantly more HIV-infected women chose to EBF (73%) than replacement feeding (9%), which was appropriate given that most women did not have access to sufficient resources to support replacement feeding (Bland et al., 2007).

2.5.3 Early cessation of breastfeeding

Since prolonged breastfeeding carries a cumulative risk of infection (Leroy et al., 2004), early breastfeeding cessation has been advocated, however there is concern over the morbidity and mortality risk associated with abrupt, early weaning. Growth faltering and a high risk of infant morbidities such as diarrhea and respiratory infections have resulted from early cessation of breastfeeding (WHO, 2000a). For mothers, breast pathology and other lactation issues have been identified as being related to stopping breastfeeding rapidly (De Paoli et al., 2006).

Hence, the recommendation of early breastfeeding cessation as a strategy for decreasing the cumulative risk of HIV infection from breastfeeding has to be implemented with

much care and within the context of supporting appropriate complementary feeding practices (Piwoz, et al., 2001). Breastmilk is a significant source of nutrients and immunological protection for the infant, once discontinued the introduction of contaminants via the infants intake of complementary foods and liquids coupled with inadequate intake may work synergistically in increasing risk of developing common childhood illnesses such as diarrhea (Coutsoudis, 2006). As well, breastmilk contributes substantially to the energy intake of an infant, hence removing this source of energy and protein compromises the infant's ability to meet energy needs, especially in resource-limited settings where an appropriate nutrient dense replacement feeding is unaffordable (Dewey & Brown, 2003).

In Côte d'Ivoire, through a prospective cohort study, investigators were interested in assessing the complementary feeding and child nutritional status of children born to HIV infected mothers. As a strategy to reduce transmission of HIV (Becquet et al., 2006), mothers were counseled to EBF and then weaned around four months. Findings showed that breastfeeding was stopped completely for 77% of infants (n=262) by the first year with a median duration of four months. Introduction to complementary foods took place at about 7 months of age for most infants with formula and infant foods introduced earlier to some at about four months. Dietary diversity was low at 6 months but improved by 12 months. Complementary feeding was inadequate at 6 months and associated with growth failure for the next 12 months, with a 37% increased risk for stunting (Becquet et al., 2006).

In the Zambia Exclusive Breastfeeding Study (ZEBS), there was no significant difference found in HIV-free survival at 24 months between infants for whom breastfeeding ceased at four months versus those who continued to breastfeed (Sinkala et al., 2007). In the Kisumu Breastfeeding Study in Kenya, infants experienced increased diarrhea and hospitalization following early cessation of EBF (Thomas et al., 2007). Similarly, a Uganda study found an increased risk of gastroenteritis and infant deaths with early breastfeeding cessation (Onyango et al., 2007).

These data illustrate the potential risk associated with early breastfeeding cessation. It should be noted that studies have not attempted gradual breastfeeding cessation, coupled with increased support during the transition period. As well, none of these studies used sustainable alternate feeding as part of the complementary feeding transition phase.

In many developing countries, finding effective ways to make breastfeeding safer for HIV positive women who do not have access to safe, nutritious, and affordable breast milk substitutes and replacement foods has become a priority issue. Several feeding options have been suggested for infected mothers to use once breastfeeding ceases. One feeding option identified by WHO (WHO/UNAIDS/UNICEF, 1998), is the use of manually expressed heat-treated breast milk by HIV infected mothers, when replacement feeding is unavailable.

2.6 Use of heat-treatment to inactivate HIV-1 in breastmilk

Retroviruses are heat sensitive, thus both cellular and acellular HIV-1 can be inactivated through pasteurization at 56°C to 62.5°C for 30 minutes using the Holder pasteurization method, a process that is widely used in human milk banking (Orloff et al., 1993). This pasteurization method is unlikely to work in many community settings since it is time consuming and requires investment of equipment such as a clock, thermostat or thermometer.

In an attempt to develop an in-home heat-treatment method, two user-friendly pasteurization methods have emerged from research work in this field: the Pretoria pasteurization (PP), developed by a South African researcher, Dr B Jeffery; and Flash-heating (FH), introduced by an American researcher, Dr Caroline Chantry. Table 2.4 outlines the procedures for both pasteurization methods. Briefly, PP requires the boiling of water in a pot, and then immersing the glass jar of EBM with lid on in the boiled water for 20 minutes then cooled before infant feeding. The procedure for FH, differs in that it requires the jar of EBM (without the lid and in a jar so that the EBM measures at least

two finger levels below the water level) to be placed in a pot of water which is then heated to a rolling boil. At this point, the virus is deactivated. The jar is then removed immediately, closed with lid, cooled, and cup-fed to the infant.

The PP method involves use of passive heat transfer from a container of boiled water (Jeffery & Mercer, 2000). Initial studies to confirm inactivation of HIV by PP and detected by use of co-culture methods, demonstrated that there was no evidence of HIV replication when infected breastmilk samples were PP heat-treated. However, data indicated that the researchers were unable to detect HIV consistently in unheated controls (Jeffery et al., 2001). The PP method requires no use of temperature gauges since boiling water is the visual cue needed to assess readiness, but it does require use of a clock (for timing the length of time jar of EBM left in boiled water).

Flash-heating (FH) is a heating method developed to duplicate commercial flash-pasteurization that functions on the principal of using a high temperature, short-time (HTST) pasteurization method. Flash-heating was first introduced by Chantry in Puerto Rico, where she and her colleagues illustrated that FH effectively destroyed HIV-1 (Chantry et al., 2000) This method depends on visual evidence that the water is boiling and requires no measurement of time or temperature. The simplicity and lack of fragile equipment, renders FH as user friendly and adaptable for in-home heat-treatment of expressed breastmilk (HTEBM) by HIV-infected mothers.

2.6.1 Comparison of Pretoria pasteurization and Flash-heating: methods to inactivate HIV in human milk

Using HIV spiked breastmilk samples from healthy women, researchers tested the ability of PP and FH to inactivate cell-free HIV, and their impact on selected proteins and vitamins (B-12, vitamin A, vitamin C, riboflavin, folate and antimicrobial proteins lactoferrin and lysozyme). In addition, the bacterial safety of both pasteurization methods

was investigated by assessing the ability of FH and PP to eliminate *Escherichia coli* and *Staphylococcus aureus* from breastmilk samples (Israel-Ballard et al., 2005).

Findings from this study showed that FH eliminated cell-free HIV whereas HIV activity was still detectable with PP. Both methods were effective in destroying bacteria, with no significant impact on the composition of the chosen nutrients (listed above) in breastmilk, using either method. Natural antibacterial activity was retained more effectively following FH than with PP (Israel-Ballard et al., 2005).

2.7 Analysis of Flash-heated breastmilk: expressed breastmilk from HIV-infected women

Some of the studies on the impact of FH on expressed breastmilk (EBM) are limited by the source of the EBM. Hence, data on the impact of FH on EBM experiments were conducted on HIV-1 spiked EBM from uninfected mothers, rather than on naturally infected samples. However, more recently, comparison data on the effect of FH on EBM from HIV-infected women has been conducted. These studies were carried out in South Africa by Israel-Ballard and colleagues - the same community that the research for this thesis was conducted.

2.7.1 Safety studies of Flash-heated breastmilk: viral and bacteriological analysis

To verify that FH does inactivate the HIV virus in human milk, the EBM of 84 HIV-infected mothers (providing 98 samples of breastmilk), from a settlement community in South Africa were used. Using FH and unheated controls, analyses that differentiated active versus inactivated cell-free HIV were undertaken using reverse transcriptase (RT) enzymatic activity assay (Israel-Ballard et al., 2007). Cellular infection requires functional RT and therefore the presence of active HIV was determined using an assay that provides a quantitative measurement of RT activity. This analysis method correlates

well with RNA quantification of HIV (Greengrass et al., 2005; Jenning et al., 2005; Malmsten et al., 2003).

All FH samples showed undetectable levels of HIV when compared to unheated controls (Fig. 2.2), a difference that was statistically significant ($p<0.001$). Detectable HIV was found in the breastmilk of 31% (26/84) of the mothers. There was a significant association found between detectable virus in breastmilk and lower breastmilk volume and low maternal $CD4^+$ lymphocyte counts (OR=0.20; 95% CI 0.53-0.74) and lower volumes of breastmilk expressed (OR=0.90; 95% CI 0.83-0.97) (Israel-Ballard et al., 2007).

The impact of FH on the anti-microbial properties of breastmilk is important to understand, particularly in settings where refrigeration is absent and the possibility of contaminants is high. To determine the bacteriological safety of EBM post heat-treatment, samples of breastmilk from HIV-infected mothers (n=38), all from the same community as the virology study, underwent FH. Heated samples were compared to unheated controls after storage at room temperature for zero, 2, 6, 8 hours. Total colony counts following 24 hours of incubation, resulted in the identification of *E. coli, S. aureus* and Group A and Group B *Streptococci*. There was a significantly higher amount of growth ($p<0.001$) and greater bacterial propagation ($p<0.005$) in the unheated samples at each time point compared to FH samples (Fig. 2.3). Flash-heated samples did not contain any pathogenic growth, however *E. coli* (n=1) and *S. aureus* (n=6) were observed in unheated samples (Israel-Ballard et al., 2006).

Although, this study did not specifically determine the anti-microbial activity of FH breastmilk by introducing specific contaminants to evaluate activity remaining post heat-treatment, the data show that FH does eliminate pathogenic and non-pathogenic bacteria. Most significantly, the study demonstrated that FH breastmilk does not have significant bacterial growth up to 8 hours at room temperature. Hence, HTEBM is safe for infant consumption for a specified period even in households without refrigeration, which might be the settings where HTEBM is the most beneficial.

2.7.3 Nutrient analysis of Flash-heated expressed breastmilk.

Analysis of the impact of FH on the vitamin content of EBM was investigated, using the EBM samples of 50 HIV-infected mothers from an urban South African community. The FH samples were analyzed for vitamin A, vitamin C, riboflavin, vitamin B_6, folate and vitamin B_{12} (Israel-Ballard et al., 2008). While FH did not significantly affect vitamin A content, concentrations of Vitamin C, B_{12} and folate were significantly increased when compared to unheated samples. Riboflavin was decreased to 59% (95% CI 44-81) of content of unheated breastmilk while B_6 decreased to 96% (95% CI 92-99) of control. (Israel-Ballard et al., 2008).

The increased vitamin C, folate and B_{12} content of the heated samples was attributed to the release of the vitamins from their binding proteins because of the heating process. The bioavailability of these increased nutrients has yet to be determined. The results concerning the nutritional quality of the breastmilk undergoing heat-treatment are encouraging as there was minimal decline in B_6 following FH, and riboflavin was the only vitamin with a substantial decrease in concentration. The authors of the study do point out that previous studies have not found a significant decrease in riboflavin content using similar pasteurization methods. As well imputed riboflavin values were used for analysis, which could vary results by approximately 25% (Israel-Ballard et al., 2008). Although, considered heat-stable, studies have yet to be conducted on trace minerals.

2.7.4 Impact of FH on immunological properties of breastmilk.

Given the immunological protection infants receive from breastmilk, it is important to measure the effect of FH on the immune properties of breastmilk. Using the EBM of urban South African HIV-infected mothers (n=50), the total immunoglobulin levels (IgA and IgG), and immunoglobulin activity, measured by specific antigen binding capacity for HIV gp 120 (glycoprotein on HIV envelope that binds the virus to the CD4 protein), influenza and poli viruses, and *Salmonella*, were analyzed (Chantry et al., 2008). Results

showed a significant decrease ($p<0.001$) in concentration for both IgA and IgG of 20% (95% CI 15-25) and 33% (95% CI 27-39), respectively. Decreases were also observed for the antigen-specific binding capacity immunoglobulins, with anti-HIV gp 120 IgG decreased by 26% (95% CI 18-33) and anti-pneumococcal polysaccharide by 30% (95% CI 21-38) -all significant changes ($p<0.001$). The largest decrease was observed with anti-poliovirus IgA which declined by 34% (95% CI 26-41). An increase was found in the immunoglubulins binding to influenza, with IgA binding increasing by 13% ($p=0.029$) and IgG by 15% ($p=0.025$). An increase observed for anti-*Salmonella* IgA did not reach significance, $p=0.13$ (Chantry et al., 2008). Despite these changes, heat-treatment is necessary and is even used when feeding very high-risk premature infants in industrialized countries (Holder method used for pasteurizing EBM form milk banks), as the advantage of breastmilk outweighs the disadvantages.

Overall, the study showed that despite the decreases observed in the FH samples when compared to controls, the FH breastmilk retained most of the concentration and activity of the immunoglobulins and the specific antigens investigated. The study is limited, however, in that it does not measure other immunoprotective components of breastmilk. This identifies a focus for future studies.

In summary, the above studies illustrate that FH successfully deactivates cell-free HIV virus, providing pasteurized breastmilk that is safe to keep at room temperature up to 8 hours. Moreover, measurements of the effect of FH on specific vitamins and immunolglobulin levels and activity suggest that HTEBM, as suggested by WHO policy, is a viable feeding option for use by HIV-infected breastfeeding mothers from resource poor settings.

What remains to be evaluated is the feasibility of mothers living with HIV being able to carry out FH of their EBM, and to use the HTEBM to feed their infants.

2.8 Challenges of community based HIV initiatives

Several factors will affect whether heat-treatment of breast milk can become a viable infant feeding option for HIV-infected women. The central issue is whether women are willing to EBF for 6 months, rapidly wean their infants by using expressed breastmilk that is heat-treated, and then use it as a feeding option for their infants. Beyond the mother's belief system and her personal perceptions, is the attitude of her family, friends and community. Thus, the social context of women's lives will influence the success of this modified feeding strategy. Many HIV community-based prevention interventions have been criticized for failing to take into consideration the socio-cultural determinants of health-enhancing behaviour change at both the individual and community levels (Campbell, 2003). Often these programs have a biomedical focus with emphasis on individual behaviour, excluding social and community level factors that affect an individual's life (Campbell, 2003). Traditional approaches focusing on providing health enhancing information often influence a small proportion (approximately 25%) of the intended population, usually those who have more financial resources and education (Gillies, 1998). Participation of HIV infected women and their community to inform and collaborate with researchers may foster community ownership over a strategy, and create a more relevant community-based initiative and study.

2.9 Qualitative research

Qualitative and quantitative research methods operate on divergent assumptions, based on differing theoretical paradigms. Although aspects of each method can be borrowed by the other, there are dominant underpinnings for each research method. Quantitative methods are deductive in nature, comprised of formulation of a priori hypothesis, generating numerical data that are analyzed using mathematical approaches. Therefore, quantitative methods are dependent on adequate numbers of subjects (sample size) in order to draw conclusions from statistical analysis of data and seek to generalize findings (Creswell, 1994).

In contrast, qualitative research is inductive, identifying data as emanating from a variety of sources, such as a participant's words from interviews, participant observations, pictures or artifacts (Glesne & Peshkin, 1992). Since qualitative methods function on the premise that human behaviour is complex and difficult to measure, qualitative methodological tools exist to facilitate examination of participant's words and actions. Consequently, qualitative research provides rich descriptive data, facilitating the understanding of people's experiences and construction of situations in their world (Patton, 2002). Sampling in this methodology is based on saturation point where the intention of qualitative research is to form unique interpretations of events and not to generalize findings (external validity). The ability to generalize is limited to areas like emerging themes from data (Merriam, 1988). As a result, in qualitative research, the quality of data is judged on trustworthiness through a verification process (i.e. member checks) in contrast to validity and reliability measures, which are central to quantitative research (Lincoln & Guba, 1985).

The benefit of qualitative research is its provision to explore effectively an area that has not been previously studied. For example, the aim is to examine the context in which people live and to increase understanding of how cultural beliefs influence participants' interactions with their surroundings (Creswell, 2003). Interventions emerging from qualitative research are therefore developed based on listening to participants and building a strategy based on their input.

Both qualitative and quantitative methods have benefits and limitations. Collecting numerical data and applying statistical analysis has great value in developing science and advancing understanding of fields of study. Understanding human experiences provides valuable information on the indicators of behaviour and conditions for modifying or influencing human beliefs, attitudes and practices. However, in isolation the two research methods are limited and hence, integration of qualitative and quantitative methods, as in the mixed methods approach, combines the benefits of both research paradigms.

2.9.1 Mixed methods

The combining of qualitative and quantitative methods within a single study has a potentially valuable function for exploring research questions, deriving benefit from the strength of both research methods. Triangulation of methods adds breath and scope to a research topic, particularly formative research. Given that qualitative and quantitative research methods have differing underlying considerations and process (Table 2.7), use of both methods should be implemented in a systematic manner, as in the use of simultaneous triangulation. Simultaneous triangulation operates on the premise that both qualitative and quantitative data can be collected together and thus findings are presented both in themes or voices of the participants and with statistically analyzed data (Morse, 1991).

Mixed methods, due to the diverse data they present, can be used effectively in the development of health strategies that inform policy, and as such, may prove particularly beneficial in the area of public health and health promotion.

SUMMARY AND STUDY RATIONALE

Given that breastmilk in developing areas of the world is critical for optimal development and survival of the child, exclusive breastfeeding then becomes an essential infant feeding practice. For infants born to HIV infected mothers, breastfeeding cessation at six months of age has the dual benefit of minimizing HIV transmission risk, while promoting infant health, therefore improving HIV-free infant survival. However, in resource poor settings, breastmilk is an important source of energy and nutrients for the developing child, since it contributes up to half of the required energy intake for 6 to 9 month old infants. As a result, transitioning to complementary feeding is a potentially high risk period for growth faltering, within such settings. Continued safe use of breastmilk in the infant's diet thus becomes an advantageous public health strategy. Studies have confirmed that Flash-heating deactivates the HIV virus in the breastmilk of HIV-infected South African women, without destroying the majority of vitamins and immunoglobins. What remains to be elucidated is the acceptability and feasibility of mothers living with HIV, being able to successfully use heat-treated expressed breastmilk in feeding their infants.

ACCEPTABILITY AND FEASIBILITY STUDIES

Research questions

1. Within a specified resource poor urban South African setting, how acceptable, at the individual, family and community levels, is the use of heat-treated expressed breastmilk to feed infants born to HIV-1 infected mothers using heat-treated expressed breastmilk ?

2. How feasible is this proposed modified breastfeeding strategy (HTEBM as component of complementary feeding, following 6 months of EBF) for HIV-infected mothers to implement ?

Objectives

(i) To use qualitative research methods to determine the *acceptability* of HTEBM use as a viable alternate feeding from an individual, family and community wide perspective, in an urban HIV affected, resource poor setting in South Africa.

(ii) To use the data from the acceptability study as a guide in the development of the study intervention carried out in the feasibility study.

(iii) To implement the intervention with HIV-1 infected women from the targeted community, providing support to the mothers through home visits by trained breastfeeding counselors.

(iv) To evaluate the *feasibility* of the intervention utilizing a mixed methods approach, capturing quantitative and lived experience, qualitative data.

(v) To evaluate nutritional status and immune profile of breastfeeding, HIV-1 infected mothers.

This thesis addressed the above research questions and objectives through the implementation of three studies. The acceptability study is discussed in manuscripts detailed in chapters 5 and 6, with the feasibility research presented in the manuscript contained in chapter 7. The final manuscript of chapter 8 presents the study on maternal nutritional and immune status of HIV-infected breastfeeding women.

Table 2.1 Transmission route, point and risk estimates for mother-to-child transmission of HIV, without antiretroviral prophylaxis

	Risk of transmission (%)		
Transmission point	Without breastfeeding	Breastfeeding for 6 months	Breastfeeding for 18 to 24 months
Pregnancy	5-10	5-10	5-10
Labour and Delivery	10-20	10-20	10-20
Postnatal: breastfeeding			
< 2 months		2-10	2-10
>2 months		1-5	5-10
Total risk	15-30	25-35	30-45

Source (De Cock et al., 2000)

Table 2.2 Definitions of breastfeeding patterns

Exclusive Breastfeeding Infant receives breastmilk only, infant can receive drops, syrups (vitamins, minerals, medicines). Infant receives nothing else.
Predominant Breastfeeding Infant receives breastmilk as the predominant source of nourishment. Infant also receives liquids (water, and water-based drinks, fruit, juice, oral rehydration solution), ritual fluids and drops or syrups (vitamins, minerals, medicines). Infant receives nothing else
Mixed Feeding Infant receives breastmilk plus foods and or other fluids.

Source: (WHO, 2001)

Table 2.3 Components of the current WHO/UNICEF/UNAIDS infant feeding guidelines for HIV infected mothers.

The most appropriate infant feeding option for an HIV-infected mother should continue to depend on her individual circumstances, including her health status and the local situation, but should take greater consideration of the health services available and the counselling and support she is likely to receive.
Exclusive breastfeeding is recommended for HIV-infected women for the first 6 months of life unless replacement feeding is acceptable, feasible, affordable, sustainable and safe for them and their infants before that time.
When replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding by HIV-infected women is recommended.
At six months, if replacement feeding is still not acceptable, feasible, affordable, sustainable and safe, continuation of breastfeeding with additional complementary foods is recommended, while the mother and baby continue to be regularly assessed. All breastfeeding should stop once a nutritionally adequate and safe diet without breast milk can be provided.
Whatever the feeding decision, health services should follow-up all HIV-exposed infants, and continue to offer infant feeding counseling and support, particularly at key points when feeding decisions may be reconsidered, such as the time of early infant diagnosis and at six months of age.
Breastfeeding mothers of infants and young children who are known to be HIV-infected should be strongly encouraged to continue breastfeeding.
Governments and other stakeholders should re-vitalize breastfeeding protection, promotion and support in the general population. They should also actively support HIV-infected mothers who choose to exclusively breastfeed, and take measures to make replacement feeding safer for HIV-infected women who choose that option.

Source:(WHO, 2006)

Table 2.4 Maternal and infant risk factors associated with postnatal transmission of HIV

Maternal factors	Infant factors
• Immune status • Plasma viral load • Breastmilk virus • Breast infection • New HIV infection • Viral characteristics	• Non-exclusive BF • Breastfeeding duration • Age • Lesions in mouth, intestine • Prematurity • Infant immune response

Source: (E. Piwoz & Preble, 2000)

Table 2.5 Bioactive constituents found in breast milk

Constituent	Action
B Lymphocytes	Provide antibodies, targeted against specific pathogens.
Macrophages	Kill microbes in infant gut, also produce lysozyme and activate other components of the immune system.
Neutrophils	Act as phagocytes.
T lymphocytes	Kill infected cells directly or send out to mobilize other defenses, proliferate in the presence of pathogens.
Antibodies of secretory IgA	Bind to microbes in infant gut, thereby prevent them from passing through walls of the gut into body's tissues.
B12 binding protein	Reduces amount of vitamin B-2, which bacteria need in order to grow.
Bifidus factor	Promotes growth of *Lactobacillus bifidus*, harmless bacterium, in infant gut. Growth of such nonpathogenic bacteria helps to crowd out dangerous varieties.
Fatty acids	Disrupts membranes surrounding some viruses and destroys them.
Fibronectin	Increases antimicrobial activity of macrophages; helps to repair tissues that have been damaged by immune reactions in infant gut.
γ-interferon	Enhances antimicrobial activity of immune cells.
Hormones & growth factors	Stimulate infant gut to mature more rapidly, as "leaky" infant gut matures, infants become less vulnerable to microorganisms.
Lactoferrin	Binds to iron (many bacteria use to survive), thus decreasing amount of iron pathogenic bacteria need.
Lysozyme	Disrupts cell walls of bacteria.
Mucins	Stick to bacteria and viruses, thus keeping them from attaching to mucosal surfaces.
Oligosaccharides	Binds to microbes and stops them from attaching to mucosal surfaces.

Source: (Hanson, 2004)

Table 2.6 Contrast and comparison between Flash-heat and Pretoria pasteurization heat-treatment of expressed breastmilk methods.

Flash-heating*	**Pretoria pasteurization****
Breastmilk is manually expressed into an uncovered clean glass jar (i.e. peanut butter jar).	Breastmilk is manually expressed into a clean glass jar (i.e. peanut butter jar), then covered with the lid.
Water is poured into an aluminum pot and the jar of expressed breastmilk placed in the water.	Water is poured into an aluminum pot and heated to boiling. Once boiled, the pot is removed from the heat.
Breastmilk and water are heated together over a high flame until the water reaches a rolling boil.	The covered peanut butter jar of expressed breastmilk is immediately placed in the pot of boiled water for 20 minutes.
Jar with breastmilk is immediately removed from the pot, covered, and cooled.	Once completed, jar of breastmilk is cooled.
Once the heat-treated breastmilk is cooled, it can be cup fed (or spoon fed) to the infant.	Once cooled, the heat-treated breastmilk can be cup fed (or spoon fed) to the infant.

* amounts of breastmilk and water are detailed on pamphlet in the appendix VII.
** amounts of breastmilk and water are detailed in (Jeffery et al. 2000).

Table 2.7 Comparison of key considerations and the process of quantitative and qualitative research

Quantitative research	Qualitative research
• Knowledge required to develop research question and or test hypothesis • Selecting concepts to test hypothesis or research question • Operationally defining concepts • Scientific theory to explain data • Interpreting results and report in accepted format for quant-itative research	• Discovering what informants know about culture and phenomenon under study • Examining how informants classify experiences • Determining how informants define these concepts • How do informants explain their experiences • Translating informant cultural knowledge and experiences into description for qualitative publication

Source: (Spradley, 1979)

Burden of HIV in sub-Saharan Africa

90% of the epidemic burden

10 % of the world population

10% of world earning economic power

Infection rate women: 61.% of the infected African population

Infection rate children: 90% of world's pediatric infections

Infection rate in pregnancy: highest antenatal infection rates in the

South Africa

Total population: 47.4 million

Diverse racial composition and distribution:

Black African:	79.5%
White:	9.2%
Mixed Race:	8.9%
Indian/Asian:	2.5

Burden of HIV in South Africa

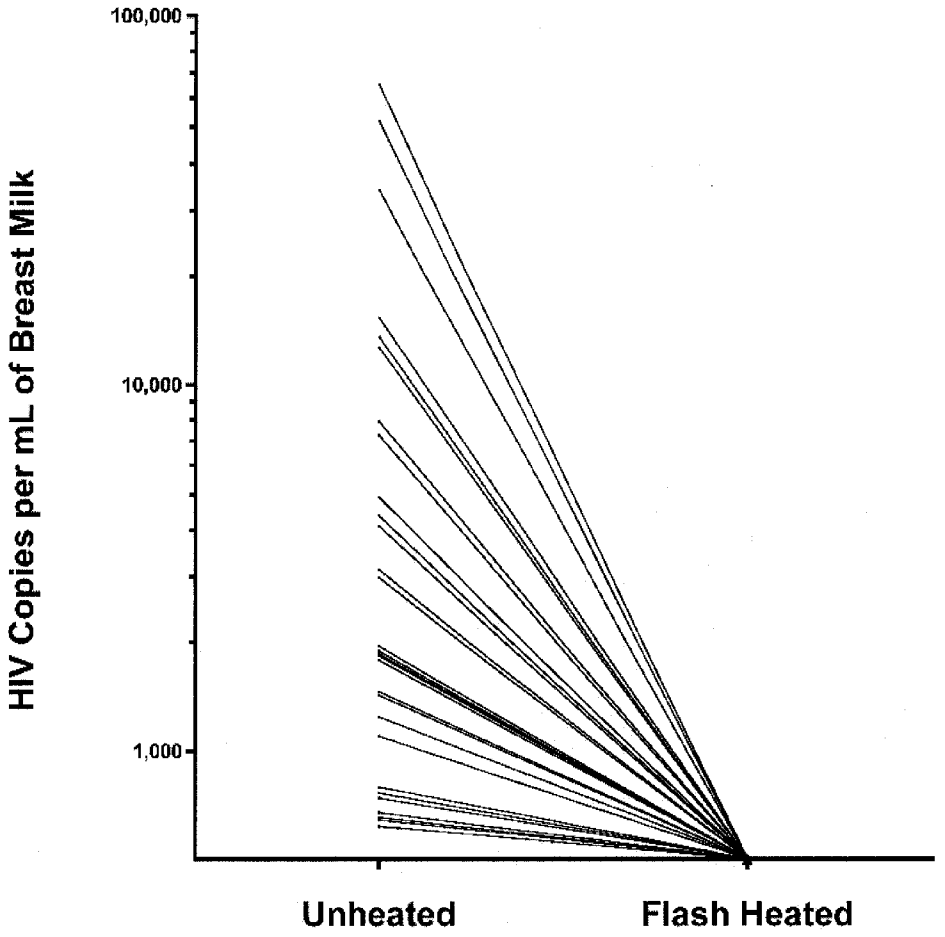
National prevalence: 11%

Distribution of HIV prevalence in 2005 (>2 years old).

Males	8.2%
Females	13.3%
Africans	13.3%
White	0.6%
Mixed race	1.9%
Asian	1.6%

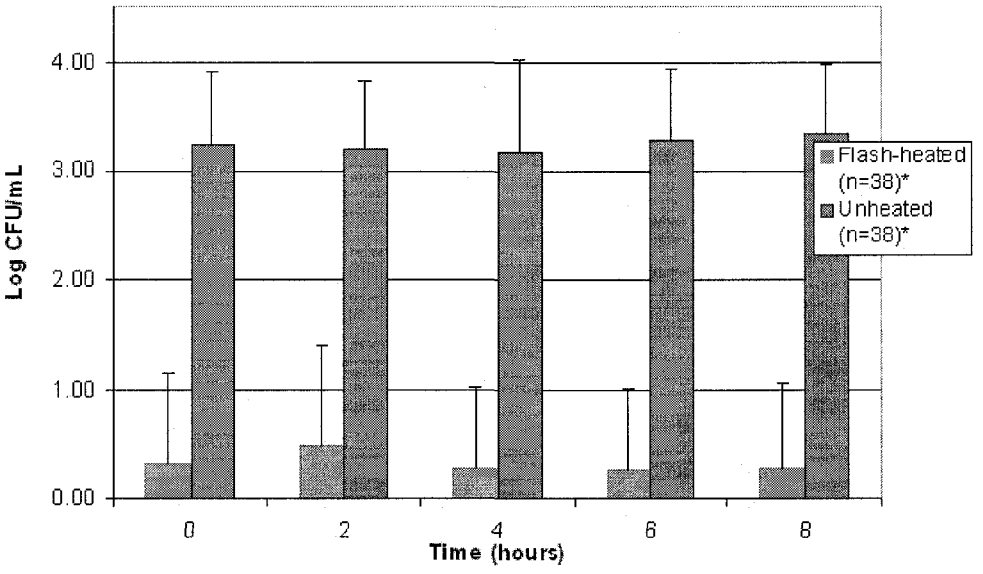
Source : (Department of Health, 2007b; UNAIDS, 2007)

Figure 2.1 Sub-Saharan Africa and South Africa population demographics and HIV burden



Source: (Israel-Ballard et al., 2007)

Figure 2.2 Detection of cell free HIV-1, following Flash-heating of expressed breastmilk compared to unheated controls (n=30).



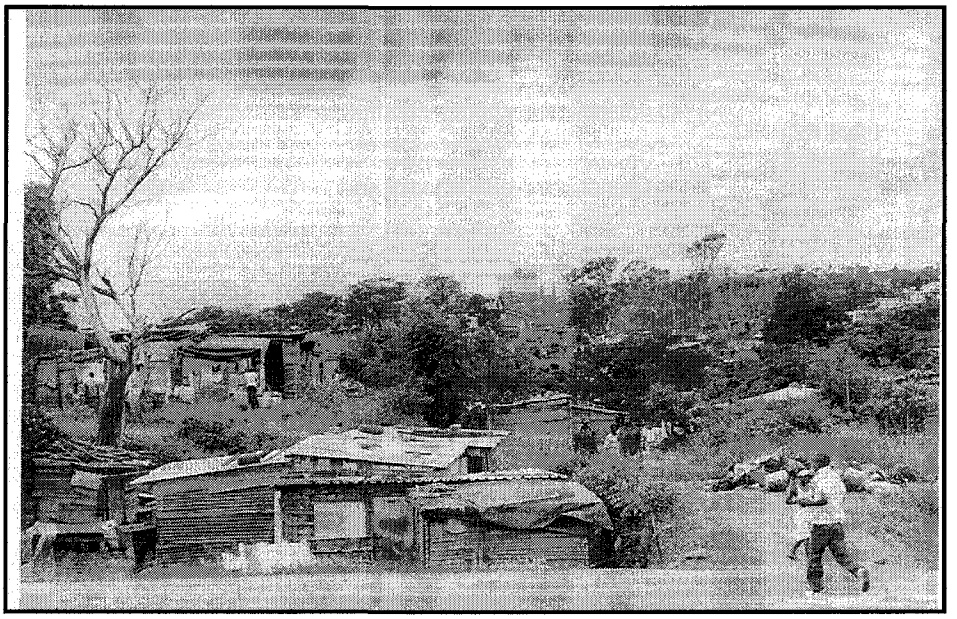
Source : (Israel-Ballard et al., 2006)

Figure 2.3 Comparison of non-pathogenic growth in Flash-heated expressed breastmilk and unheated controls, from mothers infected with HIV-1, at 0 to 8 hours storage time ($p<0.0001$).

*Samples labeled to numerous to count (TNTC, colony counts above 200) were set to 6,166 CFU/mL (log value =3.79), which may underestimate the actual bacterial growth in these samples. However, no FH samples were TNTC at any time point, while in the controls 4,5,7,13, and 16 samples were considered TNTC at 0,2,4,6, and 8 hours, respectively.

CHAPTER 3

3.1 Research site



Although the majority of informal settlements in South Africa are the vestiges of the Apartheid system, often called Townships, these settlements continue to thrive in the new democratic South Africa. As the country prospers economically and urbanization takes place, an increasing number of rural dwellers leave their homes and seek employment in urban centers. However, South Africa has one of the highest level of inequity in the world, with the gap between the economically advantaged and the poor widening. Food security, morbidity and mortality are also impacted by poverty mainly due to lack of basic income for transportation to access health care, food and clothing. Current estimates indicate that 37% of the population live on less than a R1000 per month (approximately $140.00 CDN) and 60% of the poor receive no social transfers (Wolard, 2002).

The concentration of poverty in the African population is striking, despite representing 79% of the national population, black Africans comprise 95% of the poor and overall 50% of all those living in poverty in South Africa. With poverty comes poor access to basic services such as electricity, piped water, and adequate sanitation (Wolard, 2002).

The province of KwaZulu-Natal (KZN) has been heavily affected by South Africa's HIV epidemic. A little more than 20% of the national population live in KZN and with an antenatal infection rate of 39.1%, KZN has the highest rate of HIV infected pregnant women for all provinces (Department of Health, 2007). At 21.6%, KZN has the highest proportion of births in the country of which 8.6% are infected, the highest percentage of infected children in the country. In terms of child mortality, KZN again performs poorly having the worst rates, with an infant mortality rate of 63 per 1000 birth compared to the national rate of 48/1000 births. Life expectancy at birth is currently estimated to be 43 years (Dorrington et al., 2006) .

The study site for this research is located within an urban settlement in KZN, with a population of approximately 170,000 residents, most being Zulus. Since this is a resource poor setting, the community is characterized by the factors described above.

The community is comprised of both formal and informal dwellings, with an estimated 40% of the residents living in shacks. These shacks are rented at a monthly rate of R50 to R80, approximately two-thirds of a person's income. Shacks do not have electricity and provide free communal sources of water. Formal homes are often single rooms and difficult to access, due to the services they *may* have (electricity and piped water). Rental rates range from R200 to R400 per month. In most places the rent includes water and electricity, some cases an electricity card is purchased (R50 per month). There is a 200 liter water limit in the more formal area, once the limit is reached, the resident does not have access until the next morning (5 a.m.). The above are all conditions described from conversations with community members at the time of this research study.

This is a community that is highly affected by the province's HIV epidemic with An informal community survey conducted in 1999 estimating close to half of the pregnant women attending the clinic were HIV infected (personal communication).

3.2 Study set-up

This setting was ideal for assessing a modified breastfeeding infant feeding strategy since most families are not able to purchase formula. At the time of the study a free formula program was available for use by HIV infected mothers. However, the program was fraught with sustainability and supply issues, and is now no longer available. As well, some women did not access the program for socio-cultural reasons and concerns over the stigma of formula feeding in a breastfeeding community.

The research idea to evaluate the acceptability and feasibility of mothers in a high HIV prevalence setting in South Africa originated from the candidate, who developed the concept and contacted Dr Anna Coutsoudis of the University of KwaZulu-Natal because of her bench mark studies elucidating the benefits of EBF on infant health and the minimal associated transmission risk. Upon securing a collaborative agreement with Dr Coutsoudis, the candidate completed grant applications to Canadian funding bodies (CIHR and IDRC) and was successful in receiving funding from both sources. Collaboration was also established with Dr Caroline Chantry from the University of California (Davis) who had developed the FH method and her study coordinator Kiersten Israel-Ballard.

The WHO policy guidelines of AFASS for infant feeding RF, were used as the framework and basis of this research. Essentially, the research encompassed three phases (safety, acceptability and feasibility evaluations) and involved the undertaking of six studies within the 3 phases. The candidate took the lead on 4 of the 6 studies. Manuscripts have been completed for all the studies and are all included in this thesis.

Prior to study implementation, a number of tasks and activities were undertaken by the candidate in preparation for the study, all outlined in the following sections.

3.3 Training and capacity building

Capacity building, an essential component of population health research, allows for members of a community within a study site to gain valuable and tangible benefit from the research taking place within their community. For the study undertaken in this thesis work, capacity building took place in the form of training offered to community members by the candidate. The candidate developed and delivered a breastfeeding counselor training course (16 hour certification course), training peer breastfeeding counselors in theoretical and practical breastfeeding skills (syllabus in appendix V).

Six women from the community completed the training, all receiving a passing grade (passing grade set at 65%). Trainee assessment was provided in the form of a pre and post written test and an oral test.

Although the study employed 1 counselor (plus one back-up), the other women participated in the training to gain skills and become certified (all received certification verifying training as peer breastfeeding counselors), increasing their opportunity for employment, wherever lay peer breastfeeding counselors are needed.

The study's breastfeeding peer counselor was mentored by the candidate through home visits, developing her skills to the point that she was able to become a trainer at the clinic. The peer counselor was also trained on data collection and data entry (by the candidate), skills that have kept her employed in other studies, once this study was completed.

The candidate trained the peer breastfeeding counselor to also deliver a 10 minute presentation at the antenatal clinic for a pregnant audience, promoting optimal breastfeeding. This activity was conducted to increase the visibility of the peer counselor and to actively promote EBF.

The candidate developed and delivered in-service presentations for staff at the local hospital to foster collaboration in supporting breastfeeding mothers to initiate breastfeeding successfully (syllabus in appendix VI). The peer counselor was also trained to deliver these presentations.

Along with the above study set-up activities, the candidate developed the study protocol for both the acceptability and feasibility intervention studies, as well as the data collection instruments (see appendices for examples).
The candidate conducted the field study over a period of 14 months.

3.4 Participatory resource development

One of the outcomes of the acceptability study was the identification by mothers of the need for resource developed to promote HTEBM, using images that were representative of the women in the community. The initial resource developed was a pamphlet in isiZulu, illustrating the process for HTEBM pictorially (Appendix VII). Mothers volunteered to work collaboratively with the candidate in the pamphlet development, providing input on what worked and consenting to being photographed for the pamphlet. This resource and the process of development was presented, by the peer counselor (who attended from South Africa), at the International AIDS conference in Toronto. It is now used as a counseling tool at the community clinic for mothers interested in using HTEBM.

Likewise, mothers suggested the development of a representative video promoting EBF and HTEBM. The candidate developed the concept, worked with the two mothers who volunteered to discuss EBF and demonstrate HTEBM on film, directed and helped produce the video. Again this video is used in the clinic for counseling mothers. Both resources have been requested by other sites with interest in modifying them for use within their settings. (Appendix XI for English translation of video content).

CHAPTER 4

METHODOLOGY

Three studies comprise the body of work for this thesis. Methodology used for recruitment, sampling, selection criteria, data collection and analysis for all studies undertaken will be discussed. The chosen methodology demonstrates why a particular method was used to address study objectives, thereby elucidating the study's research question or hypothesis.

4.1 ACCEPTABILITY STUDY

4.1.1 Gaining entry into the community and participant recruitment

The acceptability study used qualitative research methods to examine the receptivity to HTEBM, at the individual (mothers), family (partners, extended family) and societal levels (traditional healers, clinic counselors). Through this process, data are collected on the proposed intervention from different perspectives, identifying potential barriers to HTEBM, as well as the facilitating factors. The acceptability study was conducted first since it provided feedback on HTEBM, as well as guided the development of the infant feeding intervention to be examined in the follow up feasibility study.

The study site was selected because it is a high HIV prevalent urban settlement community, a setting where AFASS conditions do not exist, and therefore, a safe and nutritious feeding option for infants born to infected mothers, is needed.

Gaining trust within the community was a critical component of the study due to the stigma and discrimination associated with HIV and concomitant sensitivity and suspicion that discussions on the subject may raise. Gaining access and acceptance in the

community was achieved through the candidate's increased activities in the clinic, meeting key community stakeholders and leaders and through community mapping activities, all processes that provided increased visibility within the community. Most critical to this process was partnering with a community resident who was employed and trained by the candidate, to collaborate on the study as a community research partner (CRP).

4.1.2 Selection criteria and sampling

Mothers were eligible for the study if they were residents of the community and had an index child a year old and younger. These were mothers who would have recent infant feeding experiences, reflecting current relevant issues within the community.

The sample of mothers were purposefully selected, a procedure that selects for information-rich cases that provide in-depth understanding of the issue of inquiry, a goal of qualitative research. This is a different approach from selecting a statistically representative sample, a procedure that generates empirical generalizations of findings from a sample, to a population (Merriam, 1998)

The study sample size (n=31) was guided by reaching a point of saturation, achieved through content analysis of both individual and group interviews. Point of saturation is reached when no new emergent themes or issues appear, as additional participants are interviewed. This saturation point has been estimated to occur from interviews of 12 to 20 participants in the targeted population (Lincoln & Guba, 1985).

Within the clinic, it was easier to identify mothers to participate in the study, through the assistance of clinic staff. Not wanting to restrict our sampling only to mothers who attended the clinic, we also went into the community to recruit mothers. Wanting input from women living under different conditions, mothers form both shack and semi-formal dwellings were identified and participated in the study.

One of the strategies used to recruit within the community, was to approach homes with diapers hung out on a washing line, indicating the presence of an infant within the household.

A snowball approach was used to recruit additional study participants. In snowball sampling, the targeted participant (the mothers) identifies other individuals to interview. In this case mothers indicated who was an important in their infant feeding decision making and practices (i.e. partners, grandmothers, mothers-in-law).

The remainder of the sample was comprised of relevant individuals (informants), who may influence the mother's infant feeding knowledge, attitudes and behaviour, but are external to their immediate social circle (i.e. clinic counselors, traditional healers, health care workers).

4.1.3 Data collection instruments

In qualitative research the individual is the primary source of data, interpretations and constructs of their world as they see it are obtained through interviews and observations (Lincoln & Guba, 1985). The in-depth interviews for this were conducted in isi-Zulu by the trained CRP, in the presence of the candidate. Interviews were tape recorded with additional note taking by the candidate. Group interviews were done prior to individual interviews with mothers?

The general interview guide approach was used for all interviews. This form of interviewing is open-ended in nature, with a prepared interview guide identifying basic issues to be explored with each person or group interviewed. Flexibility is provided within the interview to probe, explore and question the participant, focusing on the subject of interest (Patton, 2002). The line of inquiry outlined in the guide asked mothers to reflect on their infant feeding experiences and based on those experiences, to discuss their opinions and insights on the potential use of HTEBM. The guide for the informants

focused on infant practices in the community and identification of barriers and support for mothers using HTEBM.

Individual interviews were conducted after a group interview. Initiating the study with group interviews had a dual purpose, firstly, for the mothers, it promoted a level of comfort with the interview process, helping to identify key infant feeding issues. As well, this order of interviewing provided a means of validating emerging themes, allowing for further exploration of issues raised in the initial group interview. On average group interviews took an hour, while one to one interviews took approximately 45 minutes.

The majority of interviews took place outside of the clinic, within homes or various locations in the community. This minimized participant travel burden and allowed discussions to occur within familiar surroundings. Note taking of observations in the clinic and within the community were undertaken by the candidate.

4.1.4 Data analysis and trustworthiness

The initial step in analysis was to transcribe the interviews, which were then translated to English. This produced a large amount of unstructured data in the form of text. The text then had to undergo an established structured process of analysis, whereby the text was sorted into categories and themes emerged from the text. The qualitative content analysis method (Cavanagh, 1997) was chosen to analyze the data from the transcript, followed by triangulation (merging of different sources of data) with the recorded observations.

Content analysis initially involved several readings of a few transcripts, with repeated words, comments and concepts highlighted. These highlighted components were used to develop codes, with the codes then used for grouping the remaining transcripts. New codes were developed when new concepts emerged. Once coding was completed, all codes were collapsed into subcategories.

This procedure was duplicated independently by a separate individual, followed by a discussion comparing subcategories. Consensus on thematic categories was reached and checked whether it described the data appropriately. Member checks, a process of checking with participants if interpretations of their views are accurate, was then carried out. The challenge inherent with member checks is finding the participant and securing time for the "checking" discussion.

4.2 FEASIBILITY STUDY

This is the first feasibility study of HIV-infected mothers exclusively breastfeeding their infants for six months, ceasing breastfeeding and transitioning to feeding their infants complementary foods plus HTEBM. Being the first study, it was essential to collect data that are informative in terms of what is feasible (quantitative data), as well as document the lived experience (qualitative data of women participating in the intervention. For this reason, mixed methods research was used. Methodologically, this involved triangulation of qualitative and quantitative methods from a simultaneous triangulation perspective (Morse, 1991). Simultaneous triangulation operates on the premise that quantitative and qualitative inquiries can be answered at the same time in a single study.

The lack of field or program experience in using HTEBM, although a WHO recommend-dation, dictated that this study was developed as a pilot project, to develop a clear understanding of the components of implementing such a strategy and thus inform larger studies that can be powered to investigate infant and maternal health outcomes.

4.2.1 Selection criteria and sampling

Pregnant women, in their third trimester (gestational age $\geq$ 34 weeks), with known seropositive HIV status were recruited in the prenatal period during their antenatal clinic (ANC) visit at the community primary health clinic. The selection criteria included ensuring that the mothers were residents of the community, planning to give birth at the

hospital serving that community, intended to breastfeed and planned to return to the community following birth of their infants. These criteria were essential, since this was a breastfeeding intervention that included a hospital visit at birth and postnatal follow-up home visits. No other inclusion criteria were needed. There were no exclusion criteria.

Clinic counselors provide counseling, which includes infant feeding options, for pregnant women during their ANC visit once HIV status has been confirmed. Infected mothers choosing to breastfeed were approached to participate in the study. Participants entered the study once informed signed consent was obtained.

4.2.2 Study intervention and data collection

At the time of this study, in the KZN province, pregnant women received NVP prophylaxis during the intrapartum period, as a means of preventing MTCT of HIV, with their infant's NVP prophylaxis dose administered at the hospital, as per protocol.

Participant mothers were registered in the HIV treatment and care program in the clinic, which provided relevant services for themselves and household members as needed.

Follow-up visits were conducted by the candidate, a board certified lactation consultant and dietitian and by the CRP, who was rained as breastfeeding peer counselor In the prenatal period, each woman was visited to confirm the exact location of their residence, as well as to counsel the mother on EBF. The mother's name and expected due date was then provided to a counselor located at the hospital. Once the mother arrived for delivery she received a visit within 24 hours of delivery. This visit was to ensure successful initiation of breastfeeding and to support the mother

Upon discharged, follow-up visits were conducted according to the protocol developed by the candidate. Support of the mothers was ensured through the provision of scheduled follow-up home visits. At each visit, observations were documented and data collection

took place. The data collection was guided by a structured questionnaire and protocol (examples in appendices I X to X I). The visits schedule and their purpose are outlined in the manuscript contained in chapter 7, (Table 7.1).

At each visit the mothers were asked how they breastfed their infant in the past 24 hours, probing for foods and liquids that may have been used. These data were used to categorize infant feeding into EBF, PB or MF groups.

A full description of the flash-heating of expressed breastmilk method is described elsewhere (Israel-Ballard et al., 2005). As adapted for home use the procedure involves the expressing of breast milk into a clean glass jar (i.e., peanut butter or jam jar). For the purpose of the study all participants were issued a commercial glass jar. The jar is then placed in a small aluminum pan (which most households have) with the water level in the pan measured to 2 finger levels above the quantity of expressed breastmilk Thus the pan forms a water bath for the expressed milk in the jar. The pan is then placed on a hot flame of the cooking equipment used in the home (most homes in the community use a kerosene stove). Water and milk are heated together until the water reaches 100°C and there is a rolling boil. At this point the jar with breast milk is immediately removed from the pan, capped with a lid and allowed to cool. Flash-heated breastmilk typically reaches temperatures above 56.0°C for 6 min 15 sec and peaks at 72.9°C, temperature where the virus is deactivated (Israel-Ballard et al., 2007). Each mother received the HTEBM pamphlet, developed in collaboration with participants (appendix VII). Mothers were encouraged to build up to expressing approximately 250 ml daily, which would be added to the complementary diet.

The mothers received training on FH and practiced expressing breastmilk, cup feeding and FH of EBM according to a protocol developed by the candidate (Table 7.1).

Close to the breastfeeding cessation period, mothers were counseled on the importance of complementary foods to infant health, and on the use of appropriate local foods to use, including quantities and frequency. Broad qualitative data (kept broad to decrease

respondent burden) on foods mothers fed their infants were collected and categorized according to WHO classification (WHO, 2000b).

4.2.3 Qualitative data: interviews of mothers

A variety of qualitative data was collected, including taped interviews of structures open ended questions, observations and note taking from visits. The qualitative data were triangulated with quantitative data through the use of quotes embedded in results of this study which are detailed in the manuscript of chapter 7.

4.2.4 Data analysis

Quantitative data were analyzed using independent t-tests, on continuous maternal variables and Chi-square analysis on categorical data. Descriptive statistics were also summarized. All statistical analyses were conducted using SPSS 12.0 software.

Qualitative analysis involved transcribing interviews which were then translated from Zulu to English. The text data was analyzed using content analysis procedure, with member checks conducted to ensure content of interview reflected mother's meaning of their experiences. Notes taken during visits were triangulated with the other data, to provide a more in-depth interpretation of the feasibility of the intervention.

The feasibility study followed 66 women (61 from the antenatal period plus 5 additional in postnatal stage). No sample size was calculated given that this was a feasibility study but recruitment lasted for a period of 5 months.

4.3 NUTRITIONAL STATUS AND IMMUNE PROFILE STUDY

As data on the nutritional status and immune profile of seropositive breastfeeding African women within urban settings is lacking, the study also sought to document these data and to explore the relationship between the mothers' immune status and her nutritional status.

4.3.1 Selection criteria, recruitment and sample size

Mothers (n=84) participating in this cross-sectional study also participated in the studies investigating the safety of Flash-heating of breastmilk. These were HIV-infected mothers, not currently receiving ART or antibiotics and were breastfeeding at the time of recruitment.

Recruitment took place when the mothers attended postnatal clinic visits at the HIV treatment and care clinic, located within the urban settlement in KZN, South Africa. Recruitment period was between October 2004 and July 2005.

Sample size was determined for the safety studies, based on Flash-heat destruction of HIV in the EBM. A 90% confidence interval was constructed, containing the postulated result of 0 (complete absence of HIV as result of FH). Using at 90% CI width of 1.1%, a sample size of 75 mothers was calculated. The sample size was increased by 13% to account for a few samples from the mothers without detectable virus (since HIV is shed intermittently in breastmilk). Eighty four mothers participated in the study (providing 98 samples for the safety studies).

4.3.2 Data collection methods

The study took place between October and December 2004. Anthropometric maternal measurements conducted by a trained dietitian, employing standard procedures, measured

height (m) without shoes, using a wall mounted measure with an attached sliding headpiece. Weight (Kg) was measured without outer-wear or shoes. To assess percentage body fat, triceps skinfold thickness (TSF) was measured in cm, using standard calipers. The National Health and Nutrition Examination Surveys (NHANES III 1988-1994) for African American women were used as reference values for TSF, with depletion defined as values < 5^{th} percentile.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). For BMI assessment, WHO standards of : <18.5 (underweight), 18.5-24.9 (average) and ≥25.0 (overweight), were used.

Blood samples were sent to a local lab for determination of $CD4^+$ T lymphocyte cell count, using MultiTEST reagent and TruCOUNT tubes in BD FACSCalibur flow cytometre (Becton-Dickinson, New Jersey, USA). Three categorized were used to asses participant's immnune status, $CD4^+$ T cell count levels <200 (compromised), 200-500 (moderate) and > 500 (high).

Hemoglobin (Hb) was measured on venous blood samples collected into EDTA vacutainers for hematological analysis, with anemia defined as Hb< 12g/dL.

Breast milk samples (75 to 150 ml) provided by the mothers were stored at -80° C, then shipped frozen for analysis of viral load at the California Department of Health Services Viral Rickettsial Disease Laboratory. All samples were analyzed for levels of detectable HIV-1 using TaqMan Real Time-RNA-PCR (TaqMan RT-PCR, Applied Biosystems, Foster City, CA).

Maternal breastfeeding practices were categorized into three categories: exclusive breastfeeding, predominately breastfeeding and mixed feeding. Maternal socio-demographic data were also collected (maternal age, presence of electricity and piped water in the house, as well as type of cooking fuel used).

4.3.3 Data analysis

The relationships between nutritional status variables and immunological variables by postpartum period categories (0-7 weeks; 8-15 weeks; >15 weeks) and $CD4^+$ T cell count groupings, were analyzed using ANOVA. Tukey's post hoc test was used when the association between variables reached significance. Relationships between categorical data by postpartum periods and $CD4^+$ T cell count groupings were evaluated using the Chi-square test. Independent Student t-test was used to examine differences in variables for mothers with detectable versus undetectable breastmilk viral load. Significance was set at $p<0.05$ for all analyses. Breastmilk viral loads were not normally distributed and therefore were log transformed. SPSS 12.0. was used for the analysis.

CHAPTER 5

HEAT-TREATED EXPRESSED BREAST MILK: AN ACCEPTABLE FEEDING OPTION FOR USE BY URBAN AFRICAN MOTHERS LIVING WITH HIV

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5.1 Abstract

The majority of informants from a settlement community in South Africa, with a high HIV prevalence, found use of heat-treated expressed breastmilk (HTEBM) an acceptable feeding option when used to supplement infant intake during complementary feeding. Utilizing a modified conceptual framework of factors affecting infant feeding practices, we interpreted our findings considering the influence of individual, group and societal level factors on a mother's ability to use HTEBM. Mothers living with HIV identified support from a partner or family member combined with voluntary disclosure of HIV status as optimal conditions required to build maternal confidence and facilitate successful practice of HTEBM. Fear of excessive infant nighttime crying following early cessation of breastfeeding was a primary child-centered concern for mothers. Mothers provided viable strategic solutions to potential barriers associated with HTEBM use, but were unable to offer guidance to alleviate possible negative infant response to breastfeeding discontinuation. Within this community, infant health care seeking practices involve the combined use of traditional healers along with contemporary health care services. Traditional healers identified a need for receiving training in infant feeding guidelines, particularly in the context of HIV, to facilitate consistency of messages delivered to mothers. Collaboration between traditional and contemporary health service providers may play an important role in promoting optimal feeding practices. The fact that our data is collected from a broad sample of community informants residing in a settlement area, provides a rich source of information that can guide development of public health initiatives aimed at promoting safe breastfeeding modifications within resource constrained settings. Additionally, our findings point out resources and conditions needed in place to enable maternal use of HTEBM as a viable feeding option for breastfeeding women living with HIV.

Keywords: HIV, South Africa, exclusive breastfeeding, heat-treated breastmilk, complementary feeding, acceptability study, qualitative methods.

5.2 Introduction

Well into the third decade of the global HIV epidemic, close to 34 million people are living with HIV/AIDS, of whom two-third are from sub-Saharan Africa (UNAIDS, 2007), the epicenter of the epidemic and a region of the world that is home to some of the poorest and most marginalized people on the globe. Further, infections of women are on the rise, with an estimated 15.4 million women living with the virus, of whom 75% are African women (UNAIDS, 2007). High infection rates of women contributes to increased infections in the pediatric population, most through mother to child transmission (MTCT) of HIV, resulting in the infection of approximately 700,000 children around the world, in a single year (UNAIDS/WHO, 2005). Prolonged breastfeeding in an African setting can account up to 50% of HIV infections in infants and children (De Cock et al., 2000). Sub-Saharan Africa bears the brunt of the pediatric HIV burden, consequently maternal and child infections are considered a critical public health concern and challenge in this part of the world.

South Africa, with one of the most severe HIV epidemics in the world, has an infection rate of 29.1% in the pregnant population. The province of KwaZulu-Natal (KZN) carries the bulk of the antenatal HIV burden, with the province having experienced a doubling in infections of pregnant women within a decade, from 19.9% in 1996 to 39.1% by 2006 (Department of Health, 2007a). Consequently, South African MTCT rates are high and estimated, within a single year, to have resulted in the infection of approximately 26,000 children, through extended breastfeeding (Dorrington et al., 2006).

While U.N. guidelines advocate for the avoidance of breastfeeding by HIV infected mothers under conditions where replacement feedings are acceptable, feasible, affordable, sustainable and safe (AFASS) (WHO, 2006), these stipulated preconditions rarely exist in developing areas of the world. Moreover, Africa has a breastfeeding culture, hence even women living with HIV still choose to breastfeed for personal, economic and socio-cultural reasons.

Given the child survival benefits of breastfeeding in developing areas of the world, elucidating safe breastfeeding practices for HIV infected women has emerged as an important public health priority. The groundbreaking work conducted by Coutsoudis and colleagues (Coutsoudis et al., 1999 ; Coutsoudis et al., 2001) in South Africa, provided the first data on the impact of breastfeeding on transmission risk. Findings of the study indicated that exclusive breastfeeding (EBF) decreased infant risk of postnatal HIV infection and that infants who were EBF for a minimum of 3 months had no excess risk of HIV infection at six months of age compared to infants who were formula fed. Further, that mixed feeding (breastfeeding plus other foods and liquids) increased risk of infection. The most recent evidence to confirm the above findings comes from a large South African cohort prospective intervention study, designed to asses HIV transmission risk as a function of infant feeding. Results present an estimated transmission risk rate of 4.04% (CI 2.29-5.76%), following 6 months of EBF. Breastmilk plus formula led to a doubling of transmission risk over EBF, whereas breastfeeding, formula and solid foods resulted in a 11 fold increased risk of infection. Additionally, mortality for 3 month old EBF infants was lower (6.1%) compared to infants who received breastmilk plus solid foods or formula (15.1%) (Coovadia et al.,2007). These findings provide strong evidence for women in resource constrained settings to EBF their infants for the first 6 months of life. The need then arises to find an affordable, safe and sustainable replacement feeding that can be used when complementary feeding is initiated. One example of a WHO sanctioned replacement feeding option recommended for infants born to mothers living with HIV is heat-treated expressed breastmilk (HTEBM) (WHO/UNAIDS/UNICEF, 1998). However, to date there have been no studies published on the field application of this feeding strategy.

Two home-based methods of heat-treatment currently exist, Pretoria pasteurization (Jeffery & Mercer, 2000) and Flash-Heating (FH) (Israel-Ballard et al., 2005), both have been shown to successfully inactivate HIV. Flash-heating is a simple method of pasteurization that can be easily carried out in a home setting, within a resource-constrained environment, utilizing common kitchen equipment (small pot for water bath and glass jar, like a peanut butter jar, for the expressed milk). Through FH, temperatures

greater than 56° C for 6 min 15 sec are commonly attained, peaking at 72.9°C, adequate temperatures to inactivate heat sensitive HIV (Israel-Ballard et al., 2007). Moreover, bacteriological safety data indicates that FH breastmilk can be safely stored at room temperature for up to eight hours (Israel-Ballard et al., 2006), a critical benefit for families without access to refrigeration.

There are areas of the world where the availability of a safe, inexpensive and sustainable replacement feeding such as HTEBM holds great promise for a breastfeeding mother and her infant. However, whether this is an acceptable infant feeding choice at the community level has not been fully examined. Hence, we undertook a qualitative study with the purpose of assessing the acceptability of HTEBM as a viable infant feeding option within a resource scarce setting in KZN province, South Africa, where breastfeeding is still the norm and HIV antenatal prevalence rate is high.

Employing a qualitative research approach provides an effective method of examining the phenomenon of interest. Through qualitative methods, a context in which people live is developed thereby increasing understanding of how cultural beliefs and norms influence individual's interactions with their surroundings (Creswell, 2003).

Although promotion of HTEBM use will take place at the individual level, successful implementation of this feeding option requires a more holistic examination of the range of factors impacting maternal feeding choices, since mothers are part of a greater community and social group and therefore their behaviours and practices are influenced by forces within these environments. Many HIV community-based prevention interventions have been criticized for failing to take into consideration the socio-cultural determinants of health-enhancing behaviour change at both the individual and community levels. Hence, often these programs have a biomedical focus with emphasis on individual behaviour, excluding social and community level factors (Campbell, 2003).

To develop an understanding of the influences and inter-related factors relevant to whether use of HTEBM would be an acceptable infant feeding option, we needed to

employ a systematic approach that would facilitate a more complete examination of our research question. To achieve this objective we adapted the *conceptual framework of factors affecting breastfeeding* (Fig.1), a breastfeeding practices framework developed by Australian public health researchers (Hector, King, & Webb, 2005). The framework incorporates elements of theories used to examine and understand factors influencing health behaviours. The framework points out that infant feeding practices are influenced by factors from three different aspects: Individual, group and societal levels. Individual level factors include mother's knowledge, skills, parenting experience, health and risk status of both mother and infant and mother-infant interaction. At the group level home and peer environment factors such as partner attitudes and support are key along with community issues like health services policies, community norms and public policy related to infant feeding. Society level factors encompass issues of societal acceptance and expectations related to infant feeding practices including economic considerations of breastmilk substitutes use, as well as complementary foods in the food system. Group and societal levels interact either positively or negatively with issues at the individual level. Therefore, interventions to introduce new health practices cannot simply focus on educating and building maternal skills but must also take into consideration multiple factors from all 3 levels (Hector et al., 2005).

5.3 The Study

Setting and focus of inquiry:

This study took place in an urban informal settlement with a population of approximately 120,000, located within KwaZulu-Natal, South Africa. As in many settlements, the unemployment rate is high within this community with many of its inhabitants having moved from rural areas in search of employment.

By engaging a wide variety of community residents in discussions, we sought to address the purpose of this study, which was twofold: (i) to assess the acceptability of using HTEBM as a infant replacement feeding at the complementary feeding stage and (ii) to

gain comprehensive insight of the factors that would facilitate use of HTEBM by mothers within this community.

In-depth interviews were conducted using the general interview guide approach (Patton, 2002), whereby the interviewer has a set of issues to be explored with the participant during the interview. Interviews were open-ended and conversational with the interviewer using participant's comments to probe for further information. A similar format was used during group interviews, with the interviewer facilitating inclusive discussions while avoiding monopolization of sessions.

Interviews of mothers (group and individual) set the stage for the study. Overall, mothers were asked to reflect on their previous infant feeding experiences and based on those experiences, to discuss their opinions and insights on the potential use of HTEBM. We were interested in identifying perceived advantages, challenges and support systems, if any, that the women could pinpoint as well as to hear their thoughts on any other emergent issues. Outcome of these interviews were used to guide subsequent interviews with other participants.

Sample selection and data collection:

Our sample of mothers was purposefully selected, approaching women both at the local clinic as well as in the greater community. The study took place between October 2004 and March 2005, with interviews conducted mainly in isiZulu (main language spoken in the community) by a community research partner (SN), a resident from the study locale, who was recruited and trained by the researcher (LS). Having a resident of the community as part of the research team greatly facilitated our ability to speak to mothers in the community. All interviews took place in the presence of the researcher LS, a South African nutritionist and lactation consultant with a sufficient understanding of Zulu to follow conversation and interact during the interviews. The researcher spent a couple of months, prior to study initiation, being visible in the community thus becoming a familiar site to residents.

Following the obtainment of verbal consent, the study was initiated with a small group interview of 3 mothers. Individual interviews of additional mothers (n=8) were conducted after the group interview, a process we carried out to hear from individual mothers as well as a means of validating and further exploring issues raised in the initial group interview. Participating mothers ranged in age from 18 to 32, all with an index child under one year of age.

A snowball approach was used to recruit additional study participants (Figure 5.2), whereby mothers identified individuals in their lives that were crucial in providing support or perceived as potential barriers to implementing new infant feeding practices (i.e., partners, grandmothers, mothers-in-law). Also interviewed, were people from the community but external to the women's immediate social circle, however with the potential to influence their infant feeding knowledge, attitudes and practices.

Participants were drawn from different areas of the settlement thus providing variation in living conditions, as reflected by housing types (shack versus formal housing). Most interviews took place within homes or in the community which minimized participant travel burden and facilitated a more comfortable and non-judgmental setting. In total 31 individuals participated in the study. Our sample size was guided by the process of identifying repeat themes through content analysis of both individual and group interviews. Once there were no new emergent issues, as additional participants were interviewed, point of saturation was achieved. Saturation point has been estimated to be in the range of 12-20 participants (Lincoln & Guba, 1985).

Throughout this article anonymity of those interviewed will be maintained by not using participant's names as well as by not identifying the specific study site. Ethics approval for the study was obtained from both the University of KwaZulu-Natal Research Ethics Board and the McGill University Institutional Review Board.

Data analysis and trustworthiness:

Interviews were tape recorded in addition to note taking during interviews. Interviews were transcribed in isiZulu and then translated into English. The qualitative content analysis method (Cavanagh, 1997) was employed to analyze the text data generated from the transcripts. This process was initiated by LS and involved several readings of a few transcripts, highlighting repeated words, comments and concepts. The words and concepts were used to formulate codes and in turn the codes were used to group concepts in the remaining transcripts. When new concepts and keywords emerged a new code would be developed. The codes were then collapsed and sorted by clustering all related codes, placing them under specific subcategories. Additionally, a research assistant independently examined the interview texts, analyzing for concepts, emerging codes and subcategories. Through discussions, the final phase of analysis involved the collapsing of related subcategories into single thematic categories, care was taken to ensure that the final reported findings accurately matched the data. When possible, member checks were conducted to confirm interpretation of data through conversations to ensure we interpreted correctly what informants were telling us.

Other sources of data include observational notes from encounters and informal discussions at the clinic and in the community have been incorporated into the results and discussion.

5.4 Results

The results of this study are discussed under four thematic categories: *influences on acceptance of HTEBM, family, traditional healers and using HTEBM outside the home.* The data is presented in narrative form with quotes used to represent participants' 'voices' and their use of language.

We did not ask mothers to identify their HIV status, however, most mothers addressed HIV in their discussions with a few choosing to disclose or allude to their positive status.

Influences on acceptance of HTEBM:

"if I do this [HTEBM], the milk will be there and we don't have to buy it and it will be clean [from HIV]" (mother).

All mothers interviewed were unfamiliar with HTEBM, which initially created an atmosphere of skepticism. However with information and the ensuing discussions that followed, mothers became more responsive and accepting of HTEBM as a possible feeding option. Unanimously, mothers cited the destruction of HIV in breastmilk as the most important benefit of HTEBM (Table 5.1). Affordability was another highlighted benefit of this feeding option. Given that most households purchase fuel for meal preparation and therefore regard this as a necessary household expenditure, mothers envisioned being able to incorporate HTEBM without increased costs (i.e., additional fuel, feeding bottles or teats) to the family, which was seen as attractive.

As we introduced the concept of HTEBM to our participants, a shift from uncertainty and doubt, to one of support and acceptance, was a common participant reaction. This also held true in informal discussions within different settings in the community and at the local clinic:

"we need to stop this illness, if the mother can heat her breastmilk and kill the virus then we have to help mothers to do this because we know our babies like milk from their mother's breast" (grandmother)

"It will take a lot of hard work[promoting heat- treated breastmilk] but once one mother does it the others will try it too, especially if the babies drink the milk" (counselor)

However, respondents also pointed out their concerns and regardless of opinions, there was consensus that the uncommonness of this feeding option placed it at a distinct disadvantage for easy acceptance amongst family members and therefore would present some challenge for mothers to put into practice:

"I can try it but what will the father say and what if the baby does not like it, babies like to take the milk from the breast" (mother)

"we have tried to help mothers to express their milk and heat it. A few do it but many say they can't. But I think it is because they do it when the baby is too young" (counselor)

"you have to come and tell my partner [about HTEBM] yourself because you are from the clinic, he will listen to you and it will help me "(mother)

When discussing the practical aspects of implementing HTEBM, mothers were interested in a wide range of mother and infant centered issues (Table 5.1), with most mothers addressing the time commitment associated with the entire HTEBM process, additionally they were also concerned over their ability to express adequate quantities of breastmilk. Mostly, mothers had a tendency to focus more on issues centered on their infants as they related to the process of breastfeeding cessation. In particular, mothers communicated some anxiety around not being able to breastfeed at night and the anticipated infant crying that may result. Most mothers stated that they used breastfeeding to comfort a crying child:

"I am afraid the baby will cry when I stop giving him my breast "(mother)

"I think if the mothers can do this [HTEBM] when they are weaning it will work better. The trouble will be babies crying at night" (counselor)

We were interested in pursuing mothers thoughts on possible solutions of the potential barriers they may encounter with incorporating HTEBM into the infant's complementary diet. Education and knowledge acquisition, although identified as being useful and important in providing information and reason to try HTEBM, it was nonetheless viewed as insufficient motivation. The most essential requirement identified as key to a mother's success in using HTEBM was support. Support from a partner was viewed as the ideal,

however, support from family or friends was also deemed as a significant alternative. Mothers further elaborated that critical to the establishment of successful support, is the need for a mother to address the complex and challenging issue of HIV status disclosure and the associated stigma. Some mothers explained that the act of an infected mother disclosing her HIV status to someone she trusts, although difficult, facilitates many tasks, making life easier. However, they also pointed out that in some instances disclosure may act as a significant barrier since it is a process that may take time in identifying and securing a trustworthy support person they can confide in.

Despite some of these reservations, overall the mothers were optimistic about HTEBM since they felt that they could overcome most identified challenges, if they were sufficiently motivated. In this context, mothers related various stories of other challenges they had previously encountered. A relevant example cited were the experiences of the mothers who had practiced EBF, a behaviour that they said was initially viewed with suspicion, inciting questions and comments from family and friends. Both mothers who practiced EBF, did so following contact with clinic staff. Independently these mothers cited a supportive home environment as critical in maintaining EBF. In both cases, creative solutions were used to manage unsupportive family members and friends, thereby avoiding offending or alienating them:

"me, the money my mother gave me to buy formula , I buy food, meat for us....not to make her angry [her mother] I keep a small tin[of formula] for her to see, then she thinks I am breastfeeding and giving tin milk, but I was only giving the breast" (mother).

The other mothers recounted use of similar strategies in a variety of situations and felt that once they believe in HTEBM, acceptance within their environment could be managed

Family:

"I don't mind if the breast milk is heated and the baby drinks it in the cup...but you [looks at partner]have to boil your milk when my mother is not in the house otherwise she will trouble us" (father)

Discussions with family members were critical to building an appreciation of the composition of the mother's social network and to understand to what extent these individuals may impact her infant feeding practices. Male partners who wanted and encouraged their partners to breastfeed were more open to the idea of using heat-treated milk. However, they were concerned over the perceptions and opinions of family members and neighbors:

"if we boil breastmilk we can save and use the money for food in the house, but wont people think I am not looking after my baby then [if he does not purchase formula when mother discontinues breastfeeding] ?"(father)

These were also the fathers who were concerned that not breastfeeding would arouse suspicion and is also likely to result in family discord, mostly in the form of disapproval from female relatives, such as mother-in laws or grandmothers.

Partners favoring formula feeding were less in favor of HTEBM, seeing no need for it given that they could use formula. Being able to purchase formula for one father meant he would be perceived by his partner and family as capable of caring for his family.

Some of the mothers lived with their grandmothers, who were eager to speak to us. Common to these discussions was the expression of disappointment resulting from what the grandmothers saw as a growing trend of young women rejecting breastfeeding and by extension, of tradition:

"I see these girls are forgetting our ways to breastfeed our babies. They run after the milk in the tins. The people who made the tins sell the tin-milk and go, they wont be here when that baby is sick because he is not drinking milk from the mother's breast" (grandmother).

Grandmothers living in a household with children are often an integral part of childcare, including feeding. From our conversations, it was clear that whether breastfed or fed formula, an infant under the care of a grandmother would most likely receive other fluids (water, Rooibos- An indigenous bush tea) and at times, modified solid foods. The concept of exclusivity- breastfeeding or formula feeding, was an unfamiliar one.

Grandmothers spoke of HIV being a major problem in the community, everyone was aware of it, but no one spoke of it. Within this context, they communicated their concern and confusion regarding infant feeding. In cases where they suspected or knew a family member was infected, they were uncertain whether they should discourage breastfeeding, wanting to know if breastfeeding was contraindicated for infected mothers:

"they say the HIV can be in the mother's milk, but I hear they can still breastfeed, is this the truth?..... we just don't know anymore" (grandmother)

The idea of promoting safe breastfeeding practices through EBF followed by use of HTEBM, although both received as new concepts, were well supported by grandmothers interviewed. The support seemed to be motivated by a feeling that use of expressed breastmilk in the proposed manner will encourage and preserve breastfeeding within the community.

We were able to interview one mother-in-law, under the direction not to discuss HTEBM in the context of HIV, instead to keep to the message that it is an option for anyone wishing to pasteurize their expressed breastmilk once an infant is eating family foods.

Initially the mother-in-law completely rejected the idea of using HTEBM, stating:

"I have not heard of this [HTEBM], I don't want my grandchild to be hungry" (mother-in-law)

She felt that formula could be used as a replacement feeding, but it was clear that the idea of using formula was a not a straight forward decision either, there seemed to be undertones of concern:

"I can buy them formula but if I do they [neighbours] will think she [daughter-in- law] is sick, you know how people are...so she must continue to breastfeed, she can give some formula and food so the baby is not hungry and crying " (mother-in-law)

With further discussion involving the mother and partner, addressing health and economic perspectives, the mother-in-law felt she may be open to the idea on the condition that the whole process of expressing and heat-treatment should not be practiced openly. She also felt she needed assurance that the infant would take the milk:

"if I see that the baby is growing, I will be happy and will not trouble them [son and daughter-in-law]" (mother-in-law)

Traditional healers:

"the mothers go to the hospital and the clinic and then they want us to help their babies who are still sick" (traditional healer)

Several mothers suggested we speak to traditional healers, to hear their thoughts of using the proposed feeding option. This suggestion seemed to be motivated by the mother's health seeking behaviour, that of consulting traditional healers on a variety of issues, including infant feeding.

Within this community the traditional healers are a well organized group that meets on a regular basis. The leader and one other healer, representing their group, agreed to being interviewed. Again, similar to other participants they had never heard of HTEBM, but were supportive, cautioning that the heated breastmilk should be used only for the baby. Probed on this issue, it was explained that as a body fluid, breastmilk is potent for use in spells.

Similar to the grandmothers, the healers felt HTEBM would help preserve breastfeeding since the mothers would be using their own milk. They too complained of decreased breastfeeding in the community. They viewed this as a negative development because of frequent visits from mothers seeking help with sick infants:

"the mothers bring their child when the baby is very sick...we shout at them and ask them why they did not breastfeed and they tell us because they want the free formula but the clinic has no more milk or it is finished at home" (traditional healer)

When asked whether, during consultations with mothers, if they specified the type of breastfeeding mothers should practice, it was clear that exclusivity was not emphasized, mainly due to an unawareness issue rather than an ideological reason. The healers were surprised that EBF meant the exclusion of traditional medicines but this issue and other infant feeding recommendations were not received contentiously. On the contrary, the healers seemed receptive to information sharing, pointing out that HIV/AIDS brought confusion to the issue of infant feeding. It was suggested by the lead healer that since mothers often use both the services of the clinic and of the traditional healers, there was a need for working collectively:

"With this HIV....we have a lot of it around now..... we also must learn what to tell the mothers, you must train us too" (traditional healer)

When asked how mothers could balance their wish to use traditional remedies but still practice safe breastfeeding like EBF, they explained that as healers they can use different forms of medicines. For instance, external medicines and amulets can be used to address common infant issues, as opposed to oral preparations, which they pointed out can be delayed to when the infant is older and eating family foods.

Using HTEBM outside the home:

"at the end of the month mothers bring babies to us with no milk [formula]- they say they forgot the milk at home, but really we know they have no more milk at home. What can we do...ayeebo...the only thing we can do is we take milk [formula] from mothers who bring a lot of milk for their babies just to help those who have nothing"" (crèche director)

We interviewed principals from two crèches (daycare facilities) separately at the suggestion of the traditional healers, given that they provide childcare and may influence infant feeding behaviour. Mothers were also curious about the portability of expressed breastmilk:

"what if I have to go somewhere with the baby, what do I do with the milk...will the milk smell if I go somewhere with it?" (mother)

Initially, both principals were not receptive to mothers bringing their expressed breast milk to the crèche. Concerns were related to a perception that breastmilk would be messy and have an offensive odor which would be off-putting to staff. Despite these reservations, the principals became more open to the concept of HTEBM, particularly in the case of mothers who struggled with supplying formula. In this regard, the principals discussed their experiences with sub-optimal infant feeding practices. One principal spoke extensively of caring for infants that were consistently ill due to inappropriate feeding since these would also be the infants being left with diluted infant formula. It

was also not uncommon for mothers to leave their children at the crèche without any formula because they had run out:

"sometimes I have to give my baby weak milk because I know the tin will finish soon"(mother)

It was made clear that if HTEBM were to be used within a crèche setting, it should be brought in a bottle, facilitating easier feeding. Cup feeding of expressed milk was deemed unsuitable for a crèche setting, particularly since cups could not be propped up for self feeding (which was practiced with bottle feeding). This attitude was consistent with the mothers' perceptions, they were more in favor of using feeding bottles (than cups) to transport and feed milk outside of the home. The principals were also concerned whether mothers would be able to bring adequate quantities of expressed breastmilk, on a regular basis, it was felt that would require a dedicated mother to accomplish such a task.

Most mothers admitted taking expressed breastmilk to a crèche seemed cumbersome, preferring instead to use formula in that particular situation.

5.5 Discussion

Heat-Treated expressed breastmilk is a WHO recommended feeding option suggested for use by breastfeeding women living with HIV within resource limited settings (WHO/UNAIDS/UNICEF, 1998), yet the receptivity to this feeding alternative has not been fully explored. Hence ours is the first South African based study to examine the acceptability of HTEBM as a supplement to complementary feeding once an infant has been weaned from the breast following 6 months of EBF.

Poverty is a common characteristic of South African urban settlements (Wolard, 2002) and therefore AFASS conditions within such settings are difficult to attain. Breastmilk provides food security for an infant, however if breastfeeding is discontinued at six

months, as is the recommendation for HIV infected breastfeeding women (WHO, 2006), a challenge arises for families living under resource poor conditions. Breastfeeding cessation results in a significant decrease of energy intake for the infant since breastmilk supplies an estimated average of 54% of the total daily energy intake of a 6 month old, a point when complementary feeding should be initiated (Dewey & Brown, 2003). Consequently, the weaning period is a high risk period of transition if mothers do not have a sustainable and safe replacement feeding, infants then become vulnerable to malnutrition, growth faltering and childhood infections other than HIV, as a result of a poor quality diet or inadequate intake. Addition of HTEBM to an infant's intake as an adjunct to complementary foods can supplement the diet, contributing substantially to an infant's nutritional and overall energy intake. A recent Ugandan trial showed a relationship between early cessation of breastfeeding and increased risk of serious gastroenteritis as well as increase in infant mortality 3 months post breastfeeding cessation (Onyango et al., 2007) Other studies confirm the high risk of morbidity and mortality infants face in the absence of appropriate replacement feeds, once breastfeeding has been discontinued (Becquet et al., 2006; Piwoz, et al., 2001).

We interpreted our findings by employing a modified version of the conceptual framework of factors affecting breastfeeding (Figure 5.1). This conceptual framework identifies factors that influence infant feeding practices at the individual, group and society levels.

Individual level: maternal and child factors. At the individual level, factors are directly connected to the mother's own attitudes and beliefs, infant characteristics including health and childcare concerns, as well as the interaction that occurs between the mother-infant pair.

Throughout our discussions, mothers showed receptivity to HTEBM use, once they developed an understanding of the benefit it would present to their infants, providing HTEBM was not in conflict with their immediate environment. Support was singled out as a key factor that could enable mothers to overcome potential conflicts, as a result of

using HTEBM, within their immediate surroundings. Support was viewed as more important than information and knowledge, in motivating mothers to try HTEBM. Cognitive knowledge has been identified as being less important for some groups for performing skills, than embodied knowledge which involves exposure to the desired behaviour, in addition to other social factors, such as a supportive environment (Hoddinott & Pill, 1999).

Programs that have embarked on promoting EBF as a child survival strategy, have faced similar challenges. Although EBF is evidence-based optimal feeding, it is not the cultural norm of most communities throughout the world and therefore historically, has been perceived as impossible to implement by both the scientific community and health care service providers. However, several projects have successfully promoted EBF in a variety of settings, within all these initiatives, maternal support has been consistently identified as an essential ingredient in facilitating the successful practice of EBF (Haider, et al., 2000; Nankunda, et al., 2006; Bland et al., 2007).

The context in which mothers formulate infant feeding decisions is replete with confusing messages, particularly against a backdrop of an HIV epidemic. Despite the lack of clarity mothers encounter, we learned that many strategies were used by women to offer the best to their children, a predisposition that contributed to their general receptivity to discussing HTEBM. Although discussed positively, mother and infant centered concerns related to the practice of HTEBM were raised. Mothers offered viable solutions to potential problem situations they may encounter, mostly around family acceptance. However, generally mothers were challenged with problem solving or offering of suggestions for child-centered concerns, such as infant acceptability of heat-treated breastmilk and concern over excessive nighttime crying from infants not ready to be weaned from the breast at six months of age. It was anticipated that night feeds would be the most challenging feeds to discontinue.

Group level factors: family, community and health services. At the group level, relevant factors relate to the environment surrounding the mother and infant, including support at

home, community or local health care services. Through our conversations with a diverse group of informants comprised of mothers, fathers, HIV care counselors, grandmothers, mother-in-law, crèche principals, traditional healers and community health workers, we gained insight into some of the ways culture, social norms and family expectations interrelate in influencing infant feeding practices. Given that HIV stigma remains a significant issue in South Africa (Steinberg & Johnson, 2002), disclosure of maternal HIV status to a carefully chosen individual who is able to act as support was found to be an important factor related to the mother's interaction with her environment. Although the process of disclosure was described as difficult, due to the inherent danger in exposing oneself to rejection, it was also recognized by some as having many benefits.

The presence of a partner in the home has a very strong influence on a mother's choices in feeding her infant, since mothers often have to negotiate feeding practices with their partners. Mother-in laws and other family members are less influential forces in situations where a mother living with HIV has disclosed her status to a partner, these family members then become easier to manage when infant feeding practice diverge from expectations. Mothers practicing unfamiliar feeding behaviours navigate through family expectations and discord by using fabricated stories. These findings are in agreement with those of (Varga, Sherman, & Jones, 2006) who reported that 70% of mothers who disclosed their positive HIV status, disclosed to a partner or husband motivated by need for adequate infant care and avoidance of MTCT of HIV.

Initial doubt and suspicion from family members and service provider informants did not serve as a barrier to the acceptance of HTEBM as a viable infant feeding option, regardless of gender or age. Highlighted attributes of HTEBM were related to important key factors of safety, affordability and ease of preparation. Similarly, in a Zimbabwean study, it was reported that respondents from 13 focus groups, following education and discussion on HTEBM, were more accepting of the idea than at initial contact (Israel-Ballard et al., 2006).

Discussions with providers of child care services provided some important insight to challenges some families experience in securing a sustainable and safe source of formula, even when employed. Some mothers choosing to formula feed, despite earning an income, experience difficulty in procuring adequate amounts of infant formula, resulting inappropriate formula feeding which in turn contributes to infant morbidities witnessed by daycare centre staff. This alludes to the complexities of achieving AFASS conditions to enable safe formula feeding.

Portability of HTEBM was a less favourable concept to the mothers, particularly in settings like daycare centers. Additional explorations would be required in order to make the idea practical and safe for mothers and their infants.

Societal level factors: breastfeeding and feeding norms. Society level factors address the societal acceptance and expectations related to feeding practices including overall cultural attitudes, beliefs and practices. Breastfeeding continues to be a highly valued practice in communities such as the setting for this study, to the extent that mothers spoke of the dangers of discrimination and stigma associated with not breastfeeding. However, the concerns associated with not breastfeeding were not consistent, at some levels of community life, there seems to be an emerging trend of a decline in breastfeeding, an observation recounted by many of our informants (grandmothers, counselors, crèche staff, clinic staff). It was felt that the decrease in breastfeeding was mitigated largely by concern over MTCT of HIV. Our observations and discussions around the clinic and in the community indicated that in some instances, particularly with younger women, there was a trend towards more use of formula with breastfeeding abandoned in favor of what is perceived as a more modern mode of feeding, bottle feeding using formula.

The HIV epidemic is a priority public health issue in most of sub-Saharan Africa, however, there still remains a larger proportion of infants and children who are not HIV infected. If HTEBM helps to protect breastfeeding as the traditional healers and grandmothers believe, then the strategy has the potential to lessen a possible 'spill over' use of formula in the non-infected population. Prolonged breastfeeding in the non-

infected population protects children from morbidities and mortality from illnesses common in African settings (Jones et al., 2003).

Inter-relationship of individual, group and societal factors: Traditional healers and health care services. Interaction between group and society levels can impact the individual either in a positive manner or negatively. Utilization of polarized health care services, one at the level of greater society the other at community level, can be a disadvantage if the messages are inconsistent. Mothers do not limit their health care seeking behaviour to conventional health services, but also consult traditional healers, with both services identified as influential in their infant care practices. Traditional healers, although having their own beliefs and practices, not only seemed receptive to accepted feeding guidelines as well as use of HTEBM, but also identified the need for collaboration between traditional healers and health care services. Recognition for developing corporation between conventional and traditional medicine was a focus of a recent WHO meeting, where countries were encouraged to incorporate traditional health practitioners as partners in the health care system (WHO/AFRO, 2006).

Our data is limited in that it presents perspective from a single study setting, which is that of an urban, resource limited settlement. However, these findings clearly demonstrate ways that social and cultural factors interact with local environment to influence infant feeding practices, conditions that have been identified as important to explore in infant feeding work (Van Esterik, 2002).

5.6 Conclusion

Use of HTEBM at the complementary stage of feeding, when EBF would be discontinued was received as an acceptable feeding option by a broad sample of community informants within a South African setting, with a high HIV prevalence. Support and disclosure of HIV status were identified by mothers as important requisite conditions in facilitating the successfully practice of HTEBM. These findings have

implications for the planning of public health programs. Health care workers can enable the implementation of HTEBM during complementary feeding by providing consistent, clear information and education to build maternal knowledge, skills and confidence. Additionally, health workers can be trained to provide counseling and support to mothers living with HIV with the process of disclosure, potentially empowering mothers to successfully mange other aspects of their lives, other than infant feeding. Incorporating HTEBM into an infant's, diet following six months of EBF, provides a reliable food source to a developing infant while minimizing HIV transmission risk and has potential to preserve breastfeeding within the community. Findings of this work guided our study examining the feasibility of HTEBM use of mothers living with HIV from an urban settlement community.

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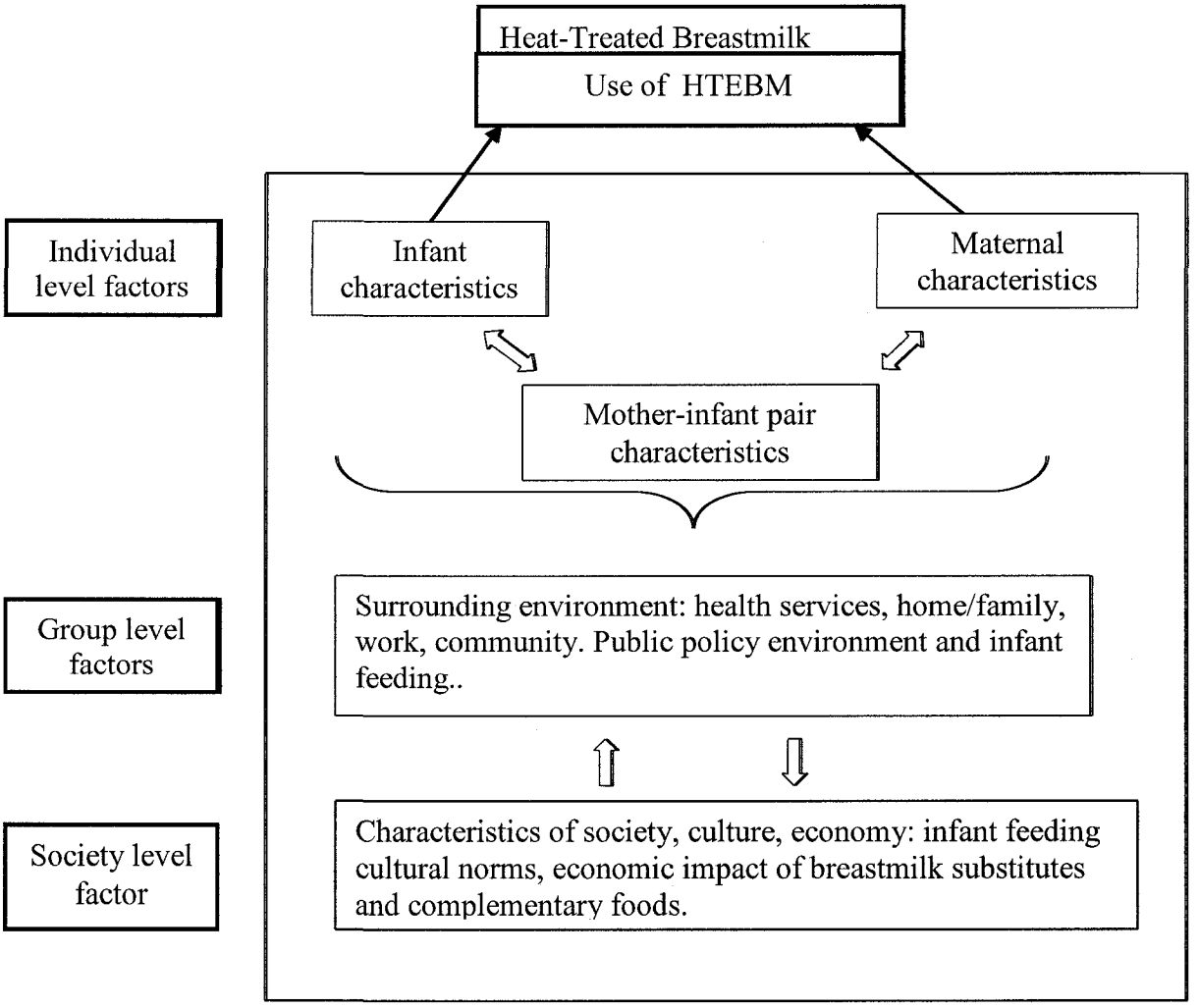
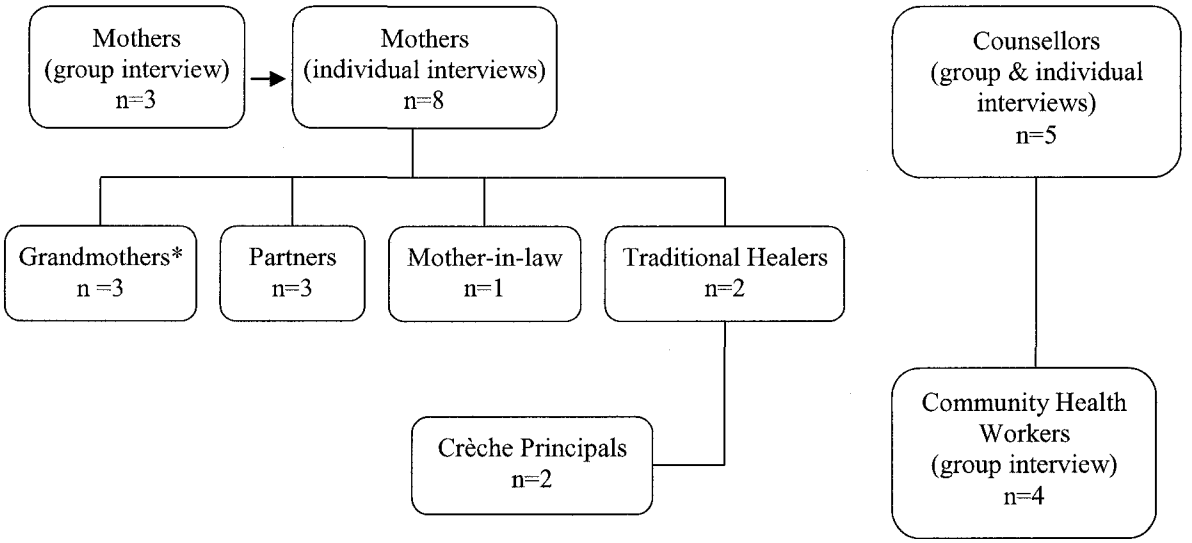


Adapted from Hector et al., 2005

Figure 5. 1 Conceptual framework outlining factors influencing infant feeding practices: heat-treated expressed breastmilk.



* mother's grandmother

Figure 5. 2. Sample selection using snowball method for recruiting participants (n=31). On the left, sample generated from mothers, on the right separate sampling from health system.

Table 5.1. Perceived advantages and challenges of using heat-treated expressed breast milk identified by mothers from an urban settlement.

Advantages	Challenges
• Destruction of virus	• Crying infant when breastfeeding is discontinued, especially at night
• Economic benefits	• Disclosure of HIV status
• Promotes breastfeeding	• Acceptance by partner, family or friends
• Health benefit of breastmilk	• Time commitment to express breastmilk
	• Expressing adequate quantities of breastmilk
	• Infant acceptance

LINK STATEMENT

The main aim of the qualitative study detailed in Chapter 5, was to assess the acceptability of HTEBM use by HIV-infected mothers from an urban resource poor settlement, in a province of South Africa with a high prevalence of HIV-1. The receptivity to using HTEBM was examined from three perspectives, the individual (mothers), the family (i.e., partners, grandmothers) and the community (i.e., traditional healers, clinic counselors). This form of inquiry acknowledges that successful promotion and implementation of a new feeding behaviour requires a contextual understanding of the mother's environment and the forces that influence her infant feeding beliefs, attitudes and practices. As a result, this study was comprised of in-depth interviews with mothers and a range of informants (n=31) from the community, identified by mothers as influential in their decision making process and maintenance of infant feeding practices. Findings from the study indicated that overall HTEBM was an acceptable feeding behaviour, providing the mother could effectively address potential challenges, such as stigma. Discrimination related to seropositive status was identified as a major concern, consequently voluntary disclosure of HIV status to a trusted individual, preferably a partner, would greatly facilitate the successful use of HTEBM.

An ethnographic study of some of the mothers would have added an informative layer to the understanding of maternal infant feeding practices taking into consideration the complex levels of interactions that take place within the household and the community. However, the in-depth interviews and observations did provide the information needed to inform the intervention introduced in this community.

Use of qualitative methods provided rich data, prompting further exploration of the participant mother's full spectrum of infant feeding experiences, including their encounters with health care workers and services, previous challenges and successes. Therefore the objective of the following study was to probe mothers further, gaining insight into the factors that would contribute to the success of the research intervention, the ultimate goal of this thesis research.

CHAPTER 6

MOTHERS' INFANT FEEDING EXPERIENCES:
constraints and supports for optimal feeding in a HIV impacted urban community in South Africa.

Under review at Public Health Nutrition
Signed co-author waiver in Appendix III

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Authors' contributions

Lindiwe Sibeko developed the study concept and study design. She conducted recruitment of participants, data collection, data analysis, data interpretation, and undertook the lead in the manuscript preparation. Dr. Anna Coutsoudis collaborated in the study conception and study design, and assisted in recruitment, data interpretation and manuscript preparation. S'phindile Nzuza conducted participant recruitment, data collection and participated in interpretation and manuscript preparation. Dr Katherine Gray-Donald participated in the study conception and development, study design, data interpretation, and manuscript preparation.

No conflict of interest is associated with this research study.

Running title: HIV and feeding experiences of mothers.

6.1 Abstract

Objective: There is an urgent need to better understand the enabling and challenging factors impacting on infant feeding practices in communities with a high HIV prevalence.

Design: Qualitative study employing the snowball method of sampling with data collected through analysis of in-depth interviews and observations of mothers and other participants.

Setting: Urban settlement in the province of KwaZulu-Natal, South Africa.

Participants: A total of 31 participants, consisting of 11 mothers plus male partners, grandmothers, mothers'-in-law, community and clinic counselors, traditional healers and health care and daycare centre staff.

Findings: Emerging from discussions with mothers were the challenges they faced in feeding their infants, encompassing three areas: stigma and disclosure of HIV; confusion and coercion; diarrhea, sickness and free formula. Mothers rarely received quality infant feeding counseling and consequently mixed feeding, a high risk for HIV transmission, was a common feeding practice. In this resource poor setting, the optimal feeding practice of exclusive breastfeeding (EBF) was best practiced with support and following disclosure of HIV. Availability of free formula did not guarantee exclusive formula feeding (EFF) but instead facilitated inappropriate feeding practices and may have contributed to increased infant morbidities.

Recommendations: There is a need for health professionals to recognize the importance of disclosure of HIV status, and facilitate optimal infant feeding by providing accurate information and support to mothers around EBF or EFF, depending on their situation.

Keywords: HIV, infant feeding practices, exclusive breastfeeding, stigma and disclosure, qualitative data, South Africa.

6.2 Introduction

The African continent is home to approximately 25 million adults and children living with HIV/AIDS, substantially burdening an already marginalized region of the world to bear the brunt of the global HIV epidemic. Furthermore, by the end of 2006, pediatric infections accounted for an estimated 2.3 million of the world's children living with HIV, 90% of whom are infected through mother-to-child transmission (MTCT) (UNAIDS, 2006).

Non-exclusive breastfeeding is associated with the infection of about 300,000 children annually, (De Cock, Fowler et al. 2000) however, breastfeeding in resource poor countries is a powerful child survival tool with the potential of preventing an estimated 13% of child deaths (Jones et al., 2003). Evidence now exists illustrating the role of exclusive breastfeeding in minimizing transmission risk of HIV while promoting child health, resulting in greater HIV-free child survival (Coutsoudis et al. 2001; Iliff et al. 2005; Newell, 2006). Recently, an African based prospective study with feeding data on 1276 infants, found a low transmission risk (4%) following 6 months of exclusive breastfeeding (EBF), with mortality at 3 months more than double in formula fed babies compared to EBF infants (Coovadia et al., 2007).

Although formula is the recommended infant feeding option for HIV positive mothers in developed countries (WHO/UNAIDS/UNICEF, 1998), this is rarely feasible or a preferred choice for women living in resource-poor communities due to the prohibitive cost of formula, lack of safe water, infrastructure to ensure consistent formula availability, socio-cultural values and the stigma associated with formula feeding. Hence, under conditions where replacement feedings are not acceptable, feasible, affordable, safe or sustainable (AFASS), UNAIDS/UNICEF/WHO guidelines encourage mothers living with HIV to breastfeed for 6 months at which point breastfeeding cessation is only recommended if a replacement milk compliant to AFASS conditions is available, to be combined with complementary foods (WHO, 2006). Operational interpretation of these guidelines requires counseling of mothers by trained health care staff.

Lack of disclosure of HIV status can serve as a significant impediment to maternal implementation of these optimal infant feeding guidelines. Despite the fear of discrimination associated with disclosure of HIV status, mothers living with HIV who disclose their status have been found to experience positive outcomes (Rutenberg, 2003). Varga and colleagues (Varga et al., 2006), challenge the dualistic approach to disclosure, where an individual either discloses or refrains. Instead they view disclosure as a dynamic process (Table 6.1) which can manifest in four different forms on the basis of voluntary or involuntary disclosure, and direct or indirect disclosure (disclosure by actions).

Translation and implementation of infant feeding policy guidelines is of great public health importance, hence gaining insight into the real experiences and challenges of mothers who are recipients of infant feeding information is crucial feedback for program development.

Here we report on data that emerged from a qualitative study aimed primarily at examining the receptivity of mothers and other community members to the use of heat-treated expressed breastmilk as a alternate feeding (Sibeko, Coutsoudis, Nzuza, & Gray-Donald, 2007a). However, data from the study also provided a rich representation of mother's perspectives on ways in which disclosure is tightly intertwined with a variety of issues and experiences related to infant feeding and how collectively these factors impact on feeding choices and practices. These factors are the focus of this paper.

6.3 Methods

This study is set in an urban informal settlement, in the province of KwaZulu-Natal, South Africa. Kwazulu-Natal has the highest antenatal HIV burden in the country, with current prevalence estimated at 39.1% (Department of Health, 2007). It is a setting in which AFASS conditions rarely exist and, hence, safe breastfeeding practices are particularly important.

Participant recruitment and interviews took place from October 2004 to March 2005, conducted by a trained community research partner, in collaboration with the researcher (LS). Our sample of mothers were purposefully selected, recruitment took place both at the local clinic as well as within the community. We were interested in interviewing mothers with young infants given that infant feeding would still be an integral part of their lives. Serostatus was not a selection criteria for study participants.

Once the purpose of the study was explained and consent given, in-depth interviews were conducted in isi-Zulu utilizing Patton's (Patton, 2002) general interview guide approach which advocates an open ended conversational interview format. Mean age of participating mothers (n=11) was 25 years with average age of index infant of 3 months. Using a snowball approach, mothers directed us to whom they felt we needed to speak to within their world of influence. Hence, our total sample of 31 participants included family members such as partners, grandmothers and mothers-in-law, with additional participants drawn from service providers in the community and within the clinic (community health care workers, counselors, traditional healers and child care professionals). Sample size was based on reaching the point of saturation, where no new emergent issues were identified with additional participant interviews.

Data collection was achieved through tape recording of in-depth interviews plus notes made during and shortly following interviews. All interviews were transcribed and then translated into English by the community research partner and researcher. The technique used for data analysis is described in detail elsewhere (Sibeko et al., 2007a). Briefly, text data from transcripts and notes were evaluated using the content analysis method (Cavanagh, 1997), whereby codes were developed based on repeat concepts and keywords identified from the text. Codes were categorized and eventually streamlined into thematic codes. The process was conducted independently by the researcher and research assistant and agreement reached through discussions. Confirmation of interpretation of data was achieved through member checks with mothers, clinic and community participants, where possible.

Notes resulting from observations and informal conversations within the community and at the clinic were used as a source of additional data to help validate our findings and to establish trustworthiness of data (Lincoln & Guba, 1985).

Precise study location is not specified to maintain anonymity of those participating in the study. Ethics approval was obtained from the University of KwaZulu-Natal Research Ethics Board and the McGill University Institutional Review Board.

6.4 Results

Although seropositive status of the mothers was not required for participation in this study, the majority did identify themselves as HIV-infected. The feeding practices of the 11 mothers were comprised of 4 formula feeders, 5 mixed feeders and 2 mothers who exclusively breastfed. Of the mothers who chose to formula feed their infants, only one was able to practice exclusive formula feeding (EFF) of her 3 month old infant. Three weeks to 2 months was the range of duration of EFF for the other 3 mothers using formula. Factors enabling the only mother who practiced EFF included being employed (informal work sector), use of the free formula program and disclosure of her HIV status to her partner. None of the other three mothers mentioned disclosure of HIV status, but they did report a reliance on assistance from family in addition to the free formula program as their sources for formula. Regardless of sources, these mothers reported formula supply to be inconsistent, which facilitated inappropriate feeding (use of cow's milk or other fluids) including over dilution of formula.

The process to access the free formula for a six month period from the clinic (based on our observations and conversations with staff and clients) required women living with HIV to self-identify themselves at the clinic, where mothers were then placed on a list entitling them to a monthly supply of powdered formula. Free formula is not available immediately and therefore mothers initially require an alternate source of formula

allotment, until they had access to the free supply (waiting period could be as long as a month).

Mothers who were mix breastfeeding added other fluids and or small quantities of foods to their breastfeeds. Asked why they fed in this manner, two reported being advised to do so by family members while another indicated she mixed fed to promote infant satiety and night sleeping. The remaining two mothers reported being directed to formula feed following delivery at the local hospital because of their positive HIV status. However, both mothers began mixed feeding following discharge from hospital: the first mother added breastfeeding to the formula feeds, motivated by her partner and her own desire to breastfeed. The second mother reported not having access to formula, she instead breastfed and added diluted solids, a practice based on past experiences and her perception that breastmilk was insufficient to satisfy her newborn.

Only two mothers were practicing EBF, one of the mothers described the events leading to her breastfeeding exclusively:

> "I saw that my baby was not full from my milk when I got home from the hospital. So I went out and got a small tin of milk [formula] and gripe water, because he was crying at night. But when I took the baby to the clinic, they told me I cannot mix feed. They explained that the baby is satisfied with my breastmilk. So I continued just breastfeeding and I can see I have enough milk and my baby is ok. My partner is ok with me only breastfeeding because he knows my problem [seropositive status]. People try to feed the baby, they shout at me and ask me why I only breastfeed? I told them if they give food to my baby, the baby will be sick. I am not going to visit my family in the farm (rural area) until my baby is 6 months, at that time the baby will be eating food".

The second mother had been breastfeeding her four month old exclusively since birth. Her initial motivation to EBF was as a result of viewing a breastfeeding promotion video at the antenatal clinic (ANC). This mother lived with her own mother and grandmother

who were both very supportive of EBF, with the grandmother informing us she cup fed expressed breastmilk made available by the infant's mother on occasions she was not home. Both relatives attributed their support of these uncommon feeding practices to their awareness of the mother's positive serostatus, her sharing of advice and information given by counselors at the clinic as well as their witnessing healthy growth of the infant.

Stigma and disclosure of HIV status:

In their discussions of feeding practices, most mothers singled out stigma and the challenge of disclosure as significant considerations in their infant feeding, decision making process. It was clear from our discussions that fear of reprisal from family members or friends as well as from the community at large, can serve as a barrier for mothers to disclose their HIV status. Mothers consistently informed us that in many instances lack of disclosure and the concomitant lack of support, may lead some women to follow practices they do not necessarily agree with. One mother pointed out: "If you have a partner who doesn't know your status it's a big problem with breastfeeding for a mother. The father wants to buy formula because he thinks our baby needs tin milk and breastfeeding [to be well fed], which the counselor said I should not do because my milk is enough..... I know, but what can I do?"

This climate of HIV stigma and discrimination at times contributed to some mothers being fearful of exposure when they picked up their formula supply from public spaces, such as a clinic. These concerns were not isolated to external environments but would also influence dynamics within the home, whereby a formula feeding mother might hide the infant feeding bottle from specific individuals, giving instead the impression she was a breast feeder. As was the experience of one mother: "I fed the baby before she [relative] came and then when we sat and talked I put the baby near my breast, so that she thinks I breastfeed the baby".

Mothers who had disclosed their HIV status were less concerned with stigma and often felt they had support to feed their infants according to their intentions.

Confusion and coercion:

Emerging from our conversations was the confusion and anxiety, infant feeding in the context of HIV, elicited from most study participants, particularly mothers. Feeding newborns presents mothers with much conflict that relates to both infant and maternal focused concerns. On the one hand, if a mother wishes to breastfeed, she grapples with her fear of infecting her infant, conversely, were she to choose to formula feed she runs the risk of arousing suspicion regarding her HIV status; additionally, in many instances, use of formula, particularly in early infancy, is often in disagreement with a partner or family member's feeding expectations:

> Mother: "but now I did not know what to do because I did not want to give this sickness [HIV] to my child, but my mother and the father [of the child] would ask me why I was not breastfeeding this child".

Most mothers did not recall receiving informative infant feeding counseling prior to the birth of their child, instead their experiences consisted of being recipients of inconsistent and confusing advice and direction from various health care workers at the delivery of their newborn. Some mothers shared their experiences of being directed by hospital staff to formula feed with breastfeeding strongly discouraged. This directive was given in the absence of an assessment of the mother's ability to feed formula safely. Mothers reported being cautioned by staff that to breastfeed was equivalent to transmitting the virus to their infants, which they were told was unnecessary since the government provided free formula for use by infected mothers. None of the mothers advised to formula feed recalled hospital staff providing counseling on how to use formula appropriately (i.e. exclusive formula feeding, not adding solids and food to infant's diet under 6 month of age, appropriate formula preparation).

This coercion to formula feed served as a source of confusion for some mothers, since the directive was in conflict with their understanding that breastfeeding remained a viable option for HIV infected mothers:

> Mother: "the nurse told me that those who say I can breastfeed are playing with me, she said I will infect my child using my own milk. I should be a good mother and use the baby tin milk [formula]"

Some of the mothers directed to use the free formula program were quick to point out that the program presented its own problems, the primary one being inconsistency in the availability of formula:

> Mother: "I was afraid to breastfeed because of other things.... this sickness [HIV]; but the tin milk cost too much and the nurses at the clinic sometimes tell us they have no milk to give us"

This mother's reaction to unavailability of the free formula was to engage in inappropriate feeding of her young infant, using sugar water plus solid food, until the clinic received the formula supply.

Coercion to use formula was not limited to health care workers, but also came from other sources:

> Counselor: "Sometimes girls do not breastfeed because her partner says that their child only drinks baby milk [formula]. But sometimes the partner is not around, so she borrows milk to feed the baby until the father comes back. But what happens if the father disappears...then you cannot pay that baby milk back, you have a hungry crying baby with no food and the father who is long gone*"

Given the level of pressure some infected mothers received to use formula, we were interested in experiences of women who were able to resist yielding to this pressure.

These were women who still initiated breastfeeding despite being counseled to formula feed, mostly motivated by a fear of scrutiny from family members and friends, as a result of not breastfeeding. Health benefits and affordability were secondary considerations in choosing to breastfeed: Mother: "babies feeding from the breast grow well and look big and healthy...but really, nobody asks funny questions when you breastfeed". These were also the mothers who ended up mixed feeding. The two mothers practicing EBF, although both felt pressured in the hospital to use formula, reported a strong commitment to their prenatal decision to EBF (even though one mother used formula briefly due to infant satiety concerns) and felt supported by family to do so.

* A partner that disappears is of concern since some mothers are dependent on their partners for financial support.

Diarrhea, sickness and free formula:

Infant centered concerns focused on morbidity issues, emerged as an area readily discussed by almost all participants interviewed. Diarrhea was the most commonly cited child illness of concern:

> Mother: "now that I am not breastfeeding I am worried; my other babies had my milk and there was no diarrhea, not like my sister-in-law , she works and she buys tin milk for her children and they had a lot of diarrhea...I know, I used to see them".

Both counselors and community health workers reported that they had observed an increased presentation at the clinic of children suffering from malnutrition, failure to thrive, diarrhea, and dehydration. They felt that the increase in these morbidities occurred shortly after the availability of free formula. Prior to the introduction of the program there was a stronger commitment from both health care workers and mothers to breastfeeding and as a result there were more women breastfeeding in the community and in their

opinion less of these childhood morbidities. This observation was confirmed through discussions with other health workers at the community clinic:

> Health care worker: "first we were happy with the free formula because we thought our problems with the infected mothers were over.....but now we see it is trouble, really it is a lot of trouble for the baby...diarrhea, sickness and not growing well".

Counselors also spoke of the increased frequency of mothers presenting at the clinic with a sick infant as a result of being fed a sugar-water solution for an extended period due to the mother running out of her formula allotment or there being no formula available for pick-up at the clinic. One counselor recounted her experience of working with a mother who had fed a sugar water solution to her infant in excess of a month, as a result of not having formula in the house. The child presented with diarrhea and was severely dehydrated, however, the mother thought she was doing the right thing since she observed that at the hospital infants are routinely fed sugar water. This was not an isolated incident, since health workers, staff at daycare centers and traditional healers all relayed similar encounters with mothers who had no access to appropriate breastmilk substitutes.

One mother provided a poignant example of the complexities of access to free formula for families with competing needs: "many mothers sell the tin milk from the clinic..... because you have to have food in the house".

6.5 Discussion

Lack of disclosure of HIV status as a result of existing stigma and discrimination associated with HIV seropositive status emerged as an important consideration in the infant feeding, decision making process. In formulating infant feeding decisions, women enter a risk benefit type of decision making dilemma, caught between a desire to feed

their infant in a manner that optimizes infant health while respecting feeding expectations of partners and family members. These factors interact against a backdrop of cultural norms that value breastfeeding. Hence, although most infant feeding strategies render women the bearers of responsibility for feeding their children, practices are rarely the sole decision of the mothers.

Our findings indicate that seropositve status adds an additional level of complexity to the issue of infant feeding practices. In the context of HIV, mothers identified two factors that facilitate optimal feeding; the first involves sharing what is perceived as the risk (i.e possible infection of an infant and or exposure to stigma) associated with a selected feeding practice with a support person, often a partner, family members or friend. The second factor being direct and voluntary disclosure of one's HIV status, preferably to the targeted support individual. Conceptually, as illustrated in Figure.6.1, both factors are interrelated since the act of disclosing facilitates a supportive connection which enables women to either EBF or EFF, providing AFASS conditions exist for formula feeding. In contrast, the framework also illustrates that free formula access, although at a policy level may seem theoretically to be an enabling factor, in reality our discussions indicate the program is a constraint, due essentially to issues of sustainability.

Our findings confirm that women who had undergone voluntary and direct disclosure of their HIV status to a partner or family member were the only women who fed their infants exclusively. In contrast for some mothers who had not disclosed their status, mixed feeding was the common practice according to their choice. Lack of disclosure seems to compromise infant health via poor feeding practices, increasing a child's risk of morbidity. Furthermore, the risk of indirect and involuntary disclosure meant women were often careful that their actions (i.e., exclusive formula feeding) did not imply positive HIV status. Moreover, mothers who had not disclosed their status engaged in creative explanations of their feeding behaviours to avoid disclosure by proxy. This concurs with other findings where it has been shown women who volunteer to disclose their HIV status, experienced significantly less negative consequences than those who

involuntarily disclosed. Also, the women who disclosed their HIV status, did so motivated by need for support of their feeding choices and practices (Varga et al., 2006).

The promotion of safe breastfeeding practices, such as EBF for the first six months of an infant's life, is the underpinning of strategies aimed at the simultaneous lowering of the transmission risk of HIV while maximizing infant survival. International infant feeding guidelines reflect this public health objective for resource poor settings, where inability to secure AFASS precludes use of replacement feedings such as formula (WHO, 2006). Settings which have discouraged breastfeeding by African mothers living with HIV have taken an approach that ignores the complex interrelationship of emotional, social, cultural and financial constraints faced by women in most of Africa, where two thirds of the world's HIV infected live. Within our study setting, where resource limited living conditions prevail and HIV prevalence is high, infant feeding practices of mothers were contrary to current policy recommendations. The nature of encounters mothers experienced with health care workers served as a key contributing factor to the existing polarization between recommendations and actual practices. A disturbing finding was the coercion mothers encountered from hospital staff following delivery, where mothers were directed to use formula as a means of avoiding vertical transmission of HIV. In these situations, no consideration was given to the personal desires or living conditions of the mother. Previously, we reported similar experiences at a large South African hospital, where 18% of mothers using formula, did so as a result of being directed by staff to not breastfeed, based solely on their HIV status, again, without any contextual assessment and discussion of personal circumstances and their abilities to practice formula feeding safely and consistently (Sibeko et al., 2005).

Inappropriate advice from frontline workers created much confusion for mothers we spoke to, consequently placing infants at risk of illness and death sue to suboptimal feeding. Indeed, we found that most mothers fed their infants inappropriately and more importantly, mothers who were not counseled properly seemed to be unaware of the dangers associated with mixed feeding, due to increased transmission risk. Mixed feeding has been consistently shown to be a highly risky feeding pattern for infants born to HIV

infected mothers (Coutsoudis et al., 1999; Iliff et al., 2005), and most recently was found to double the risk of infection of infants fed formula plus breastmilk compared to their EBF counterparts. The risk of HIV transmission increased 11 fold when solids were added to breastfeeding, in this landmark prospective study (Coovadia et al., 2007). Researchers (Doherty et al., 2007) also found that UNAIDS/WHO/UNICEF infant feeding guidelines were poorly implemented in their cohort study across three sites in South Africa. Women receiving inaccurate infant feeding counseling had inappropriate feeding practices, these were also found to be the mothers with the greatest transmission risk of infecting their infants.

Infant health emerged as an issue of great concern among most participants, both from a HIV infection risk perspective but also related to diarrhea and malnutrition morbidities, which were thought to be on the rise in the community. In most disadvantaged settings in South Africa, such as informal settlements, infant health has been found to be deteriorating, with malnutrition and diarrhea identified as highly prevalent and significant risk factors for child mortality. Food insecurity and poor living conditions are identified as underlying contributing factors to these child morbidities. Promotion, protection and support of breastfeeding continues to be the suggested strategy to address these morbidity and mortality risks, even in the era of HIV (Bourne et al., 2007).

In our resource poor study setting, breastmilk, a secure infant food, seemed to be under threat of declining according to mothers and other informants, including health services workers, who also attributed the increased childhood morbidities to decreased breastfeeding rates. The decline in breastfeeding was believed to be a consequence of the availability of a free formula program. Recipients of the free formula spoke frankly of the lack of consistent availability and the insufficiency of the supplied allotment. Our findings indicate that lack of a sustainable supply of formula often lead to poor feeding practices by mothers unable to secure alternate sources of formula. There is no evidence to support a free formula policy for mothers living with HIV within an African settings, since such an undertaking ignores the hidden costs associated with appropriate formula feeding while promoting mixed feeding, amongst other hazards (Coutsoudis et al., 2002;

Coovadia et al., 2007). Recent events in Botswana, a well resourced country, illustrate the unacceptable risks associated with free formula available to HIV infected women as per government policy (Creek et al., 2006). Government policies providing free formula to HIV infected women do not comply with international guidelines, since these policies ignore the requirements of AFASS conditions. Increased presence of formula serves as a risk for 'spillage' of formula use into the non-infected or unknown HIV status populations. As a result there can be a significantly higher risk of illness and death of infants and young children in these communities, as was evidenced in the Botswana case, where even the HIV uninfected and unexposed infants experienced greater diarrhoeal related morbidity and mortality.

The study was limited by its size, 11 mothers and 20 family and community members. However sampling was stopped because of similarity of information provided by participants. Studying one community serves as an additional limitation, although the community was felt to be representative of settings with a very high prevalence of HIV infection and similar to South African settings with limited economic resources. As community trust was essential to establish in order to be able to undertake this qualitative study our focus was placed on one community.

6.6 Conclusion

Mothers bear the responsibility of feeding infants, but decisions on practices are often not made in isolation. Hence, infant feeding strategies can be viewed as risky and divergent from cultural and social norms for these women. However, this challenge does not preclude the promotion of safe breastfeeding practices since support and voluntary and direct disclosure of HIV status greatly facilitates the uptake of optimal feeding practices. Mothers who disclosed their status and secured support were able to EBF, a recommended practice for improving HIV-free infant survival. While on the other hand, inappropriate infant feeding advice resulted in creating confusion and promoting suboptimal practices such as mixed feeding, a HIV transmission risk. Therefore, there is

an urgent need for health care services to invest in on going high quality training programs that provide support for health workers to counsel mothers in a manner that enables evidence-based optimal infant feeding. Additionally, training should include facilitation of HIV status disclosure.

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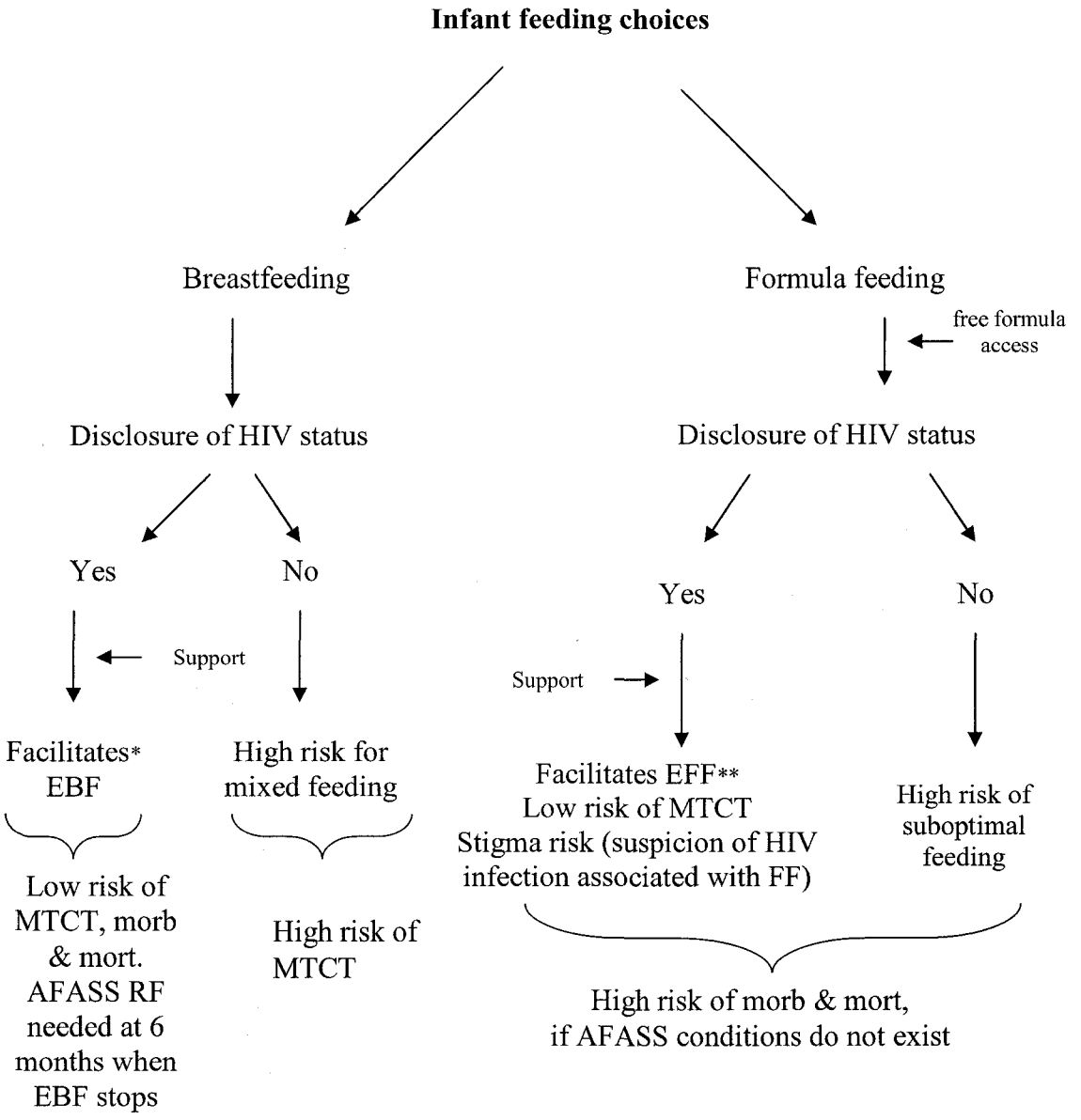

EBF= exclusive breastfeeding, EFF= exclusive formula feeding, FF= formula feeding, MTCT= mother to child transmission, AFASS= affordable, feasible, acceptable, safe and sustainable, RF= replacement feeding , morb= infant morbidity(i.e. diarrhoea, malnutrition), mort =mortality.

* optimal breastfeeding, recommended for settings without AFASS conditions
** optimal formula feeding, not recommended for settings without AFASS conditions

Figure 6. 1. A conceptual framework of the influence disclosure of HIV status exerts on infant feeding practices, highlighting the risks associated with poor feeding practices and the benefits related to optimal feeding.

Table 6.1. Characteristics and type of HIV status disclosure identified by motivation and approach.

	Type of disclosure	Disclosure characteristics
Intention of disclosure	Voluntary	decision to reveal HIV status without coercion
	Involuntary	HIV status is divulged unintentionally or without consent
Disclosure approach	Direct	straightforward approach to revealing status
	Proxy	HIV status is implied through action or association, not openly revealed

Adapted from Varga et al. 2005

LINK STATEMENT

Chapter 6 is comprised of a manuscript that elaborates on the infant feeding practices of mothers from a South African urban community, affected by a high HIV prevalence of HIV. These practices were clearly divergent from WHO/UNICEF/UNIAIDS guidelines, with mixed feeding being common. A barrier to optimal feeding practices was the interaction mothers often experienced with health care workers, where child feeding advice was delivered in a coercive manner, with formula strongly advocated. Mixed feeding, a high risk for transmission, was the inferred consequences of poor quality counseling. Mothers who had practiced EBF attributed voluntary disclosure of HIV status plus support as the key enabling factors. A final emergent issue was the free formula program available at the community clinic. Mothers perceived the free formula as a barrier to HTEBM, despite the inconsistent availability of the formula. Data from this study validated the importance of offering mothers quality counseling and support and highlighted the need for mothers to be empowered and supported to disclose their HIV status.

Collectively, the data from the acceptability work presented critical information on factors to consider and be prepared to address with the mothers choosing to participate in the feeding intervention to be piloted in the feasibility study. Having elucidated the receptivity of HTEBM within the community, the follow through action required an examination of how viable a feeding option of HTEBM could be. Hence, the purpose of following study was to investigate the feasibility of HIV infected mothers being able to use HTEBM as a component of complementary feeding. Utilizing the outcomes of the past two studies as guides, the study intervention was implemented with participants recruited during pregnancy in the later stage of their third trimester and followed from that point through six months of EBF, breastfeeding cessation at 6 months, at which point complementary feeding plus HTEBM was used. The intervention was planned to reflect current WHO recommendations, which encourage HIV-infected women EBF for 6 months. Use of HTEBM (also a WHO recommended feeding alternative), provides added nutrients and energy to the infants complementary diet, thereby promoting child health

CHAPTER 7

HEAT-TREATED EXPRESSED BREASTMILK IS A FEASIBLE FEEDING OPTION FOR SOUTH AFRICAN MOTHERS LIVING WITH HIV: A MIXED METHODS APPROACH.

To be submitted to JAIDS
Signed co-author waiver in Appendix III

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7.1 Abstract

Objective: The aim of this study was to investigate the feasibility of HIV-1 infected, urban South African mothers to exclusively breastfeed (EBF) their infants for 6 months, followed by using heat-treated expressed breastmilk (HTEBM) as a replacement feeding in conjunction with complementary foods. The study also seeks to understand the lived experience of women implementing this infant feeding strategy.

Methods: An intervention employing mixed methods, with follow up of breastfeeding mothers from the prenatal through the postpartum period for the duration of the feeding intervention.

Results: Of the 66 HIV infected participating mothers, 55 % were able to EBF for six months, and of the mothers practicing EBF, 42% used HTEBM as a replacement feeding during complementary feeding. Breastmilk expressed on a daily basis ranged from 65ml to 600 ml with the longest duration of HTEBM being 5 months. Engorgement or mastitis as a result of breastfeeding cessation was not reported or observed. Enabling factors for HTEBM use included support received through home visits and disclosure of HIV status, particularly to a partner. Cereal based foods were over represented in the infant complementary diet, with little variety in fruits and vegetables. Household finances seemed to dictate type of protein foods and overall diversity of diet.

Conclusion: Our findings demonstrate the feasibility of both EBF and HTEBM, both WHO feeding recommendations that can be implemented with support for breastfeeding mothers living with HIV. These data provide important information for resource poor areas, where safe sustainable feeding options are desperately needed for use by HIV infected women.

Keywords: heat-treated expressed breastmilk, flash-heating, feasibility, exclusive breastfeeding, mixed methods.

7.2 Introduction

In sub-Saharan Africa, the HIV/AIDS epidemic has become a burden carried mostly by women and children. Sixty one percent of the 22.5 million Africans living with HIV/AIDS being women, most in their reproductive years (UNAIDS/WHO, 2007). Each year, an estimated 700,000 of the world's children are infected with HIV, 90% occur through mother-to-child transmission (MTCT) of HIV. African children comprise the majority (90%) of these pediatric infections (UNAIDS/WHO, 2005).

South Africa, with one of the most severe HIV epidemics in the world, has 5.5 million people living with HIV/AIDS, the majority being women in their childbearing years. Nationally, the antenatal HIV infection prevalence is estimated at 29.1%, the highest rate (39.1%) being in the province of KwaZulu-Natal (KZN), consequently MTCT rates are high in KZN with an estimated 25,000 children infected annually (Department of Health, 2007).

In Africa, where breastfeeding is universal, prolonged breastfeeding is commonly practiced, but in the era of HIV it has been estimated to contribute up to 350,000 HIV infections of children a year (De Cock et al., 2000). Logically, prevention of HIV transmission would dictate the avoidance of breastfeeding, which in the developed world is possible. However, in developing regions of the world, exclusive breastfeeding (EBF) for the first 6 months of life is estimated to save the lives of approximately 6 million children a year, children who might otherwise die from common childhood infections (UNICEF, 2007).

Coutsoudis and colleagues (Coutsoudis et al. 1999; Coutsoudis et al. 2001) provided the initial evidence of an association between EBF and lower transmission risk of HIV. Additional evidence has confirmed these findings (Iliff et al., 2005), with the most recent risk estimate of 4% transmission for 6 months of EBF. Additionally, breastfeeding in addition to formula plus solid foods was found to increase the risk of infection 11 fold (Coovadia et al., 2007). Consequently, based on the mounting evidence, WHO guidelines

recommend HIV infected mothers EBF for six months unless they have access to a replacement feeding that is acceptable, feasible, affordable, sustainable and safe (AFASS) (WHO, 2006). These guidelines further caution mothers, that unless they are able to secure a replacement feeding that meets AFASS conditions, breastfeeding should continue beyond 6 months, in addition to complementary foods (WHO, 2006). These current guidelines reflect the new focus on infant feeding having the dual purpose of minimizing transmission risk while enhancing infant survival.

In developing areas of the world, breastmilk is a secure and important food for infants, and even after 6 months of age it accounts for approximately 50% of the infant's nutrient intake, thus making it a difficult food source to replace (Dewey & Brown, 2003). Finding a safe method of incorporating breastmilk as a component of the infant diet during complementary feeding thus becomes of great public health importance. Moreover, a modified breastfeeding strategy that minimizes the cumulative risk of HIV transmission via EBF cessation at 6 months, must include an affordable, safe and sustainable replacement feeding that can be combined with the complementary diet.

Heat-treatment of expressed breastmilk (HTEBM) has been identified by WHO, UNICEF and UNAIDS as a viable replacement feeding (WHO/UNICEF/UNAIDS/UNFPA, 2004). Flash-heat, a user-friendly in home pasteurization method has been shown to successfully inactivate HIV (Israel-Ballard et al., 2005; Israel-Ballard et al., 2007), as well as eliminate pathogenic and non-pathogenic microbes, allowing for safe storage at room temperature up to 8 hours, without significant increase of bacteria (Israel-Ballard et al., 2006). This is a critical feature for settings where access to refrigeration is limited.

Previously, we reported the acceptability of HTEBM within a South African setting (Sibeko et al., 2007a) similarly, acceptance was also demonstrated in a Zimbabwean (Israel-Ballard et al., 2006) based study. Despite the receptivity to this feeding practice, the feasibility of HTEBM use has never been fully studied, with no practical or programmatic experiences of HTEBM use documented.

The study employs a mixed methods approach, using a methodological triangulation of qualitative and quantitative methods from a simultaneous triangulation perspective (Morse, 1991). Simultaneous triangulation operates on the premise that quantitative and qualitative inquiries can be answered at the same time in a single study. Hence, the purpose of this intervention study is twofold. It is primarily to investigate, how feasible it is for South African mothers living with HIV-1 to EBF for 6 months, following which they use HTEBM, as a replacement feeding and integral component of the complementary infant diet (quantitative). The second aim of this research is to understand the lived experience of women implementing this infant feeding strategy (qualitative).

7.3 Methods

This research took place in an urban community in the province of KwaZulu-Natal, (from February 2005 to June 2007). The study was developed as a pilot project, to inform larger studies that can be powered to investigate infant and maternal health outcomes.

Pregnant women with known seropositive HIV status were recruited in the prenatal period (gestation age $\geq$ 34 weeks), during their antenatal clinic (ANC) visit at the community primary health clinic. Trained clinic counselors provided counseling for the women, which included infant feeding choices. Mothers who chose to breastfeed were then approached to participate in the study. Participants entered the study once informed signed consent was obtained. Unexpectedly, a few mothers from the community who had already delivered and were exclusively breastfeeding and were interested in trying HTEBM, entered the study at this point (see Figure 7.1). Inclusion criteria involved women with confirmed HIV status, living in the targeted community, intending to breastfeed their infant and indicating they will be in the community for a minimum of a year.

In order to minimize MTCT transmission during the intrapartum period, women were given nevirapine prophylaxis during the ANC visit, to be taken during labour, with their infant's prophylaxis dose administered at the hospital, as per protocol.

Each woman recruited in their final trimester was visited once during the prenatal period, to confirm the exact location of their residence as well as to review and reinforce exclusive breastfeeding. Communication with the participants was in isiZulu.
Participant mothers were registered in the HIV treatment and care program in the clinic, which provided relevant services for themselves and household members as needed.

Study intervention

The study's peer breastfeeding counselor (SN) was trained by the researcher (LS), a certified lactation consultant and dietitian, through a 16 hour breastfeeding training course developed by LS and covering a wide range of topics relevant to breastfeeding counseling, including heat-treatment and complementary feeding.

Mothers participating in the study were informed of the safe breastfeeding infant feeding strategy being used, which involved exclusive breastfeeding for six months with breastfeeding cessation taking place when the infant was 6 months of age. At this point HTEBM was used as a replacement feeding in conjunction to complementary foods.
Mothers were also encouraged to use condoms where applicable, the risk of increased viral load as a result of postnatal infection was explained.

Exclusive breastfeeding:

Exclusive breastfeeding was defined as breast milk only, no solid foods or liquids. In this study, operationally, mothers were still considered to be EBF if there was one episode of their infant receiving a fluid, but then returned to EBF. Any addition of solids to the infant's intake, even one episode, a mother would no longer be categorized as practicing EBF. At each visit mothers were asked how they breastfed their infant in the past 24 hours, probing for foods and liquids that may have been used, following which mother's were categorized on whether they are still EBF or not.

Flash-heat of expressed breastmilk procedure:

A full description of the flash-heating of expressed breastmilk method is described elsewhere (Israel-Ballard et al., 2005). As adapted for home use the procedure involves the expressing of breast milk into a clean glass jar (i.e. peanut butter or jam jar). For the purpose of the study all participants were issued a commercial glass jar. The jar is then placed in a small aluminum pan (which most households have) with the water level in the pan measured to 2 finger levels above the quantity of expressed breastmilk (a pictorial resource is available detailing the procedure). Thus the pan forms a water bath for the expressed milk in the jar. The pan is then placed on a hot flame of the cooking equipment used in the home (most homes in the community use kerosene stove). Water and milk are heated together until the water reaches 100°C and there is a rolling boil. At this point the jar with breast milk is immediately removed from the pan, capped with a lid and allowed to cool. Flash-heated breastmilk typically reaches temperatures above 56.0°C for 6 min 15 sec and peaks at 72.9°C, temperature where the virus is deactivated (Israel-Ballard et al., 2007). Amounts of breastmilk being expressed were recorded for the duration that mothers practiced HTEBM, using visual measures mothers were trained on.

Complementary foods:

Mothers were counseled on the importance of complementary foods and the appropriate local foods to use. To minimize respondent burden, data collection on the complementary diet consisted of broad qualitative information on foods mothers fed their infants. Mothers were asked about the foods they fed their infants in the past 24 hours. Reporting of data consisted of categorizing foods using the WHO (WHO, 2000) classification, system highlighting the foods most frequently mentioned during home visits.

Breastfeeding cessation and HTEBM use:

Results of the acceptability study (Sibeko et al., 2007a; Sibeko, Coutsoudis, Nzuza, & Gray-Donald, 2007b), used to inform and shape the current study, clearly indicated that implementation of HTEBM would require support of the mother. Similarly, the literature indicates that EBF success is predicated on empowering the mothers through support (Haider et al., 2000).

Support of the mothers was ensured through the provision of scheduled follow up home visits. At each visit, observations were documented and data collection took place guided by a structured questionnaire and protocol. The schedule and purpose of the visit is detailed on Table 7.2.

Follow up visits were conducted by the researcher (LS), and breastfeeding peer counselor (SN). All mothers gave birth at the same local hospital. The mother's name and expected due date was provided to a counselor located at the hospital, once the mother arrived for delivery, the researcher and peer counselor were informed and visited the mother without delay, usually within 24 hours of delivery.

Interviews of mothers:

Qualitative data consisted of taped interviews of structures open ended questions, observations and note taking from visits. The data are included within each category in the results section, represented through quotes. Socio-demographic data were also collected on participants.

Analysis

For quantitative data analysis, independent sample t-test was performed on continuous maternal variables and Chi-square analysis on categorical data. Descriptive statistics were also summarized. Statistical analysis were conducted using SPSS 12.0 software.

Qualitative analysis involved transcription of participant interviews, translated from isiZulu to English. Member checks were conducted to ensure that the content of interview reflected the mother's meaning. Notes taken at visits were also used as data. Content analysis (Cavanagh, 1997) was used to analyze the text and illustrative quotes are embedded into the text of the results.

This study was approved by the Institutional Review Board of McGill University and the ethics board of the University of Kwazulu-Natal.

7.4 Results

Of the 103 pregnant women approached for study recruitment, 61 pregnant women were eligible and agreed to participate in the study Thirty mothers did not exclusively breastfeed their infants and therefore withdrew from the study (Figure. 7.1). A little more than a third of the women who discontinued EBF, did so as a result of wanting to use the free formula program available at the local clinic. In some cases the mothers felt that breastmilk alone was insufficient and in other situations mothers did not want to be left out of receiving a free expensive product like formula. Attrition also occurred as a result of mothers gaining employment, which whether informal or formal as well as a lack of family support.

Exclusive breastfeeding for six months:

Including the mothers who entered the study in the postpartum period (n=5), a total of 36 mothers practiced exclusive breastfeeding until their infant was six months of age. Unanimously, mothers who were breastfeeding exclusively reported that the home visits by the counselors were critical in encouraging them to continue EBF and in building general confidence in their breastfeeding and belief that breastmilk alone was sufficient. The fact the mothers knew when the home visits would occur (counselors left written appointment cards) seemed to be an additional benefit since mothers felt that whatever issues arose in the interim, they could depend on an upcoming home visit to address the issue, knowledge that facilitated continuation of the feeding strategy. As explained by a 20 year old mother with a 2 1/2 month old infant: "I did not believe my baby will grow just with my milk, my mother also did not believe. Now we see it is true."

Although the follow up visits had an overall purpose of supporting mothers to continue with the intervention, a range of purposes and specific information was covered and discussed within each time frame (Table 7.1).

The most common challenge mothers encountered through the EBF period was explaining to partners, family members or friends the reasons they were not feeding their infant any other foods and fluids. These were either exchanges that took place within their household or when they would go to the rural area to visit family. The most common effective response mothers reported was to simply indicate that the way they were feeding was strongly recommend by clinic staff, to ensure health for their infant. Some mothers indicated that once they themselves believed that breastmilk alone was adequate nourishment, they were able to continue EBF. A supportive home environment helped. On many occasions, at the request of the mother, a home visit also meant, explaining EBF to partners and family friends. This was often well received.

Breast pathology (mastitis, cracked bleeding nipples) was not observed in the women and engorgement was rare. Occasionally, in the early postpartum period, a mother might report experiencing sore nipples, a condition which was resolved through correction of the infant's latch on the breast. However, thrush was not uncommon, although the incidence of thrush is common in this area. When observed in the infant and or mother, it was immediately treated by the counselors, with mothers instructed on how to recognize and treat thrush themselves, using local treatment protocol.

Mothers were also encouraged to use condoms where applicable, the risk of increased viral load as a result of postnatal infection was explained.

A comparison of the maternal characteristics, support network and living conditions of the mothers who practiced EBF only with those who proceeded to use HTEBM (Table 7.2) indicated that the HTEBM group of mothers were of similar age and CD4+ cell count.

There was a trend towards a greater proportion of the mothers who practiced HTEBM being unemployed both groups had similar levels of disclosure of their HIV status, proportion of mothers heat-treating their breastmilk who lived with their partners was higher than those not using HTEBM (p=009). The majority of the mothers in this

community lacked piped water and instead had to fetch water from communal sources, however those who did have piped water were more likely to use HTEBM.

Preparation for HTEBM:

During the preparation period leading up to HTEBM use, some mothers found the practicing of expressing breastmilk (EBM) had several benefits including, developing comfort around EBM as well as training their infants to cup feed. Additionally, the EBM practice provided the opportunity for those around the mother to become comfortable with the activity, allowing the mother to address any issues that arose. This training period also helped mothers to harness support within their environment, this was more acutely beneficial when the mother had disclosed her status, particularly to a partner.

At this point concerns mothers highlighted focused on their infant's ability to cope with discontinuation of breastfeeding. There was less concern for the infant's acceptance of HTEBM. At the 5 month mark when mothers where practicing HTEBM, none reported infant rejection of HTEBM, instead, the simplicity of HTEBM was a constant feedback from mothers.

The consistent reasons mothers cited as reason for trying HTEBM were twofold: an issue of cost and health benefits to their infant, as captured in this mother's comment "because formula is expensive sometimes you won't have money to buy it, but if you are expressing your milk and heating it, you feed the baby and the baby is growing".

Initiation and duration of HTEBM:

Mothers reported that practicing HTEBM required the initial task of finding the best time to dedicate to milk expression, describing how they incorporated the HTEBM procedure within their daily activities. One mother who used HTEBM for 3 months reported how she liked to use the 'quiet' times in her daily routine "I wake up early before everyone else and I can express the milk well and lots, and can heat the milk before the baby and

everyone is up". This mother had to have her stove on early in the morning to feed her school age daughter breakfast and so could heat-treat the milk prior to cooking the porridge. In general the manual expression became easier with time, the easier it became the more mothers reported they could express.

Mothers were counseled to cease breastfeeding gradually, decreasing frequency at the breast and instead, feeding their infant complementary foods and HTEBM. The process of breastfeeding cessation occurred over a two week period for most mothers. Only one mother took 3 weeks, she reported difficulty discontinuing the night feeds.

Night feeds were the most challenging breastfeeds to discontinue for most mothers, with some mothers resorting to using a small amount of pepper on their nipple to speed up the process, this was not a suggested strategy but one that most women knew as a traditional method for getting a child to discontinue breastfeeding. Using cup feeding more frequently also required an adjustment period for some infants, despite the learning period prior to 6 months.

Engorgement as a result of breastfeeding cessation was not reported or observed. The amount of breastmilk expressed daily varied (Figure 7.2), ranging from an average of 65 ml per day for a mother who practiced HTEBM for 1 month to a mothers expressing 600ml per day (HTEBM for 5 months). The quantity of breastmilk was a gradual build up, for instance the mother who practiced HTEBM for five months began at expressing approximately 300 ml/d, after 3 months of HTEBM she was expressing 600 ml and in the final month the quantity was decreased to 500ml per day. This mother attributed her success to the support and encouragement of the home visits.

The majority of mothers who continued to practice HTEBM, felt they were able to continue because of supportive partners, most of whom were aware of their seropositive status. This type of support may have been more readily accessible to these mothers than to mothers not using HTEBM since more mothers using HTEBM were living with their

partners. Receiving support had an additive effect, because mothers repeatedly conveyed they continued to use HTEBM because of the home visits.

Mothers who did not disclose to their partners were able to invent plausible reasons to help them continue the feeding strategy. One mother who practiced HTEBM for 4 months explained that "he asked [partner] why I was heating my milk, then I said it is because I want a job so I want the baby to get used on cup feeding, not on my breast anymore'.

There were a variety of reasons mothers discontinued HTEBM, including: a seropositive infant, becoming employed, perceiving they had an inadequate milk supply, feeling manual expression was time consuming and incompatible with their other tasks and simply feeling it was time to discontinue.

Complementary foods:

On average infants were fed solid food a minimum of twice per day, while HTEBM was fed minimally 3 times per day. Foods from the cereal group were the most frequently served food regardless of age, with soft porridge being the most common item, occasionally with a spread (margarine or occasionally peanut butter) added. Potatoes were also used often and at times combined with the porridge. There was also little diversity in the vegetables and fruits used (carrots and butternut squash; apples and bananas). Serving flesh-based protein foods was often dependent on household finances, chicken and beef being the preferences, with beans and eggs being more affordable. Although difficult to assess, given the limitations of the data collection method, there seemed to be less attention paid to the complementary diet as the infant got older. In some households, it was not uncommon for the infant to be fed porridge and potatoes, with little else. These were the households who had frequent bouts of food shortage. Water and fruit drinks (not juices) were the most common other fluids provided for infants.

7.5 Discussion

This is the first study to investigate the feasibility of using flash-heat treated manually expressed breastmilk to feed infants born to HIV-1 infected mothers. Our data from this pilot study clearly demonstrates that use of HTEBM as an alternate feeding during complementary diet phase, was feasible in this resource poor urban community in KZN, with a high prevalence of HIV infected pregnant women. Of the mothers who practiced EBF, a high proportion (47%), intended to use HTEBM with actual initiation of HTEBM remaining high 15/36 (42%). This was a remarkable finding given that both EBF and HTEBM are unfamiliar practices, particularly HTEBM. Mothers participating in the study had never heard of HTEBM prior to entering the study. Moreover, five mothers not part of the ANC recruited group indicated interest in using HTEBM and thus entered the study. Given the level of poverty in this community, having access to a safe, inexpensive replacement feeding that mothers perceived as promoting healthy growth, were reported motivators for using HTEBM.

It is important to point out that implementation of WHO recommendations (WHO, 2006) of 6 months of EBF by HIV infected mothers is possible. In our study, 55% (36/66) of the mothers were able to EBF for six months, a rate that compares favorably to a recent large South African based study (Coovadia et al., 2007), where 40% of HIV infected mothers breastfed exclusively for 6 months.

Support of the mothers through home visits proved to be the most important factor enabling mothers to maintain EBF, and continued to be of great benefit for mothers who practiced HTEBM. Having support at home was also beneficial. The ease of heat-treatment was also viewed as an enabling factor as was infant acceptance of the HTEBM. Several studies promoting EBF (Haider et al. 2000; Iliff et al., 2005; Bland et al., 2007; Coovadia et al., 2007) identify support of the mother, usually through peer counseling as critical in promoting safe breastfeeding practices.

Stigma was not identified as a concern for mothers using HTEBM, including with the mothers who did not initiate HTEBM. This might be due to the high level of disclosure of HIV status amongst the study participants. Disclosure to a partner seemed to be of particular significance in enabling the mother to persist with the feeding strategy. In our previous study (Sibeko et al., 2007b) examining the level of acceptability of HTEBM in this community, disclosure was identified as an important component of facilitating HTEBM practice.

Abrupt early cessation of breastfeeding has been associated with infant morbidity and some cases, mortality, as was the case in Uganda where infants born to HIV infected mother were EBF for 3 months to 6 months with breastfeeding abruptly stopped at 6 months. Serious gastroenteritis developed in 4% of infants at 3 months post weaning, with 5 deaths attributed to the gastroenteritis (Onyango et al., 2007). Other studies confirm the risks associated with early weaning (Johnson et al., 2006; EG Piwoz et al., 2001). This risk was taken into consideration in the development of our intervention. The use of HTEBM was strategically promoted to be used following 6 months of EBF. This approach has several advantages, mothers who EBF for 6 months have established a good milk supply and are confident of their breastfeeding skills which is a positive underpinning for introducing HTEBM. As well, because of the energy contribution of breast milk to the infant diet post 6 months, we felt that HTEBM would be most beneficial to infant health when used in conjunction to appropriate complementary foods. As a result, infant or maternal health problem were not encountered by counselors following breastfeeding cessation. This observation may be attributed to the fact that the breastfeeding cessation used in our intervention was gradual and not the rapid cessation others have used. Additionally, during home visits, mothers received counseling on appropriate complementary feeding, to the best ability of their family.

Although our data indicate that mothers in our study did provide complementary foods to their infants at the suggested period, to minimize respondent burden, we did not collect data that examined the diet in detail or quantified intake to determine adequacy of infant intake. WHO identifies adequacy as feeding that is timely, adequate, safe and appropriate

(Ruel et al., 2002). Our limited data do hint at the lack of diversity, particularly in fruits and vegetables and a reliance on cereal based foods. These findings underscore the need for future studies to examine complementary feeding practices using validated instruments that assess dietary and feeding adequacy, as well as understanding food availability from a behavioural perspective.

Discontinuing night time feeds was the most common challenge mothers experienced, which they eventually (over 2-3 weeks) overcame. Another observed challenge was the free formula program that was available at the time of this study, it served as a constant competitor to HTEBM use. This free formula is no longer available in the community, probably due to the unsustainable nature of such a policy.

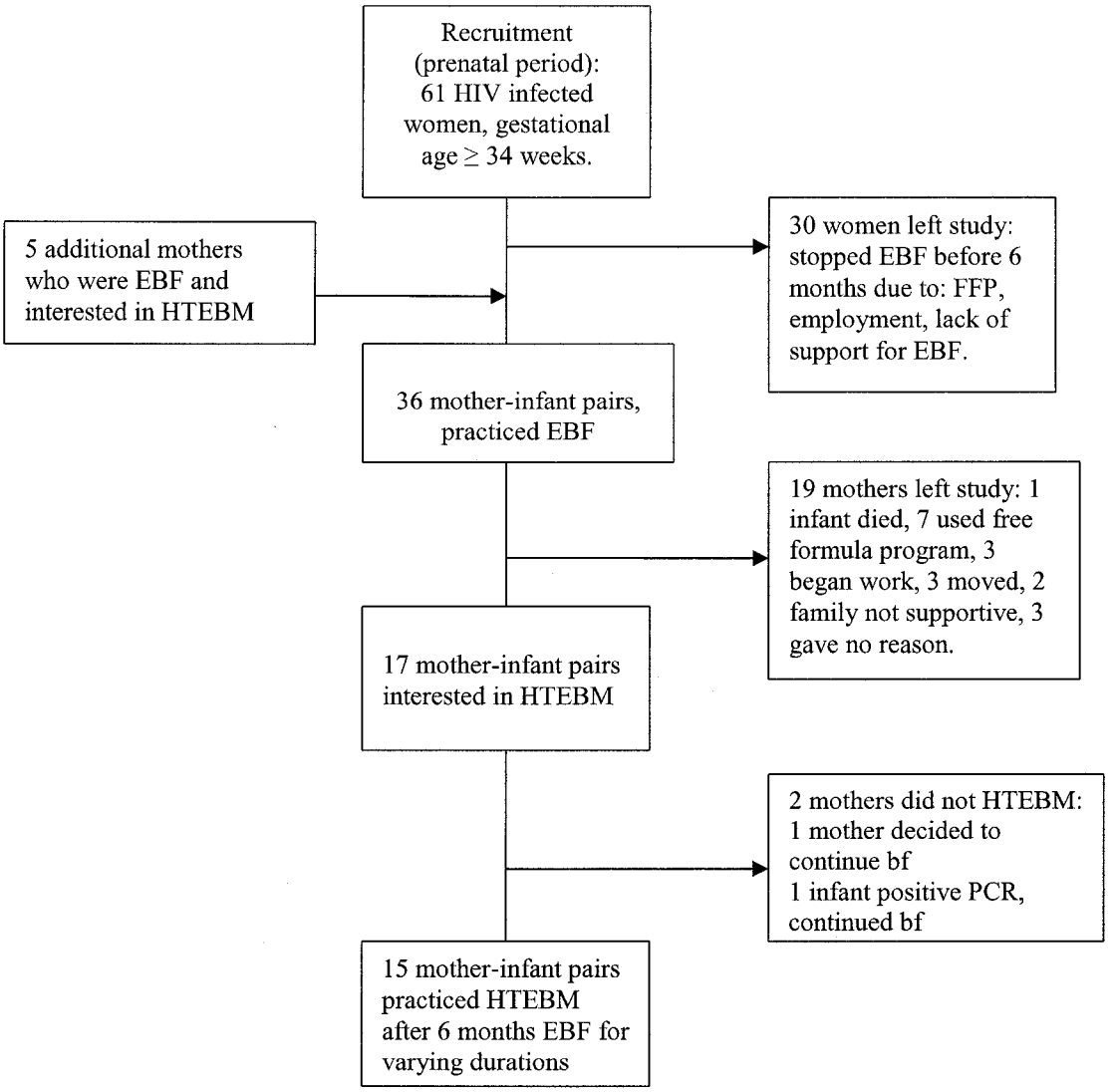
Our study was limited by its sample size, given that the overall aim was to pilot the feasibility of a safe modified breastfeeding strategy using HTEBM. Hence, we were not powered to examine the correlates between maternal characteristics and infant feeding nor to look at infant health outcomes, including transmission risk. Larger studies, informed by our work underway in different African settings, have been developed to investigate feasibility within different contexts and the above mentioned outcomes.

To summarize, our study provides important data documenting the feasibility of mothers living with HIV using HTEBM as a component of the infant complementary diet. This is an important finding since it implies that mothers who are supported can be empowered to practice new feeding behaviours that benefit their infants. Through HTEBM use HIV-infected mothers have a mother controlled, inexpensive, safe and sustainable feeding option. Moreover, despite that the South African EBF rate has been estimated at 10.5 %, this study has shown that high quality counseling and support of women plays an essential role in empowering mothers living with HIV to implement safe breastfeeding practices.

Based on the experiences and data from this study, HTEBM is now one of the feeding options offered to breastfeeding HIV infected mothers from the community at the study

site. Additionally, resources developed, in collaboration with study participants (pictorial pamphlet depicting the process of HTEBM and a video demonstrating the procedure), are used as teaching tools.

Acknowledgements: we are grateful for the many women who participated in this study, providing important information for the benefit of other mothers. We also thank the staff in the ANC clinic and the staff in the HIV treatment and care clinic for their assistance and support of this work. Gratitude is also extended to Kiersten Israel Ballard and Dr Caroline Chantry for all their work on the flash-heating procedure. We thank our funders, the Canadian Institute of Health Research (CIHR) and the International Development Research Centre (IDRC).



Bf = breastfeeding
FFP= free formula program
HTEBM= heat-treated expressed breastmilk
PCR= polymerase chain reaction

Figure 7.1 Flow diagram of participant recruited for feasibility of HTEBM study.

Table 7.1 Follow up visits of mothers at different infant ages, the purpose and content of visits in the continuum from pregnancy to breastfeeding cessation and use of HTEBM.

Time and Infant Age	Frequency of visits	Purpose of visits	Information covered by counselors
Prenatal	1 visit	To confirm the exact location of residence Assess support	Review, reinforce EBF
Hospital visit	1 visit	Ensure successful Initiation of breastfeeding	Assessed breast health and infant at breast, checking latch build mother's confidence
Postpartum Period			
0-2 months	weekly	Support mother to continue with EBF	Check breast health address concerns Encourage EBF
2-4 months	biweekly	Introduce manual EBM	Continue to support to EBF Discuss EBM and cup feeding
4 months	biweekly	Practice EBM and accustom infant to cup feeding	Observe mother manually EBM and practice cup feeding Encouraged to practice often
5 months	weekly	Practice HTEBM	Observe mother with HTEBM, encourage to practice often beginning with once/week; mother is supported to continue EBF address arising issues; counseled on complementary foods; preparation for bf cessation through discussions
6 months	weekly	Breastfeeding cessation, feed complementary foods and HTEBM	Mother counseled and supported on gradual breastfeeding cessation, use of HTEBM + complementary foods
7 months +	monthly	Support	Continued support of mother for duration of HTEBM

EBF exclusive breastfeeding, EBM expressed breastmilk, HTEBM heat-treated breastmilk; bf breast-feeding.

Table 7.2. Characteristics of HIV-1 infected mothers who practiced EBF for 6 months compared with those who practiced EBF and HTEBM.

Characteristics	Mothers who practiced EBF + HTEBM (n=15)	Mothers who practiced EBF (n=21)	P-value
Maternal characteristics			
Age of mother (yrs)	24.7 [4.0]	27.9 [6.1]	0.114[1]
CD4+ cell count (cells/mm^3)	452.6 [153.9]	381.0 [204.4]	0.335
Education*			
None	3 (21)	1 (5)	0.551
1-5	0	2 (10)	
6-10	9 (64)	10(50)	
>10	2(14)	7(35)	
Employment			
	1(8)	7 (33)	0.107
Support network			
Feel supported	8 (67)	17 (81)	0.357
Disclosed HIV status			
If yes, who	10 (67)	14(67)	1.000
Partner			0.192
Mother	6 (40)	3(14)	
Sister	2 (18)	1(5)	
Other	1(7)	3(14)	
	1(7)	7(34)	
Living conditions			
Living with (adults)			
Alone	2(17)	13(62)	
Partner	7 (58)	8(38)	0.009
Family member(s)	3(25)		
Children in household			
None	2(17)	7(35)	
≤2	9(75)	11(55)	0.451
≥3	1(8)	2(10)	
Type of housing			
Mixed materials	6(43)	6(30)	
Plank	3(21)	6(30)	0.974
Mud	2(14)	1(5)	
Bricks	4(29)	7(35)	
Access to electricity	3(21)	7(35)	0.660
Water source			
Piped	5(45)	6((30)	0.032
Public + yard	6(55)	14(70)	
Sanitation			
Pit	11(71)	11(55)	0.641
flushed	4(29)	9(45)	
Fuel			
Paraffin	13(86)	15(75)	0.664
Electricity	2(14)	5(25)	

[1] Equal variances are assumed

Continuous variable mean (standard deviation), categorical variables are numbers (%).
EBF= exclusive breastfeeding, HTEBM= heat-treatment of expressed breastmilk.

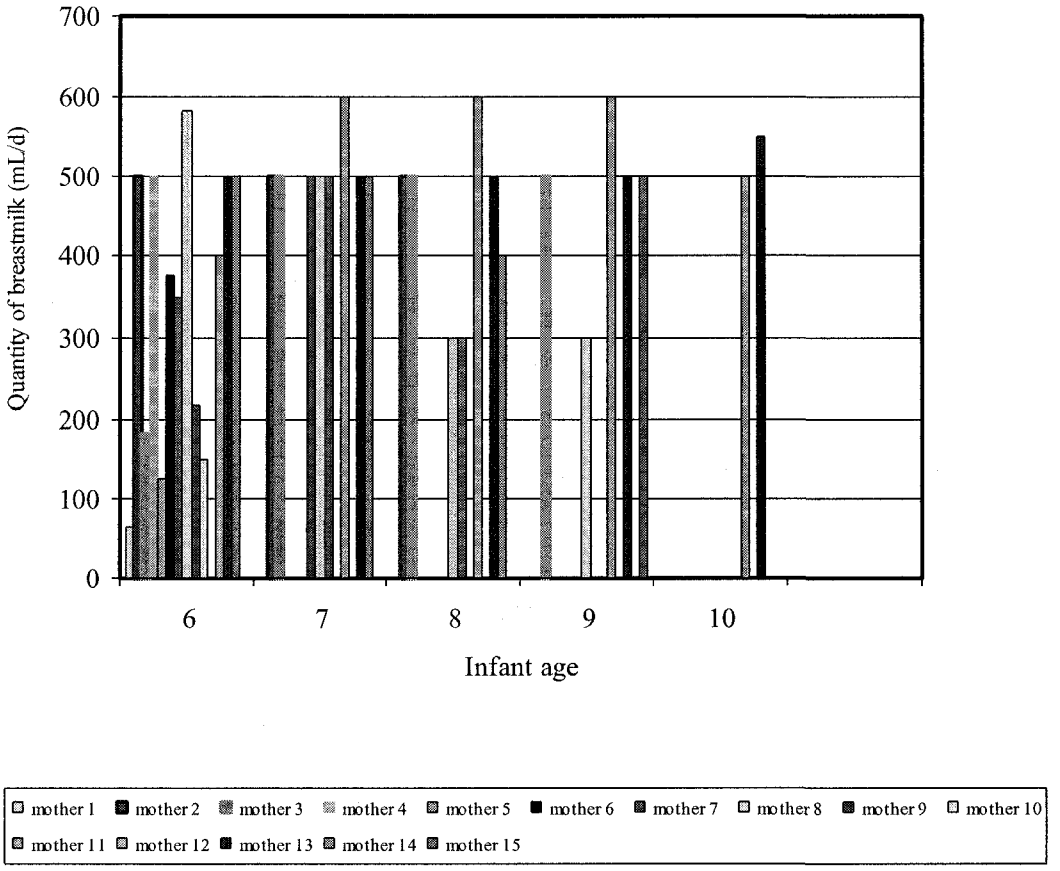


Figure 7.2. Amount of breastmilk expressed each month by mothers practicing HTEBM

LINK STATEMENT

Through EBF for the first 6 months of the infant's life, mothers provided a high quality, food secure, infant diet associated with minimal HIV infection. It should be recognized that EBF is not a common feeding pattern in the community, instead mixed feeding, a highly risky feeding practice, is the usual practice. Therefore, the fact that 55 % of the mothers participating in the study were able to breastfeed their infants exclusively is an important outcome. Similar to other initiatives to promote EBF, support of the mother, enables optimal infant feeding.

This is the first study to provide evidence of the feasibility of HTEBM use after 6 months of EBF, by HIV infected women. Several factors contributed to the success of this feeding intervention. Additional enabling factors included taking a gradual breastfeeding cessation (2 weeks) approach, as opposed to rapid weaning. Mothers participating in the acceptability study identified disclosure and support, especially from a partner as being critical to the success of the feeding intervention. Our findings did show that mothers who were able to practice HTEBM were more likely to live with a partner ($p=<009$) as well as have access to piped water ($p=<032$) when compared to mothers who did not use HTEBM. Disclosure was common to both groups and may have facilitated the earlier component of the intervention, since both groups practiced EBF for a 6 month period. The outcome of this pilot study provides much needed data for a variety of settings, including areas where there is a desperate need for affordable and safe feeding choices for infected women, once they cease breastfeeding.

The nutritional (using BMI, TSF and Hb) and immune profile (CD4+ and breastmilk viral load) of HIV+ mothers ($n=84$) was determined through a cross-sectional study. Mothers seemed mostly healthy, as illustrated by the nutritional and immune status indicator used. Anemia is present with severely immunocompromised mothers, as was high breastmilk viral load. Implications for practice and policy are suggested in the paper presented in Chapter 8.

CHAPTER 8

NUTRITIONAL STATUS AND IMMUNOLOGICAL PROFIL OF LACTATING HIV-INFECTED SOUTH AFRICAN URBAN WOMEN

To be submitted to Maternal and Child Nutrition

Signed co-author waiver in Appendix III

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8.1 Abstract

Data are lacking on the nutritional status and immune profile of urban dwelling, HIV-infected breastfeeding women in African settings. A cross-sectional survey was undertaken to examine the nutritional status [body mass index (BMI), triceps skinfold (TSF) and hemoglobin (Hb)], of breastfeeding HIV-1 infected mothers from an urban South African settlement. Breastfeeding mothers (n=84), average age of 26 years, had an index child in the 4 to 68 week age range. Overall, there was no indication of compromised maternal nutritional status, as illustrated by mean BMI of 26.8 (4.0), TSF 14.8 (5.50) and Hb level of 11.6 (1.49)). Type of or duration of breastfeeding were not related to nutritional or immune status. However, mothers with $CD4^+$ T cell counts < 200 cells/mm^3 were more likely to be anemic. Additionally, breastmilk viral loads were also higher in mothers with lower $CD4^+$ T cell counts. Urban HIV infected women from a resource poor community, who chose to breastfeed their children were well nourished, however those who were severely immunosupressed were at increased risk of anemia and had higher breastmilk viral loads, which increases the risk of infection for their children I thought this is important to add but your call here. . This offers further evidence that breastfeeding (exclusive must be encouraged) mothers in this setting are not nutritionally compromised.

Keywords: breastfeeding, anthropometry, $CD4^+$ cell count, breast milk viral load, HIV-1, South Africa.

8.2 Introduction

On a global scale, mother-to-child transmission (MTCT) of HIV accounts for the infection of approximately 700,000 children, 90% of whom are African children (UNAIDS/WHO, 2005). In South Africa, where HIV is highly prevalent, an estimated 29.5% of pregnant women are HIV infected, (Department of Health, 2007). As a result, nationally MTCT has become an urgent public health concern (Dorrington et al., 2006).

Exclusive breastfeeding (EBF) for six months has been estimated to have minimal (4%) HIV transmission risk, with mortality rates found to be double for formula fed infants compared to those who are breastfed exclusively (Coovadia et al., 2007). Hence, in Africa, where breastfeeding is universal, the ability of a mother living with HIV to breastfeed without compromising her health is critical for improving HIV-free infant survival.

HIV infection has been associated with poor nutritional status due to the increased demands placed on the body as a result of the infection (Bogden et al., 2000). The additional stress imposed on the immune system as a consequence of poor nutritional health may further deplete nutritional stores and may contribute to the progression of HIV, thereby increasing mortality risk (Hecker & Kotler, 1990).

In resource poor environments, infected mothers could be at a greater risk for deteriorating health since food insecurity and malnutrition are underlying living conditions. Within these settings mothers choosing to breastfeed are guided to do so exclusively (WHO, 2006), however, some concern has been raised over the health implication or burden that breastfeeding may impose on HIV infected mothers (Nduati et al. 2001.). The inference being that breastfeeding may lead to progression of HIV infection as evidenced by unintentional weight loss or wasting and decreased $CD4^+$ T cell count level. However, a recent cohort study investigating disease progression and lactation of infected women, comparing breastfeeding to non-breastfeeding women, did not show breastfeeding to be associated with HIV-1 progression, measured by either time

to death, low $CD4^+$ T count, anemia or wasting. They concluded that there was insufficient evidence to indicate that breastfeeding was a risk to the health of HIV infected women (Sedgh et al., 2004).

African based studies have shown a strong independent association between body mass index (BMI) of people living with HIV and survival. The strength of the association between survival and BMI has a similar predictive value to $CD4^+$ T cell count under 200, which is the accepted standard for severely compromised immune status level (van der Sande et al., 2004). Furthermore, in a Zambian based study, poor maternal health was found to be the most influential factor contributing to increased breastmilk viral load, a risk factor for MTCT of HIV (Phiri et al., 2006). In some cases, anemia in HIV infected women has been shown to be an independent marker of disease progression and mortality (Moore, 1999; O'Brien et al., 2005).

There is a paucity of data on the nutritional profile of seropositive breastfeeding African women within urban settings. Therefore, the primary objective of this study was to explore the relationship between breastfeeding mothers' immune status and their nutritional status. A secondary objective was to measure the link between maternal immune status and the viral load of the breastmilk.

8.3 Materials and methods

Setting and subjects:

This cross-sectional study involved the recruitment of 84 breastfeeding mothers living with HIV, with infants ranging in age from 4 to 68 weeks, attending a community clinic located within a settlement in Durban, South Africa. Recruitment took place between October 2004 and July 2005. None of the mothers had initiated antiretroviral treatment.

Anthropometric and biochemical measurements

After obtaining signed consent, anthropometric measurements were done on the 84 participating mothers by a trained dietitian, employing standard procedures. Height was measured without shoes using a 2 m wall mounted measure with an attached sliding headpiece in duplicate to the nearest 0.1 cm (mean of measures used). Weight was measured without outer-wear or shoes, to the nearest 100 g using an electronic scale. To assess percentage body fat, triceps skinfold thickness (TSF) was measured at the nearest 0.1cm, using standard calipers. Body mass index was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Norms derived from The National Health and Nutrition Examination Surveys (NHANES III 1988-1994) for African American women were used as reference values for TSF, with depletion defined as values < 5^{th} percentile (Gibson, 2005). Categories for BMI assessment are according to WHO standards of : <18.5 (underweight), 18.5-24.9 (average) and ≥25.0 (overweight).

Determination of $CD4^+$ T lymphocyte cell count ($CD4^+$ cell count) was conducted using MultiTEST reagent and TruCOUNT tubes in BD FACSCalibur flow cytometre (Becton-Dickinson, New Jersey, USA). Study participants were categorized into three $CD4^+$ T cell count levels <200 (compromised), 200-500 (moderate) and > 500 (high). Hemoglobin (Hb) was measured on venous blood samples collected into EDTA vacutainers for hematological analysis, with anemia defined as Hb< 12g/dL.

Breast milk samples

Participants were instructed to wash their hands using soap and water prior to manual expression of 75 – 150 mL of breastmilk into sterile glass jars. Assistance was provided by a Lactation Consultant if mothers required assistance. The jar was immediately stored on ice in insulated containers and transported, within two hours to the laboratory. Breastmilk samples were divided into 1.5 mL aliquots and immediately stored at -80° C. All samples were shipped frozen on dry ice to the California Department of Health Services Viral Rickettsial Disease Laboratory. Samples were analyzed for levels of detectable HIV-1 using TaqMan Real Time-RNA-PCR (TaqMan RT-PCR, Applied Biosystems, Foster City, CA), the method is described elsewhere(Yun, Fredriksson, & Sonnerborg, 2002) and has a sensitivity of 50 RNA copies/mL on the breastmilk samples. Extraction of RNA was performed using Viral RNA Kit (Qiagen, Valencia, CA). The RT assay limit of quantification was 400 copies/mL; therefore levels less than the assay limit were replaced by a value of 400 copies/mL to quantify the undetectable levels of HIV at a conservative level.

Feeding practices

Breastfeeding practices were categorized into exclusive breastfeeding (EBF = breastmilk only, no other fluids or solid food), predominately breastfeeding (PB = breastmilk plus non- nutritive fluids) and mixed feeding (MF = breastmilk plus other fluids and or solid foods). Socio-demographic data were also collected (maternal age, presence of electricity and piped water in the house, as well as type of cooking fuel used).

Statistical analysis

ANOVA was used to examine the relationships between nutritional status variables and immunological variables by postpartum period categories (0-7 weeks; 8-15 weeks; >15 weeks) and $CD4^+$ T cell count groupings. Tukey's post hoc test was used when variables were significantly associated, overall.

Chi-square was used to examine the relationships between categorical data by postpartum periods and $CD4^+$ T cell count groupings. Independent Student t-tests were used to

examine differences in variables for mothers with detectable versus undetectable breastmilk viral load. Significance was set at $p< 0.05$ for all analyses.

Breastmilk viral loads were not normally distributed and therefore log transformed, however, this paper presents the untransformed breastmilk viral load values for ease of comprehension. All statistical analyses were conducted using the statistical software SPSS 12.0.

The study was approved by the ethics committee of the University of KwaZulu-Natal and the McGill University Institutional Review Board.

8.4 Results

Characteristics of the 84 mothers and children aged 0-7 weeks (n= 30), 8-15 weeks (n=26) and >16 weeks (n=28) are described in Table 8.1. None of the mothers had a BMI less than 18.5 and mean BMI was in the overweight range. Breastfeeding mothers with infants of different ages were not different in terms of BMI and TSF, hemoglobin, CD4+ cell count, or breast milk viral loads. Moreover, there were no differences in demographic variables amongst women with different aged children in this sample. Most women participating in the study were not taking multivitamins or prophylactic antibiotics. More than 75% of the infants were being exclusively breastfed, while less than a quarter were being mixed fed. Younger infants were more likely to be EBF.

In order to explore the relationship between mothers' immune status and nutritional status, women were grouped into 3 CD4+ cell count levels, comprised of CD4+ level < 200, 200 ≤ CD4 < 500 and CD4 ≥ 500. Less than 10% of the sample of mothers had severely compromised immune status (CD4 levels <200), (Table 8.2). Moreover, the immune status of these women was not related to any of the anthropometric measures such as weight, BMI or TSF. Hemoglobin was however, lower in the immunosupressed mothers (p= 0.007), at a mean hemoglobin level of 9 g/dL. Mothers' CD4+ cell count was not related to type of infant feeding practiced.

Overall, 26 (31%) of the mothers had detectable breastmilk viral load. These mothers were older than mother with undetectable breastmilk viral load (p=0.015) and were more likely to be in the most immunologically compromised group. Those with the lowest $CD4^+$ T cell count had the highest breast milk viral load (p=0.002) (Table 8.2).

8.5 Discussion

Breastfeeding mothers living with HIV, all from similar living conditions within an urban setting of South Africa, did not present with compromised nutritional status. None of the mothers had a BMI <18.5, which is of significance since BMI has been shown to be a valid and strong correlate of survival, (van der Sande et al., 2004). Although immune status was not associated with poorer weight, anemia was significantly associated with the presence of severe immunosupression. Similar findings were also reported in a study of HIV infected African pregnant women (Antelman et al., 2000), with an association between anemia and CD4+ cell count < 200 observed. The researchers hypothesized that their data illustrated a state of chronic infection, reflected in the poor immune status, which would result in impaired erythropoiesis and concomitant decreased hemoglobin level. Correction of anemia in HIV infected individuals has been associated with improved survival (Moore et al., 1998).

The low number of mothers with detectable breastmilk viral load in this study may be a reflection of the high quality of breastfeeding counseling women from this community attending the clinic (study site) receive. None of the participating mothers presented with mastitis or breast engorgement, both conditions are often indicative of poor breastfeeding management. This is important as mastitis has been associated with increased breastmilk viral load (Semba et al., 1999; (Willumsen et al., 2003).

Our findings on the higher breast milk viral load in those with a CD4 count of <200 corroborate those with a longitudinal study of infected South African women, where it was shown that mothers with CD4+ cell count level less than 200 cells/mm^3 had increased breastmilk viral loads compared to mothers with higher immune status (Willumsen et al., 2003).

Clinically, within the study community, mothers with high viral loads and poor health status are counseled to avoid breastfeeding and assisted in securing replacement feeding for their infants. Our study is limited by being cross-sectional and therefore may suffer

from survival bias, whereby mothers who are in poor health may have discontinued breastfeeding. Our participants may represent women who are in relatively good health and not experiencing breastfeeding challenges. However, all lactating women attending the clinic were approached over the 9 month study period, which would maximize the opportunity to capture a representative sample. Overall, our data indicates that infected mothers who are breastfeeding are relatively well.

A recent longitudinal study assessed body composition changes of HIV positive breastfeeding women from rural South Africa at 8 weeks and 24 weeks postpartum compared to uninfected women. At 8 weeks postpartum there were no significant differences in anthropometric measurements (BMI, Mean Upper Arm Circumference, triceps skinfold thickness), however at 24 weeks BMI and triceps skinfold thickness were significantly lower in lactating HIV-infected women compared to the uninfected women, suggesting a loss of fat mass. Even though infected mothers lost weight in the form of fat mass, the data illustrate that HIV infected mothers were still comparable to their non-infected breastfeeding counterparts in terms of fat free mass and fat mass (Papathakis et al., 2006). Our study differs in its setting in that it is of urban mothers, who had mean BMI values of 26.4 and 27.6 at 8-15 weeks and >16 weeks postpartum, respectively, however it is difficult to make a true comparison given that our study is cross-sectional.

Although a Kenyan randomized controlled trial indicated an increased risk of mortality among lactating women compared to formula feeding mothers (Nduati et al., 2001), the study was limited by the fact mothers assigned to breastfeed had higher HIV-1 RNA viral load than the ones in the formula feeding group. In contrast, an observational study of breastfeeding infected women in South Africa did not find compromised health or increased mortality risk associated with breastfeeding (Coutsoudis et al., 2001).

In summary, this study presents the nutritional profile of 84 urban lactating African mothers living with HIV, data that are lacking for this population group. Generally, our data demonstrate that HIV infected mothers who are breastfeeding for varying lengths of time, many of whom practiced EBF, are not in poor health as indicated by our nutritional

status and immune status measures. However, in the presence of severe immuno-suppression in a small number of women, anemia becomes a problem, posing a significant risk to the mother's overall health. In addition, a high viral load was present in severely immunocompromised women and needs to be recognized in terms of recommending breastfeeding to this subset of breastfeeding mothers.

Key messages

Research

1. HIV-infected mothers who breastfeed for varying durations are not nutritionally compromised, as indicated by anthropometric measures, with less than 10% being severely immunocompromised.
2. Anemia and high viral load are present in severely immunocompromised mothers.

Practice

3. The majority of HIV-infected women choosing to breastfeed seem not to have their health deteriorate as a result of breastfeeding.
4. Infected mothers with high viral loads and CD4+ counts below 200 should receive additional assistance to procure breastmilk substitutes for infant feeding.

Policy

5. Optimal breastfeeding (EBF for 6 months) should continue to be promoted and supported for HIV-1 infected mothers.

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Table 8.1. Maternal, infant and demographic characteristics with infant feeding practices by categories of postpartum periods.

Maternal and Infant Characteristics [a,b]	Total population (n=84)	Mothers with infants 0-7 weeks old (n=30)	Mothers with infants 8-15 weeks old (n=26)	Mothers with infants >16 weeks old (n=28)	p-value
Maternal age (years)	25.8 [5.46]	25.0 [4.78]	26.0 [5.38]	26.3 [6.27]	0.634
Infant Birth weight (g)	3092 [475.9]	2961 [497.0]	3158 [510.0]	3177 [393.0]	0.166
Child sex (%male)	42 (50)	18 (60)	13 (50)	11 (39.3)	
Mother CD4 (cells/mm^3)	502 [234.8]	568 [240.3]	492 [196.8]	439 [246.4]	0.109
<200	7 (8.6)	2 (6.7)	0	5 (17.9)	
200-500	41 (50.6)	12 (40.0)	15 (65.2)	14 (50.0)	
>500	33 (40.7)	16 (53.3)	8 (34.8)	9 (32.1)	
Breast milk viral load (copies/mL)	3153 [9858.8]	2290 [6473.9]	2834 [10137.9]	4374 [12495.6]	0.714
Hemoglobin (g/dL)	11.6 [1.49]	11.3 [1.92]	11.8 [1.13]	11.6 [1.22]	0.56
Weight (kg)	66.7 [10.9]	65.3 [9.46]	64.2 [12.11]	70.5 [10.61]	0.089
Height (m)	1.58 [0.07]	1.58 [0.076]	1.56 [0.051]	1.60 [0.061]	0.096
BMI (body mass index kg/m^2)	26.8 [4.0]	26.3 [3.15]	26.4 [4.74]	27.6 [4.07]	0.422
TSF (mm)	14.8 [5.50]	13.2 [4.98]	14.9 [5.48]	16.4 [5.79]	0.114
Type of feeding					<0.001
EBF	65 (78.3)	29 (96.7)	23 (92.0)	13 (46.4)	
PBF	2 (2.4)	0	0	2 (7.1)	
MF	16 (19.3)	1 (3.3)	2 (8.0)	13 (46.4)	
Electricity	16 (34.8)	3 (23.1)	5 (38.5)	8 (40.0)	0.576
Water for household use	26 (35.1)	7 (28.0)	9 (37.5)	10 (40.0)	0.779
Cooking fuel	56 (75.7)	20 (80.0)	17 (70.8)	19 (76.0)	0.668

[a] continuous variables are reported in means ± standard deviation ; [b] categorical data is presented as n, with percentages in brackets. EBF- exclusive breastfeeding, PBF- predominant breastfeeding and MF- mixed feeding, TSF, Triceps Skinfold, N<84 is due to missing values for some variables

Table 8.2. Nutritional status variables and breast milk viral load differences by $CD4^+$ categories of HIV-1 infected mothers. (n=81)*

	CD4 < 200 (n = 7)	200 ≤ CD4 < 500 (n=41)	CD4 ≥ 500 (n=33)	p-value
Maternal age (years)	25.6 [4.47]	27.0 [5.31]	24.6 [5.70]	0.159
Birth weight(g)	3129 [520.3]	3167 [467.5]	2966-- [469]	0.198
Breastmilk viral load (copies/mL)	12,788 [23740.]	3,671 [9717.]	716 [951.0]	0.008[Υ]
Hemoglobin(g/dL)	9.9 [1.59]	11.7 [1.10]	11.8 [1.69]	0.007
Weight (kg)	62.8 [9.25]	65.2 [11.53]	69.6 [10.30]	0.161
Height (m)	1.55 [0.106]	1.58 [0.059]	1.58 [0.065]	0.404
BMI (kg/m^2)	26.3 [3.10]	26.1 [4.21]	27.8 [3.88]	0.197
TSF (mm)	14.0 [6.75]	13.6 [4.62]	16.4 [6.12]	0.113
Detectable breastmilk viral loads	4 (57%)	17 (42%)	5 (15%)	0.018
Feeding type				
EBF	5 (71.4)	31 (75.6)	26 (81.3)	0.207
PBF	1 (14.3)	0	1 (3.1)	
MF	1 (14.3)	10 (24.4)	5 (15.6)	

*Missing values for CD4=3

[Υ] p-value for breastmilk viral load analysis is based on log-transformed breast milk viral load

CHAPTER 9

SUMMARY AND CONCLUSION

More than a quarter of a century into the epidemic, HIV/AIDS has evolved into a burden shouldered mostly by women and children, the vast majority living in sub-Saharan Africa. On the African continent women and children constitute the most disenfranchised and marginalized members of society, factors that help to fuel and propagate their vulnerability to the epidemic. As a result, HIV prevalence amongst women in their child bearing years comprises the greatest proportion of those infected, contributing to high antenatal infection rates. Ninety percent of the world's approximately 700,000 children infected annually are African children, most having being infected as a consequence of MTCT (UNAIDS/WHO, 2005). It is against this grim and unacceptable backdrop that strategies for pMTCT continue to be the most critical challenges to public health.

The Southern African sub-region stands out as the epicenter of the HIV/AIDS epidemic on the continent, with South Africa having the largest number of individuals living with the virus. Nationally, the rate of infection in pregnant women is highest in KZN (39.1%) (Department of Health, 2007), site of the research undertaken for this thesis.

Breastfeeding, when practiced sub-optimally and for extended periods, can contribute significantly to pediatric HIV infections (De Cock et al., 2000). However, optimal breastfeeding (i.e. EBF), is the foundation of childhood survival in resource poor settings, with the potential of saving 13%-15% of deaths experienced annually in developing countries, as a consequence of non-infectious childhood morbidities (Jones et al., 2003). Moreover, EBF is associated with low HIV transmission risk, hence, WHO/UNICEF/UNAIDS infant feeding guidelines (WHO, 2006) combine the objectives of minimization of MTCT risk with infant survival, striving for the holistic goal of promoting HIV-free infant survival. To that end, EBF for 6 months, is emphasized when replacement feeding cannot be provided under AFASS conditions, which is the situation

in most of Africa. Clearly, HIV-infected mothers with limited economic resources lack options for securing affordable feeding alternatives, that can be used should a mother wish to cease breastfeeding when her infant turns six months of age. Heat-treated expressed breastmilk, a WHO sanctioned option, is one such suggested viable alternative infant feed. In settings where childhood morbidities are common and food insecurity is an issue, the maintenance of breastmilk in the infant's diet, once feeding at the breast has ceased, has obvious nutritional benefits. Additionally, the infant continues to receive benefit from the immunoprotective factors of breastmilk, present at some level even post FH.

However, to date, data detailing the practical experiences of mothers integrating HTEBM into the infant diet are lacking. Whether mothers can sustain breastmilk expression for any duration of time or incorporate the process of heat-treatment into their daily routine were unknown but essential to address if HTEBM is to become a usable infant feed. The promise HTEBM holds as a safe, affordable and sustainable infant feeding option, was the underpinning of this research, undertaken to provide data on the application, within households, of this infant feeding alternative.

The study site selected was purposefully chosen because of its socio-cultural and demographic characteristics, representing an urban resource poor setting that is highly affected by HIV. Evaluating a relatively inexpensive feeding option within such a setting provides data for a high need environment. All three studies comprising this dissertation, were conducted by recruiting participants residing in the study community, hence our data are representative of the women living in this setting.

Utilizing the WHO/UNICEF/UNAIDS policy AFASS parameters as a guide to assess HTEBM as a potential feeding option for infants born to HIV infected mothers, the initial research involved safety evaluations. The data on safety are detailed in the appended manuscripts.

An examination of the nutritional and immune profile of HIV-infected lactating mothers, provided important data on the health status of infected mothers who chose to breastfeed for varying durations, practicing different breastfeeding patterns. Overall these mothers showed no indication of compromised nutritional or immune status as illustrated by anthropometry (BMI of 26.8 and TSF 14.8) and hemoglobin level (11.6). Moreover, the majority of mothers had $CD4^+$ cell count levels above 200 (< 200 being the severely immunosuppressed level). Further, these health markers were unrelated to breastfeeding type or duration. Only in the severely immunosupressed mothers was anemia important. Implying that infected mothers were relatively healthy with no indication that breastfeeding imposed a burden on maternal nutritional status or immune status. In this community, women who are severely compromised would be followed more closely and assessed for initiation of HAART treatment.

Concern raised over risk of disease progression as a result of the burden breastfeeding could present to infected mothers could not be fully assessed in this study given the limitation of the study design. However, we attempted to correct this design through our recruitment wherein we approached and invited all eligible breastfeeding women in the clinic to participate in the study. These mothers were not followed longitudinally but the sample was representative of the population served by this clinic.

Acceptability of HTEBM was an important issue to explore from different aspects of household and community life to fully understand the receptivity of this feeding option. In-depth interviews with a range of community informants indicated that HTEBM is considered an acceptable new feeding practice. Mothers highlighted their sensitivity to issues of stigma and discrimination and the need for negotiation of infant feeding practices with partners and family members as important consideration in making HTEBM work. Disclosure of HIV status was also identified as a facilitating factor for behavioural change.

In many cases, mother's experiences when interfacing with health care providers, was interpreted as negative and a source of distress. Health care providers gave inaccurate

information causing a lot of confusion, resulting in the perpetuation of mixed feeding. Although formula was purportedly provided free of charge for a 6 month period to infected women, the lack of sustainability associated with formula contributed to sub-optimal dangerous feeding practices. A reported trend was the declining breastfeeding rates in the community, with concomitant rise in infant morbidity observed.

Data from the acceptability study helped to shape the development and implementation of the feeding strategy evaluated in the feasibility study. The question of the feasibility of using HTEBM in the complementary diet following 6 month of EBF was investigated employing a longitudinal intervention study. Key to the intervention was the guidance and support mothers received through home visits by trained counselors

This pilot illustrated that HTEBM use after EBF is feasible. Our findings show that the intervention was successful in encouraging 55% of the participating mothers to EBF their infants for 6 months, and 42% of the mothers who practiced EBF of their infants, to use HTEBM, for varying durations. There was a high level of disclosure of HIV status amongst the women in both groups, which mothers believed was important in enabling them to practice EBF and HTEBM. Mothers unanimously reported they were able to implement HTEBM as a direct result of the support they received from the home visits by the counselors. Additionally, our data also indicated that the HTEBM practicing mothers tended to be the most likely to live with their partners.

One of the concerns of early breastfeeding cessation is the development of breast pathology and infant and maternal health problems (De Paoli. et al., 2006; Piwoz et al., 2001). However, this was not the experience of the women participating in the feasibility study. The lack of breast pathology may be attributed to good breastfeeding management as a result of EBF and home visits. Additionally, breastfeeding cessation was implemented through a gradual transition process, with mothers closely followed and supported.

The success of .an infant feeding intervention such as the one implemented in the feasibility study requires well trained facilitators, who can provide quality care to mothers. Although, home visits may be viewed as a costly support system, however, through use of trained peer counselors, other communities have found success in promoting optimal feeding practices (Coovadia et al., 2007; Haider et al., 2002). Another approach would be to have community peer support groups. Anecdotally, a mother in the community heard of the study intervention, and due to limited resources arrived at the community clinic to receive training on HTEBM, through the use of pamphlets and the video developed as result of this study (see appendices). She was able to EBF for 6 months then use HTEBM with family foods until her child was 18 months of age. She was not receiving home visits.

The collective data emanating from this study has practical implications, since it provides much needed information for health workers, who in turn can confidently suggest to HIV infected women that feeding their babies with HTEBM, is safe and achievable. Use of HTEBM has huge financial implications for poor communities in South Africa, where 1 month's supply of commercial formula for a young infant can cost approximately R150, which is about 35% of the average family monthly income in the study community and in many other poor communities.

Besides being the first study to demonstrate the feasibility of HTEBM use by HIV infected women, these findings also illustrate that with accurate information and support HIV infected mothers were able to follow optimal feeding practices that are congruent with WHO guidelines. Moreover, this study implemented an intervention that used a modified breastfeeding strategy encompassing three unfamiliar feeding practices: EBF for 6 months, early breastfeeding cessation and HTEBM use. In view of this, the feasibility study is a successful pilot study, since more than half of the study participants practiced EBF for six months and more than a third of these women followed the entire intervention. It is these preliminary outcome data that have encouraged the development of feasibility studies at other African research sites. Indeed, our preliminary data was

requested and used as evidence of HTEBM feasibility for a successful National Institutes of Health (NIH) grant application for one of the future studies.

The limitation of the work undertaken in this research is that the intervention was carried out at a pilot level, where sample size was limited. Although it was essential to pilot the infant feeding strategy, infant outcomes in terms of growth were not measured nor were health outcomes, which would require even larger sample sizes than for growth. Although the pilot provides valuable information and addresses a gap in the literature, recommendations cannot be based on pilot outcomes. The findings of this work need confirmation from other studies.

This thesis is based on a study that succeeded in taking an international infant feeding policy from the bench to the field to practice. This is much like the historical path that EBF, as a recommended feeding practice, took from preliminary findings indicating positive health outcomes, to large cohort studies clearly demonstrating improved HIV-free survival with 6 months of EBF. The larger HTEBM studies currently underway will provide much needed data on the health outcomes of using HTEBM. Meanwhile ours is the first study to demonstrate that HTEBM provides a safe, affordable and feasible feeding option, that is a viable choice for HIV infected mothers in resource poor settings.

REFERENCES

Antelman, G., Msamanga, G. I., Spiegelman, D., Urassa, E. J. N., Narh, R., Hunter, D. J., et al. (2000). Nutritional Factors and Infectious Disease Contribute to Anemia among Pregnant Women with Human Immunodeficiency Virus in Tanzania. *J Nutr, 130*, 1950-1957.

AVERT. (2007). HIV & AIDS in South Africa. Retrieved JUNE 9, 2007 from http://www.avert.org/aidssouthafrica.html

Bahl, R., Frost, C., Kirkwood, B. R., Edmond, K., Martines, J., Bhandari, N., et al. (2005). Infant feeding patterns and risks of death and hospitalization in the first half of infancy: Multicentre cohort study. *Bulletin of the World Health Organization, 83*(6), 418-426.

Becquart, P., Hocini, H., & Levy, M. (2000). Secretory anti-human immunodeficiency virus (HIV) antibodies in colostrum milk are not a major determinants of protection of early postnatal transmission of HIV . *J Infect Dis, 181*, 532-539.

Becquet, R., Leroy, V., Ekouevi, D. K., Viho, I., Castetbon, K., Fassinou, P., et al. (2006). Complementary feeding adequacy in relation to nutritional status among early weaned breastfed children who are born to HIV-infected mothers: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. *Pediatrics,117*(4). 701-710.

BHITS. (2004). Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. The Breastfeeding and HIV International Transmission Study. *J Infect Dis, 189*, 2154–2166.

Bland, R. M., Rollins, N. C., Coovadia, H. M., Coutsoudis, A., & Newell, M. L. (2007). Infant feeding counselling for HIV-infected and uninfected women: Appropriateness of choice and practice. *Bulletin of the World Health Organization, 85*(4), 289-296.

Bogden, J. D., Kemp, F. W., Han, S., Li, W., Bruening, K., Denny, T., et al. (2000). Status of selected nutrients and progression of human immunodeficiency virus type 1 infection. *American Journal of Clinical Nutrition, 72*, 809-815.

Bourne, L. T., Hendricks, M. K., Marais, D., & Eley, B. (2007). Addressing malnutrition in young children in South Africa. Setting the national context for paediatric food-based dietary guidelines. *Maternal & Child Nutrition 3*(4), 230–238.

Campbell, C. (2003). *Letting them die: why HIV/AIDS prevention programs fail* Oxford: James Currey.

Cavanagh, S. (1997). Content analysis: concepts, methods and applications *Nurse Researcher, 4(3)*, 5-16.

Chantry, C. J., Israel-Ballard, K. A., Moldoveanu, Z., Peerson, J., Coutsoudis, A., Sibeko, L., et al. (2008). Effect of Flash-heat on Immunoglobulins in Breast Milk. *Submitted manuscript.*

Chantry, C. J., Morrison, P., Panchula, J., Rivera, C., Hillyer, G., Zorilla, C., et al. (2000). Effects of lipolysis or heat treatment on HIV-1 provirus in breast milk. *J Acquir Immune Defic Syndr, 24*(4), 325-329.

CIAC. (2006). *Rape in RSA for the period of April to March 2001/2002 to 2004/2005*: Crime Information Analysis Centre. Pretoria, South Africa.

Cooper, E. R., Charurat, M., Mofenson, L., Hanson, I. C., Pitt, J., Diaz, C., et al. (2002). Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. *Journal of Acquired Immune Deficiency Syndromes, 29*(5), 484-494.

Coovadia, H., Rollins, N., Bland, R., Little, K., Coutsoudis, A., Bennish, M., et al. (2007). Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. *Lancet, 369*(9567), 1107-1116.

Coutsoudis, A. (2003). Breastfeeding and risk of HIV transmission: an update. *Forum Nutr, 56*, 162-164.

Coutsoudis, A. (2006). New information to inform the debate around infant feeding by HIV positive mothers. *Perinatology, 8*(1), 1-8.

Coutsoudis, A., Coovadia, H., Pillay, K., & Kuhn, L. (2001). Are HIV-infected women who breastfeed at increased risk of mortality? *AIDS, 15*, 630-635.

Coutsoudis, A., Goga, A. E., Rollins, N., & Coovadia, H. M. (2002). Free formula milk for infants of HIV-infected women: Blessing or curse? *Health Policy and Planning, 17*(2), 154-160.

Coutsoudis, A., Pillay, K., Kuhn, L., Spooner, E., Tsai, W. Y., & Coovadia, H. M. (2001). Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: Prospective cohort study from Durban, South Africa. *AIDS, 15*(3), 379-387.

Coutsoudis, A., Pillay, K., Spooner, E., Kuhn, L., & Coovadia, H. M. (1999). Influence of infant-feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa: A prospective cohort study. *Lancet, 354*(9177), 471-476.

Creek, T., Arvelo, W., Kim, A., Lu, L., Bowen, A., Mach, O., et al. (2007). *A large outbreak of diarrhea among non-breastfed children in Botswana 2006 - Implications for HIV prevention strategies and child health* Paper presented at the XIV Conference on Retroviruses and Opportunistic Infections 2007. California, U.S.A.

Creek, T., Luo, C., & Quick, T. (2006). *HIV-exposed children highly affected by deadly outbreak of diarrhea and severe acute malnutrition - Botswana, 2006* Paper presented at the The 2006 HIV/AIDS Implementers Meeting of the President's Emergency Plan for AIDS Relief, Durban, South Africa.

Creswell, J. W. (1994). *Research design: Qualitative & quantitative approaches*: Thousand Oaks, CA: Sage Publications

Creswell, J. W. (2003). *Research Design: Qualitative, Quantitative, and Mixed Methods Approaches* Thousand Oaks, CA: Sage.

Dabis, F., Newell, M. L., Fowler, M., & Read, J. (2004). Prevention of HIV transmission through breastfeeding: strengthening the research agenda. *JAIDS, 35*, 167-168.

De Cock, K. M., Fowler, M. G., Mercier, E., De Vincenzi, I., Saba, J., Hoff, E., et al. (2000). Prevention of mother-to-child HIV transmission in resource-poor countries: Translating research into policy and practice. *Journal of the American Medical Association, 283*(9), 1175-1182.

De Paoli, M., Mkwanazi, D., & Rollins, N. (2006). *Rapid cessation of breastfeeding - a safe and feasible PMTC strategy?* Paper presented at the XVI International AIDS Conference August, 2006. Toronto, Canada.

Department of Health. (2000). *South African Breastfeeding Guidelines for Health Workers*. Pretoria, South Africa: South African Department of Health.

Department of Health (2007a). HIV and AIDS and STI, Strategic Plan For South Africa 2007-2011. Retrieved JANUARY 21, 2007 from http://www.doh.gov.za/docs/hivaids-progressrep.html.

Department of Health. (2007b). *National HIV and Syphilis antenatal sero-pravlence survey in South Africa, 2006.* Retrieved. JANUARY 21, 2007 FROM www.doh.gov.za/docs/hivaids.

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin, 24*(1), 5-28.

Doherty, T., Chopra, M., Jackson, D., Colvin, M., & Persson, L.A. (2007). Effectiveness of the WHO/UNICEF guidelines on infant feeding for HIV-positive women: results from a prospective cohort study in South Africa. *AIDS, 21*, 1791-1797.

Dorrington, R. E., Johnson, L. T., Bradshaw, D., & Daniel, T. (2006). *The Demographic Impact of HIV/AIDS in South Africa: National and Provincial Indicator for 2006.* Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa.

Dunn, T., Newell, M., Ades, A., & Peckham, C. (1992). Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet, 340*, 585–588.

Gibson, R., S. (2005). *Principles of Nutritional Assessment* (second edition ed.). New York: Oxford University Press, Inc.

Gillies, P. (1998). The effectiveness of alliances and partnerships for health promotion. *Health Promotion International, 13*, 99-120.

Glesne, C., & Peshkin, A. (1992). *Becoming qualitative researchers: An introduction.* White Plains, NY: Longman.

Gray, R. H., Li, X., Kigozi, G., Serwadda, D., Brahmbhatt, H., Wabwire-Mangen, F., et al. (2005). Increased risk of incident HIV during pregnancy in Rakai, Uganda: A prospective study. *Lancet, 366*(9492), 1182-1188.

Greengrass, V., Turnbull, S., Hocking, J., Dunne, A., Tachedjian, G., Corrigan, G., et al. (2005). Evaluation of a low cost reverse transcriptase assay for plasma HIV-1 viral load monitoring. *Curr HIV Res, 3*(2), 183-190.

Haider, R., Ashworth, A., Kabir, I., & Huttly, S. R. A. (2000). Effect of community-based peer counsellors on exclusive breastfeeding practices in Dhaka, Bangladesh: A randomised controlled trial. *Lancet, 356*(9242), 1643-1647.

Haider, R., Kabir, I., Huttly, S. R. A., & Ashworth, A. (2002). Training Peer Counselors to Promote and Support Exclusive Breastfeeding in Bangladesh. *Journal of Human Lactation, 18*(1), 7-12.

Hamosh, M. (1997). *Human milk: composition and function in the infant In: Fetal and Neonatal Physiology* (2 ed.). Philadelphia, PA. : W. B. Saunders.

Hanson, L. (2004). *Immunobiology of Human Milk: How breastfeeding Protects Babies.* Goteberg, Sweden: Pharmasoft Publishing.

Hanson, L., Ahlstedt, S., Carlsson, B., Fallstrom, S., Kaijser, B., Lindblad, B., et al. (1978). New knowledge in human milk immunoglobulin. *Acta Paediatr Scand 67*(5), 577-582.

Hecker, L. M., & Kotler, D. P. (1990). Malnutrition in patients with AIDS. *Nutrition Review, 48*, 393-401.

Hector, D., King, L., & Webb, K. (2005). Factors affecting breastfeeding practices: applying a conceptual framework. *N S W Public Health Bull, 16(3–4)* 52–55.

Iliff, P. J., Piwoz, E. G., Tavengwa, N. V., Zunguza, C. D., Marinda, E. T., Nathoo, K. J., et al. (2005). Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. *AIDS, 19*(7), 699-708.

Israel-Ballard, K., Chantry, C., Dewey, K., Lonnerdal, B., Sheppard, H., Donovan, R., et al. (2005). Viral, nutritional, and bacterial safety of flash-heated and pretoria-pasteurized breast milk to prevent mother-to-child transmission of HIV in resource-poor countries: A pilot study. *Journal of Acquired Immune Deficiency Syndromes, 40*(2), 175-181.

Israel-Ballard, K., Donovan, R., Chantry, C., Coutsoudis, A., Sheppard, H., Sibeko, L., et al. (2007). Flash-Heat Inactivation of HIV-1 in Human Milk: A Potential Method to Reduce Postnatal Transmission in Developing Countries. *J Acquir Immune Defic Syndr.* 45(3), 318-323.

Israel-Ballard, K., Maternowska, M. C., Abrams, B. F., Morrison, P., Chitibura, L., Chipato, T., et al. (2006). Acceptability of heat treating breast milk to prevent mother-to-child transmission of human immunodeficiency virus in Zimbabwe: a qualitative study. *J Hum Lact, 22*(1), 48-60.

Israel-Ballard, K. A., Abrams, B. F., Coutsoudis, A., Sibeko, L. N., Cheryk, L. A., & Chantry, C. J. (2008). Vitamin Content of Breastmilk from HIV-1 Infected Mothers Before and After Flash-heat Treatment. *JAIDS, in press*.

Jeffery, B., & Mercer, K. (2000). Pretoria Pasteurisation: A potential method for the reduction of postnatal mother to child transmission of the human immunodeficiency virus. *Journal of Tropical Pediatrics, 46*(4), 219-223.

Jeffery, B. S., Webber, L., Mokhondo, K. R., & Erasmus, D. (2001). Determination of the effectiveness of inactivation of human immunodeficiency virus by Pretoria pasteurization. *J Trop Pediatr, 47*(6), 345-349.

Jenning, C., Fiscus, S., Crowe, S., Danilovic, A., Morack, R., Scianna, S., et al. (2005). Comparison of two human immunodeficiency virus (HIV) RNA surrogate assays to the standard HIV RNA assay. *J Clin Microbiol, 43*(12), 5950-5956.

John-Stewart, G., Mbori-Ngacha, D., Ekpini, R., Janoff, E. N., Nkengasong, J., Read, J. S., et al. (2004). Breast-feeding and Transmission of HIV-1. *Journal of Acquired Immune Deficiency Syndromes, 35*(2), 196-202.

Johnson, W., Alons, C., Fidalgo, L., Piwoz, E., Kahn, S., Macombe, A., et al. (2006). *The Challenge of Providing Adequate Infant Nutrition Following Early Breatfeeding Cessation by HIV positive, Food-insecure Mozambican Mothers.* Paper presented at the 16th International AIDS Conference 2006. Toronto, Canada.

Jones, G., Steketee, R. W., Black, R. E., Bhutta, Z. A., & Morris, S. S. (2003). How many child deaths can we prevent this year? *Lancet, 362*(9377), 65-71.

Kourtis, A. P., Lee, F. K., Abrams, E., Jamieson, D. J., & Bulterys, M. (2006). Mother-to-child transmission of HIV-1: timing and implications for prevention. *Lancet Infectious Diseases, 6*(11), 726-732.

Kramer, M., & Kakuma, R. (2002). Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev, CD003517*(1).

Leroy, V., Becquet, R., Rouet, F., Ekouevi DK, Viho I, Bequet L, et al. (2004). *Postnatal transmission risk according to feeding modalities in children born to HIV-infected mothers in a PMTCT project in Abidjan, Côte d'Ivoire. DITRAME PLUS project ANRS 1201/1202.* . Paper presented at the XV International AIDS Conference 2004, Bangkok, Thailand.

Lincoln, Y., & Guba, E. (1985). *Naturalistic inquiry.* New York: Sage.

Lonnerdal, B. (1985). Biochemistry and physiological function of human milk proteins. *Am J Clin Nut, 42*, 1299–1317.

Lonnerdal, B. (2006). Recombinant human milk proteins. *Nestle Nutrition Workshop Series, Paediatric Programme.*(58), 207-215; discussion 215-207.

Malmsten, A., Shao, X., Aperia, K., Corrigan, G., Sandstrom, E., Kallander, C., et al. (2003). HIV-1 viral load determination based on reverse transcriptase activity recovered from human plasma. *J Med Virol.71*(3), 291-296.

McDougal, J. S., Pilcher, C. D., Parekh, B. S., Gershy-Damet, G., Branson, B. M., Marsh, K., et al. (2005). Surveillance for HIV-1 incidence using tests for recent infection in resource-constrained countries. *AIDS, Supplement, 19*(2), S25-S30.

Merriam, S. B. (1988). *Case study research in education: A qualitative approach*: San Francisco: Jossey-Bass.

Merriam, S. B. (1998). Designing the Study and Selecting a Sample. In *Qualitative research and case study applications in education*. San Francisco: Jossey Bass.

Moore, R. D. (1999). Human immunodeficiency virus infection, anemia, and survival. . *Clin Infect Dis., 29*, 29-33.

Morse, J. M. (1991). Approaches to qualitative-quantitative methodological triangulation. *Nursing Research, 40*(1), 120-123.

MRC. (2006). *South African National HIV Prevalence, HIV Incidence, Behaviour and Communication Survey, 2005*. Cape Town: South African Medical Research Council.

Nankunda, J., Tumwine, J. K., Soltvedt, A., Semiyaga, N., Ndeezi, G., & Tylleskar, T. (2006). Community based peer counsellors for support of exclusive breastfeeding: Experiences from rural Uganda. *International Breastfeeding Journal, 1(19) online journal.*

Nduati, R., John, G., Mbori-Ngacha, D., Richardson, B., Overbaugh, J., Mwatha, A., et al. (2000). Effect of breastfeeding and formula feeding on transmission of HIV-1: A randomized clinical trial. *Journal of the American Medical Association, 283*(9), 1167-1174.

Nduati, R., Richardson, B. A., John, G., Mbori-Ngacha, D., Mwatha, A., Ndinya-Achola, J., et al. (2001). Effect of breastfeeding on mortality among HIV-1 infected women: a randomised trial. *Lancet, 357*(9269), 1651-1655.

Newell, M.-L., Brahmbhatt, H., & Ghys, P. D. (2004). Child mortality and HIV infection in Africa: a review. *AIDS, 18 Suppl 2*, S27-S34.

Newell, M. (2006). Current issues in the prevention of mother-to-child transmission of HIV-1 infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 100*(1), 1-5.

O'Brien, M. E., Kupka, R., Msamanga, G. I., Saathoff, E., Hunter, D. J., & Fwazi, W. W. (2005). Anemia Is an Independent Predictor of Mortality and Immunologic Progression of Disease Among Women With HIV in Tanzania. *Journal of Acquired Immune Deficiency Syndromes, 40*(2), 219-225.

Onyango, C., Bagenda, D., Mubiru, M., Musoke, P., Fowler, P., Jackson, J., et al. (2007). *Early Breastfeeding Cessation among HIV-exposed Negative Infants and Risk of Serious Gastroenteritis: Findings from a Perinatal Prevention Trial in Kampala,*

Uganda. Paper presented at the in Fourteenth Conference on Retroviruses and Opportunistic Infections (CROI). California, USA.

Orloff, S.,Wallingford, J., & McDougal, J. (1993). Inactivation of human immuno-deficiency virus type 1 in human milk: effects of intrinsic factors in human milk and of pasteurization. *J Hum Lact, 9*(1), 13-17.

Papathakis, P. C., Van Loan, M. D., Rollins, N. C., Chantry, C. J., Bennish, M. L., & Brown, K. H. (2006). Body composition changes during lactation in HIV-infected and HIV-uninfected South African Women. *Journal of Acquired Immune Deficiency Syndromes, 43*, 467-474.

Patton, M. Q. (2002). *Qualitative research and evaluation methods* Thousand Oaks, Calif: Sage.

Phiri, W., Kasonka, L., Collin, S., Makasa, M., Sinkala, M., Chintu, C., et al. (2006). Factors influencing breast milk HIV RNA viral load among Zambian women. *AIDS Research and Human Retroviruses, 22*(7), 607-614.

Piwoz, E., Huffman, S., Lusk, D., Zehner, E., & O'Gara, C. (2001). *Early Breastfeeding Cessation as an Option for Reducing Postnatal Transmission of HIV in Africa.* Washington, DC: Academy for Educational Development.

Piwoz, E., & Preble, E. (2000). *HIV/AIDS and Nutrition: A Review of the Literature and Recommendations for Nutritional Care and Support in Sub-Saharan Africa.* SARA Project, AED. Washington, DC.

Piwoz, E., Ross, J., & Humphrey, J. (2004). Human immunodeficiency virus transmission during breastfeeding: knowledge, gaps, and challenges for the future. *Advances in Experimental Medicine & Biology, 554*, 195-210.

Rehle, T. M., Shisana, O., Pillay, V., Zuma, K., Puren, A., & Parker, W. (2007). National HIV incidence measures - New insights into the South African epidemic. *South African Medical Journal, 97*(3), 194-199.

Rollins, N., Meda, N., Becquet, R., Coutsoudis, A., Humphrey, J., Jeffrey, B., et al. (2004). Preventing Postnatal Transmission of HIV-1 Through Breast-feeding: Modifying Infant Feeding Practices. *Journal of Acquired Immune Deficiency Syndromes, 35*(2), 188-195.

Ross, J. S., Piwoz, E. G., & Habicht, J.-P. (2004). Is breastfeeding best for HIV-positive mothers?[comment]. *AIDS, 18*(2), 879-885.

Rutenberg, N. (2003). *HIV testing and disclosure: experiences from prevention of mother- to- child transmission programs in Kenya and Zambia. In Women's Experiences with HIV serodisclosure in Africa: implementation for VCT and PMTCT, pp. 7-11, report of a USAID, Technical meeting. USAID Office of HIV/AIDS.* Washington, DC.

Sabbaj, S., Edwards, B., & Ghosh, M. (2002). Human immunodeficiency virus-specific CD8+ T cells in human breast milk. *J of Virology, 76*, 7365-7373.

Sedgh, G., Spiegelman, D., Larsen, U., Msamanga, G., & Fawzi, W. W. (2004). Breastfeeding and maternal HIV-1 disease progression and mortality. *AIDS, 18*(7), 1043-1049.

Semba, R., Miott, i. P., & Chiphangwi, J. (1994). Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. *Lancet, 343*, 1593-1597.

Sibeko, L., Coutsoudis, A., Nzuza, S., & Gray-Donald, K. (2007a). Heat-Treated Expressed Breast Milk: An Acceptable Feeding Option For Use By Urban African Mothers Living With HIV. *Submitted manuscript*

Sibeko, L., Coutsoudis, A., Nzuza, S., & Gray-Donald, K. (2007b). Mothers' Infant Feeding Experiences: constraints and supports for optimal feeding in a HIV impacted urban community in South Africa. *Submitted manuscript.*

Sibeko, L., Dhansay, M., Charlton, K., Johns, T., & Gray-Donald, K. (2005). Beliefs, Attitudes and Practices of Breastfeeding Mothers from a Periurban Community in South Africa. *J Hum Lact, 21*(1), 31-38.

Sibeko, L., Mfeka, M., Naicker, D., Nzuza, S., Mtambo, T., Dlamini, L., et al. (2005). *Are hospital practices having a negative impact on the promotion of exclusive breastfeeding by HIV infected woman?* Paper presented at the 2nd South African AIDS Conference: Unity and Accountability 2005. Durban, South Africa

Sinkala, M., Kuhn, L., Kankasa, C., Kasonde, P., Vwalika, C., Mwiya, M., et al. (2007). *No benefit of early cessation of breastfeeding at 4 months on HIV-free survival of infants born to HIV-infected mothers in Zambia: The Zambia exclusive breastfeeding study. X.* Paper presented at the IV Conference on Retroviruses and Opportunistic Infections 2007.

Smith, M., & Kuhn, L. (2000). Exclusive breast-feeding: does it have the potential to reduce breast-feeding transmission of HIV-1? *Nutrition Review, 58*(11), 333-340.

Spradley, J. (1979). *The ethnographic interview.* Forth Worth, TX: Harcourt Brace Jovanovich College Publishers.

Steinberg, M., Johnson, S., Scheirhout, G., Ndegwa, D. (2002). *Hitting home: how households cope with the HIV/AIDS epidemic.* University of Cape Town. Henry J Kaiser Family Foundation. Menlo Park, U.S.A.

Thomas, T., Masaba, R., van Eijk, A., Nasokho, P., Thigpen, M., & Fowler, M. (2007). *Rates of diarrhea associated with early weaning among infants in Kisumu, Kenya* Paper presented at the XIV Conference on Retroviruses and Opportunistic Infections 2007. California, U.S.A.

UNAIDS. (2006). *Report on the global AIDS epidemic.* Geneva, Switzerland.WHO/ UNAIDS.

UNAIDS. (2007). *AIDS epidemic update.* Geneva, Switzerland.WHO/ UNAIDS.

UNAIDS/WHO. (2005). *AIDS epidemic update.* Geneva, Switzerland.WHO/ UNAIDS

UNICEF. (2000). *State of the World's Children 2000.* New York: UNICEF.

UNICEF. (2001). GlobalDatabase: Breastfeeding indicators. Retrieved MARCH 21, 2006 from http://www.childinfo.org/areas/breastfeeding/patternview.php?country=South Africa.

UNICEF. (2003). *State of the World's Children*. New York: UNICEF.

UNICEF. (2007). *The State Of The World's Children 2007*. New York:UNICEF.

United Nations (2000). United Nations General Assembly Special Session for Children. Retrieved MARCH 21, 2006 from http://www.un.org/av/photo/ga/sschildren02.html

US Bureau of the Census. (2001). *Recent HIV seroprevalence levels by country: June 2001*. Washington: US Bureau of the Census. Washington, DC.

van der Sande, M. A. B., Schim van der Loeff, M. F., Aveika, A. A., Sabally, S., Togun, T., Sarge-Njie, R., et al. (2004). Body Mass Index at Time of HIV Diagnosis, A Strong and Independent Predictor of Survival. *Journal of Acquired Immune Deficiency Syndromes, 37*(2), 1288-1294.

Van Esterik, P. (2002). Contemporary trends in infant feeding research. *Annual Review of Anthropology, 31*, 257-278.

Varga, C. A., Sherman, G. G., & Jones, S. A. (2006). HIV-disclosure in the context of vertical transmission: HIV-positive mothers in Johannesburg, South Africa. *AIDS Care - Psychological and Socio-Medical Aspects of AIDS/HIV, 18*(8), 952-960.

Victora, C., Smith, P., Vaughan, J., Nobre, L., Lombardi, C., Teixeira, A., et al. (1987) Evidence for protection by breast-feeding against infant diseases in Brazil. *Lancet, 2*, 319-322.

WHO. (2000a). Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality, Effect of breastfeeding on infant and child

mortality due to infectious diseases in less developed countries: a pooled analysis. *Lancet, 355*, 451-455.

WHO. (2000b). *Complementary Feeding. Family foods for breastfed children.* World Health Organization. Geneva.

WHO. (2001). *Breastfeeding and Replacement Feeding Practices In the Context Of Mother-To-Child-Transmission Of HIV: An Assessment Tool For Research.* Geneva, Switzerland: WHO.

WHO. (2005). *Nutrition risk factors throughout the life course. Nutrition for Health and Development*. Geneva: WHO.

WHO. (2006). *Consensus statement: held on behalf of the Inter-Agency Task Team (IATT) on Prevention of HIV infections in Pregnant Women, Mothers and Their Infants. Presented at: WHO HIV and Infant Feeding Technical Consultation..* Geneva. WHO.

WHO (2007a). Gender and HIV infections. Retrieved MAY 10, 2007 from http://www.who.int/gender/hiv_aids/en/

WHO. (2007b). *Towards universal access : scaling up priority HIV/AIDS interventions in the health sector : progress report, April 2007.* Switzerland: WHO.

WHO, UNICEF, UNAIDS, & UNFPA. (2004). *HIV and infant feeding: a guide for health-care managers and supervisors.* Geneva: WHO.

WHO/AFRO. (2006). WHO Urges Countries to Scale Up Collaboration Between Traditional and Conventional Health Practitioners in Prevention of HIV/AIDS Retrieved JULY 26, 2007, from http://www.afro.who.int/note_press/2006/pr20060831.html

WHO/UNAIDS/UNICEF. (1998). *Technical Consultation on HIV and Infant Feeding.* Geneva, Switzerland: WHO.

WHO/UNICEF/UNAIDS/UNFPA. (2004). *HIV and infant feeding: A guide for health-care managers and supervisors.* Geneva, Switzerland: WHO.

Willumsen, J. F., Filteau, S. M., Coutsoudis, A., Newell, M. L., Rollins, N. C., Coovadia, H. M., et al. (2003). Breastmilk RNA viral load in HIV-infected South African women: Effects of subclinical mastitis and infant feeding. *AIDS, 17*(3), 407-414.

Wolard, I. (2002). *An overview of poverty and inequality in South Africa* Southern African Regional Poverty Network (SARPN) South Africa.

Yun, Z., Fredriksson, E., & Sonnerborg, A. (2002). Quantification of human immunodeficiency virus typ 1 proviral DNA by the TaqMan real-time PCR assay. J Clin Microbiol, 40(10), 3883-3884.

APPENDIX I

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Flash-Heat Inactivation of HIV-1 in Human Milk

A Potential Method to Reduce Postnatal Transmission in Developing Countries

Kiersten Israel-Ballard, MPH, Richard Donovan, PhD,† Caroline Chantry, MD,‡ Anna Coutsoudis, PhD,§ Haynes Sheppard, PhD,† Lindiwe Sibeko, MSc,‖ and Barbara Abrams, DrPH**

Background: Up to 40% of all mother-to-child transmission of HIV occurs by means of breast-feeding; yet, in developing countries, infant formula may not be a safe option. The World Health Organization recommends heat-treated breast milk as an infant-feeding alternative. We investigated the ability of a simple method, flash-heat, to inactivate HIV in breast milk from HIV-positive mothers.

Methods: Ninety-eight breast milk samples, collected from 84 HIV-positive mothers in a periurban settlement in South Africa, were aliquoted to unheated control and flash-heating. Reverse transcriptase (RT) assays (lower detection limit of 400 HIV copies/mL) were performed to differentiate active versus inactivated cell-free HIV in unheated and flash-heated samples.

Results: We found detectable HIV in breast milk samples from 31% (26 of 84) of mothers. After adjusting for covariates, multivariate logistic regression showed a statistically significant negative association between detectable virus in breast milk and maternal $CD4^+$ T-lymphocyte count ($P = 0.045$) and volume of breast milk expressed ($P = 0.01$) and a positive association with use of multivitamins ($P = 0.03$). All flash-heated samples showed undetectable levels of cell-free HIV-1 as detected by the RT assay ($P < 0.00001$).

Conclusions: Flash-heat can inactivate HIV in naturally infected breast milk from HIV-positive women. Field studies are urgently needed to determine the feasibility of in-home flash-heating breast milk to improve infant health while reducing postnatal transmission of HIV in developing countries.

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It is estimated that approximately 40% of the 700,000 children who become infected with HIV each year contract the virus by means of prolonged breast-feeding.[1,2] Completely avoiding breast-feeding is not an option for many HIV-positive mothers in resource-poor areas, however, because of the cost of infant formula, unsafe water, unsanitary conditions, and sociocultural factors. In addition, the bioactive immune properties of breast milk confer protection to the infant, resulting in decreased rates of morbidity and mortality attributable to diarrheal, respiratory, and other infections compared with formula-fed infants.[3–6]

In light of this, current World Health Organization (WHO) recommendations state that when replacement-feeding options are acceptable, feasible, affordable, safe, and sustainable (AFASS), avoidance of all breast-feeding is recommended; otherwise, exclusive breast-feeding is recommended for the first 6 months of life, followed by weaning only if a nutritionally adequate and safe diet is maintained.[5,7,8] This recommendation is based on several studies that suggest the risk of HIV transmission among infants exclusively breast-fed was lower than that of those fed a mixture of breast milk and other liquids or solids.[9,10]

Modifications to breast-feeding are recommended by the WHO to reduce the risk of HIV transmission while providing breast milk's immune properties to protect the infant against other common childhood infections. One recommended alternative is manually expressed heat-treated breast milk.[7] The WHO lists 2 heating methods: (1) direct boiling, shown to cause significant nutritional damage,[11] and (2) pasteurization. The pasteurization method most commonly used in breast milk banks is Holder pasteurization (62.5°C for 30 minutes), which has been reported to inactivate HIV while retaining most of breast milk's protective elements;[12–15] however, it requires temperature gauges and timing devices that are unavailable in most at-risk communities.[16]

Flash-heat is a recently developed, simple pasteurization method that a mother in a developing country could implement over an outdoor fire or in her kitchen. Our pilot data suggest that the flash-heat method is capable of inactivating cell-free clade C HIV-1 in "spiked" breast milk samples from healthy

mothers in the United States, while retaining most of the milk's nutritional and antimicrobial value.[17,18] The objective of this study was to confirm that flash-heat can inactivate HIV in naturally infected breast milk from HIV-positive mothers.

METHODS

HIV-positive breast-feeding mothers not currently receiving antiretrovirals or antibiotics were recruited during postnatal clinic visits at an informal periurban settlement in Durban, South Africa, between October 2004 and July 2005. Eighty-four women agreed to participate in this study; after giving written informed consent, they provided a blood sample and gave a total of 98 breast milk samples (some women donated additional breast milk samples on subsequent visits). The women were instructed to wash their hands with soap and water and then manually express 75 to 150 mL of breast milk into sterile, locally obtained glass jars that were provided. Lactation consultants were available if mothers needed assistance with manual expression. Breast milk samples were immediately placed in an ice-water bath and transported to the laboratory. Fifty milliliters of each expressed breast milk sample was aliquoted to be flash-heated in the same glass jar, and the remaining volume was aliquoted to be used as an unheated control. Laboratory analyses of plasma and breast milk samples were performed at the Nelson Mandela School of Medicine, University of KwaZulu-Natal in Durban, South Africa, and the Viral and Rickettsial Disease Laboratory at the California Department of Health Services in Richmond, California, respectively.

The flash-heat method has been described elsewhere.[17] Briefly, 50 mL of expressed breast milk in an uncovered sterile 16-oz (455-mL) commercial glass food jar was placed in 450 mL of water in a Hart brand 1-qt aluminum pan (Hendler and Hart, Cape Town, South Africa) purchased locally. Water and milk were heated together over a single-burner butane stove, used to imitate the intense heat of a fire, until the water reached 100°C and was at a rolling boil. The breast milk was immediately removed from the water and allowed to cool to 37°C. Temperature data were collected at 15-second intervals using thermometer probes (Cole-Palmer Digi-Sense DuaLogR Thermocouple Thermometers, Vernon Hills, IL). Flash-heating typically reached temperatures greater than 56°C for 6 minutes 15 seconds and peaked at 72.9°C. Flash-heated and unheated breast milk samples were then divided into 1.5-mL aliquots and immediately stored at −80°C. Samples were shipped frozen on dry ice to the California Department of Health Services Viral and Rickettsial Disease Laboratory for viral analysis.

To determine which samples initially had detectable HIV-1, we performed TaqMan Real Time-RNA-PCR (TaqMan RT-PCR, Applied Biosystems, Foster City, CA), which has a sensitivity of 50 RNA copies/mL, on the 98 unheated breast milk samples. RNA was extracted using the Viral RNA Kit (Qiagen, Valencia, CA). The TaqMan RT-PCR assay methods have been described elsewhere.[19] Because polymerase chain reaction (PCR) assays detect only viral nucleic acid and do not distinguish between viable and nonviable HIV, we used an alternate assay to assess the inactivation of HIV remaining after heat treatment.[17] Because previous studies have shown the reverse transcriptase (RT) enzymatic activity assay to correlate well with RNA quantification[20,21] and because functional RT is required for cellular infection, we chose quantitative measurement of RT activity as a marker for the presence of viable HIV in heated versus unheated breast milk (ExaVir Quantitative HIV-Reverse Transcriptase Load Kit; Cavidi, Uppsala, Sweden). The sensitivity of this RT assay was 400 copies/mL. To apply this methodology to breast milk, the manufacturer's instructions for plasma were followed, with the exception of additional readings at 30-minute intervals starting from time 0 through 4 hours and then overnight. Plasma $CD4^+$ T-lymphocyte assays were performed.

We used the Student t test, χ^2 test of independence, and bivariate and multivariate logistic regression to analyze and compare demographic data from the 84 recruited women. We identified variables a priori that were potential predictors of breast milk viral load and volume, based on previous evidence[22] and theoretic assumptions. Mothers were categorized as having detectable or undetectable breast milk HIV. For mothers who gave multiple samples, this was based on the sample from their initial visit only, because all maternal characteristics, including $CD4^+$ T-lymphocyte count and sociodemographic data, were collected at this visit. For logistic regression, all variables were analyzed as continuous, with the exception of maternal $CD4^+$ T-lymphocyte counts, which were assigned binary categoric values of $<$ or >500 cells/mm^3. For analysis of HIV inactivation and to compare concentrations of HIV in unheated versus flash-heated breast milk, we used the Sign test. RT levels less than the assay limit of quantification (400 copies/mL) were replaced by an imputed value of 400 copies/mL to quantify the undetectable levels of HIV conservatively. All statistical analyses were performed using STATA, version 8.0 (Stata Corporation, College Station, TX).

Our study was approved by the Committees for the Protection of Human Subjects at the Universities of California at Berkeley and Davis and the University of KwaZulu-Natal, Durban, South Africa.

RESULTS

Table 1 shows the maternal characteristics and sociodemographic data from the 84 HIV-positive mothers who were enrolled in this study and compares the unadjusted associations between mothers with detectable HIV in their breast milk versus those with undetectable HIV. If women provided more than 1 breast milk sample, only the first sample provided was included for this analysis. We found detectable HIV, as determined by the RT assay, in breast milk samples from 31% (26 of 84) of mothers.

We investigated the association between mothers with detectable HIV in their breast milk (>400 HIV copies/mL) and maternal characteristics that were selected a priori. Bivariate analyses showed statistically significant associations between detectable breast milk viral load and maternal age (odds ratio [OR] = 1.10, 95% confidence interval [CI]: 1.01 to 1.21), $CD4^+$ T-lymphocyte count (OR = 0.21, 95% CI: 0.07 to 0.63), use of multivitamins (OR = 4.70, 95% CI: 1.23 to

TABLE 1. Characteristics of Mothers With and Without Detectable Breast Milk HIV in Unheated Controls

Characteristic	All Enrolled Mothers (n = 84)	Mothers With No Detectable HIV in Unheated EBM (n = 58)	Mothers With Detectable HIV in Unheated EBM (n = 26)	P*
Maternal age (y), mean (SD)	25.8 (5.4)	24.9 (5.7)	27.8 (4.5)	0.02
Maternal $CD4^+$ count (cells/mm^3), mean (SD)	502.1 (234.8)	551.1 (230.6)	398.4 (212.3)	0.01
Maternal HgB (g/dL), mean (SD)	11.6 (1.5)	11.7 (1.6)	11.2 (1.2)	0.17
Maternal weight (kg), mean (SD)	66.7 (10.8)	66.2 (9.8)	68.0 (13.0)	0.49
Maternal height (m), mean (SD)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)	0.51
Maternal BMI (kg/m^2), mean (SD)	26.8 (4.0)	26.7 (3.8)	27.1 (4.4)	0.65
Maternal triceps skinfold (mm), mean (SD)	14.8 (5.5)	14.8 (5.9)	14.8 (4.4)	0.96
Currently taking antibiotics, n (%)				0.81
Yes	4 (4.8)	3 (5.2)	1 (3.9)	
No	75 (89.3)	52 (89.7)	23 (88.5)	
Missing	5 (6.0)	3 (5.2)	2 (7.7)	
Currently taking multivitamins, n (%)				0.02
Yes	11 (13.1)	4 (6.9)	7 (26.9)	
No	70 (83.3)	51 (87.9)	19 (73.1)	
Missing	3 (3.6)	3 (5.2)	0 (0.0)	
Amount expressed (mL), mean (SD)	87.1 (17.0)	91.0 (18.4)	78.2 (7.9)	0.001
Time for manual expression (min), mean (SD)	74 (102.9)	68 (91.2)	66 (52.2)	0.92
Mode of current infant feeding, n (%)				0.55
EBF	65 (77.4)	45 (77.6)	20 (76.9)	
PBF	2 (2.4)	2 (3.5)	0 (0.0)	
MF	16 (19.1)	10 (17.2)	6 (23.1)	
Missing	1 (1.2)	1 (1.7)	0 (0.0)	
Mode of infant feeding from previous child, n				0.27
EBF	17 (20.2)	11 (19.0)	6 (23.1)	
PBF	0 (0.0)	0 (0.0)	0 (0.0)	
MF	33 (39.3)	25 (43.1)	8 (30.8)	
Formula	3 (3.6)	1 (1.7)	2 (7.7)	
Missing	31 (36.9)	21 (36.2)	10 (38.5)	
History of manual expression, n (%)				0.31
Yes	29 (34.5)	18 (31.0)	11 (42.3)	
No	45 (53.6)	33 (56.9)	12 (46.2)	
Missing	10 (11.9)	7 (12.1)	3 (11.5)	
Electricity, n (%)				0.31
Yes	16 (19.1)	12 (20.7)	4 (15.4)	
No	30 (35.7)	18 (31.0)	12 (46.2)	
Missing	38 (45.2)	28 (48.3)	10 (38.5)	
Access to a refrigerator, n (%)				0.77
Yes	15 (17.9)	11 (19.0)	4 (15.4)	
No	59 (70.2)	41 (70.7)	18 (69.2)	
Missing	10 (11.9)	6 (10.3)	4 (15.4)	
Piped water source, n (%)				0.59
Piped inside	26 (31.0)	16 (27.6)	10 (38.5)	
Public tap	24 (28.6)	16 (27.6)	8 (30.8)	
Piped in yard	24 (28.6)	18 (31.0)	6 (23.1)	
Missing	10 (11.9)	8 (13.8)	2 (7.7)	
Fuel for cooking, n (%)				0.74
Paraffin	56 (66.7)	37 (63.8)	19 (78.1)	
Electricity	17 (20.2)	12 (20.7)	5 (19.2)	
Gas	1 (1.2)	1 (1.7)	0 (0.0)	
Missing	10 (11.9)	8 (13.8)	2 (7.7)	

TABLE 1. *(continued)* Characteristics of Mothers With and Without Detectable Breast Milk HIV in Unheated Controls

Characteristic	All Enrolled Mothers (n = 84)	Mothers With No Detectable HIV in Unheated EBM (n = 58)	Mothers With Detectable HIV in Unheated EBM (n = 26)	P*
Times of cooking per day, n (%)				0.66
1 time	47 (56.0)	33 (59.9)	14 (53.9)	
2 times	18 (21.4)	14 (24.1)	4 (15.4)	
3 times	2 (2.4)	1 (1.7)	1 (3.9)	
Missing	17 (20.2)	10 (17.2)	7 (26.9)	
Infant age (wk), mean (SD)	13.4 (9.5)	12.8 (9.8)	14.6 (8.6)	0.43
Birth weight (g), mean (SD)	3091.8 (475.9)	3088.3 (516.7)	3100.0 (375.8)	0.92

*Paired Student t test for continuous variables and χ^2 test of independence for categoric variables.
BMI indicates body mass index; EBF, exclusive breast-feeding; HgB, hemoglobin; MF, mixed feeding; PBF, predominantly breast-feeding.

17.88), and volume of breast milk expressed (OR = 0.93, 95% CI: 0.88 to 0.97). These variables, along with other potential predictors of breast milk viral load, were retained in our final model. In the multivariate logistic regression analysis shown in Table 2, controlling for maternal hemoglobin, age, height, weight, triceps skinfold thickness, $CD4^+$ T-lymphocyte counts, use of multivitamins, volume of breast milk expressed, and infant age, we found a statistically significant association between the odds of detectable HIV in breast milk and a decrease in maternal $CD4^+$ T-lymphocyte count ($P = 0.045$) and the volume of breast milk expressed ($P = 0.006$) and an increase in multivitamin use ($P = 0.03$). Multivariate linear regression was used to determine if infant age or mode of infant feeding was a significant predictor of the volume of breast milk expressed; a significant association was not observed, whether or not the previous covariates were retained in the model.

Of the total 98 breast milk samples tested before heating, 3 samples were positive for HIV RNA by TaqMan RT-PCR but were less than the level of RT detection. Thirty samples were positive for HIV RNA by TaqMan RT-PCR and for RT by the RT assay. Thus, the RT assay was effective in determining the viral status of 91% of the breast milk samples known to be positive by TaqMan RT-PCR.

TABLE 2. Multivariate Logistic Regression Analysis Results Showing the Adjusted Relation Between Maternal Characteristics and Detectable HIV in Breast Milk

Breast Milk Viral Load >400 Copies/mL	OR (95% CI)
Maternal age (y)	1.03 (0.91 to 1.17)
Maternal $CD4^+$ count (>500 cells/mm^3)*	0.18 (0.03 to 0.96)
Maternal HgB (g/dL)	0.65 (0.40 to 1.05)
Maternal weight (kg)	1.07 (0.98 to 1.16)
Maternal height (cm)	1.02 (0.92 to 1.13)
Maternal triceps skin fold (mm)	0.95 (0.82 to 1.10)
Multivitamins used*	15.72 (1.26 to 195.53)
Amount of breast milk expressed (mL)*	0.90 (0.83 to 0.97)
Infant age at collection (wks)	1.04 (0.93 to 1.17)

*Statistically significant results.
HgB indicates hemoglobin.

To compare the impact of flash-heat on breast milk viral load, we assayed the unheated and flash-heated aliquots of these 30 samples using the RT assay. The 30 unheated breast milk samples had an arithmetic mean of 8266 HIV copies/mL (SD = 15,376) and a mean log of 3.45 HIV copies/mL (SD = 0.586); however, all the corresponding flash-heated samples (100%) showed undetectable levels of HIV in the RT assay (Fig. 1; see Table 2). This difference in viral loads between the 30 unheated and corresponding flash-heated breast milk samples was highly statistically significant ($P < 0.00001$).

DISCUSSION

These data demonstrate that a simple pasteurization method, flash-heat, can inactivate cell-free HIV-1 in naturally

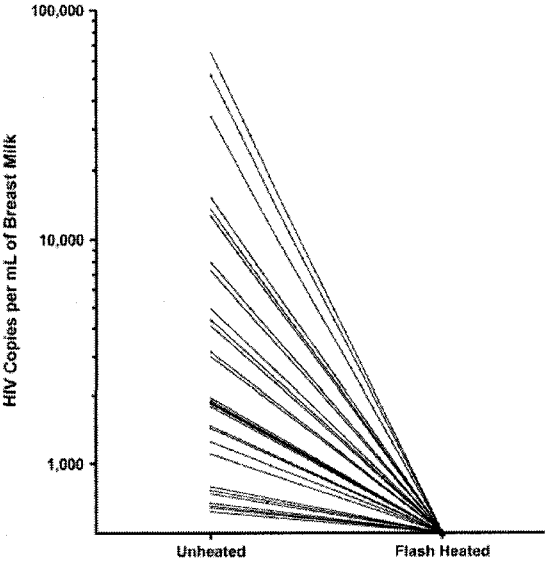

FIGURE 1. Log scale comparison of copies/mL of cell-free HIV-1 as detected by RT activity in unheated versus flash-heated naturally infected breast milk samples (n = 30).

infected breast milk from HIV-positive women. After flash-heating, HIV was undetectable by the RT assay in all 30 samples that had detectable HIV in unheated controls. This finding confirms our previous pilot data, which showed that flash-heat was capable of inactivating much greater concentrations of HIV in breast milk spiked with cell-free clade C HIV-1.[17]

We have previously shown that TaqMan RT-PCR assays are not useful in differentiating active virus versus inactive viral fragments,[17] a problem described previously in evaluating heat resistance of other viruses.[23] Similarly, studies have shown that coculture, although considered the "gold standard" for determining infectivity, is not an effective method for demonstrating inactivation of HIV in breast milk because of the assay's inherent insensitivity,[24] particularly in breast milk, because of its antiviral activity. Indeed, such data and our experience with HIV isolation suggest that only a small fraction (if any) of the 30 RT-positive samples would have been positive by HIV isolation cocultures. Thus, neither TaqMan RT-PCR nor coculture is an appropriate method for assessing HIV activity that remains after heating. The RT assay allowed us to compare a surrogate of infectious HIV in unheated and flash-heated breast milk. Although the lower limit of detection of the RT assay is 400 HIV copies/mL and, theoretically, a minimal amount of infectivity could remain in the samples showing undetectable levels of RT, the RT assay has greater sensitivity than would coculture, and was thus our method of choice. Moreover, even in samples "spiked" with high concentrations of HIV, our previous study showed undetectable levels of RT after heating.[17] The RT enzyme as a proxy for HIV infectivity could be an underestimate of viral inactivation because of the greater heat resistance of enzymes compared with surface proteins and the viral envelope, which, if disrupted, first could render the virus particle noninfectious before destruction of the RT enzyme.[25]

After controlling for other maternal characteristics, detectable breast milk viral load was significantly associated with an increased likelihood of multivitamin use and lower $CD4^+$ T-lymphocyte counts and volumes of breast milk expressed. Although we found only borderline statistical significance, previous studies have shown lower maternal $CD4^+$ T-lymphocyte counts to be predictors of increased breast milk viral load and a higher probability of transmission of HIV.[22] Likewise, previous studies have shown multivitamin intake to be protective against HIV progression;[26] yet, we found an increased risk of breast milk viral load with multivitamin use. Wide confidence intervals associated with multivitamin use, however, limit our ability to interpret these data. Factors not included in our data collection, such as fatigue and stress, may have contributed to the amount of breast milk expressed, and thus limit our understanding of the lower milk volumes expressed by some participants.[15] It is noteworthy that regardless of our findings, expressed milk volume was still substantial from mothers with a detectable breast milk viral load, with a mean of >78 mL. Moreover, there was no difference in the average time it took these mothers to express their breast milk manually, suggesting that this process was no more difficult for them than for the other mothers with less advanced disease.

Further investigation to confirm the nutritional, immunologic, and antimicrobial safety of flash-heated breast milk is needed, although preliminary data are promising.[17,27] This study investigated only 1 standardized heating protocol, using 50 mL of breast milk heated in a 450-mL water bath over a butane stove. Additional research is needed to determine if variations in milk and water volumes, jar or pan size or shape, heat source, and even altitude have an impact on the effectiveness of flash-heat. Although the WHO recommends heat treatment, if the flash-heat method is used in communities, we caution that the heating protocol described here should be strictly adhered to until additional field tests and thermal inactivation studies are completed and we better understand the margin of heating error allowable to ensure HIV destruction. Furthermore, the RT assay was limited to detection of cell-free virus only; however, recent data suggest that cell-associated HIV may play a more important role in transmission of HIV by means of breast-feeding than does cell-free virus.[28] More research is needed to address the impact of flash-heat on cell-associated HIV-1, although we hypothesize that cell-associated infectivity would be destroyed along with the cell under flash-heat conditions.

Although heat-treated breast milk may be used from birth, it may be most feasible during times of increased transmission risk, such as during mastitis[29] and, perhaps more practically, during or after the transition from exclusive breast-feeding to replacement feeds or the addition of complementary foods. Milk production would be well established at this point, and other complementary foods would then be an additional source of infant nutrition. Recent data have described several risks encountered during the weaning period. Inadequate feeding practices attributable to the lack of sufficient nutrition could result in impaired growth and malnutrition, especially without the immune protection from breast milk.[30,31] Moreover, a study in Zambia found a significant increase in breast milk viral load during the weaning period, suggesting that if a mother were to breast-feed during this time, the risk of transmission would be increased.[32] Perhaps heat-treated breast milk could be used during these high-risk times and could be viewed as a complementary food that is HIV-free, nutritious, affordable, and available.

In summary, our findings indicate that flash-heat is capable of inactivating cell-free HIV-1 in naturally infected breast milk from HIV-positive mothers. Field studies are urgently needed to determine the feasibility of flash-heating breast milk in a home setting and its ability to improve overall infant health while reducing the risk of postnatal HIV transmission in resource-poor settings.

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K. Israel-Ballard participated in study conception, study design, recruitment, data collection, data analysis, data interpretation, and manuscript preparation. R. Donovan participated in study design, virology analysis, data interpretation, and manuscript preparation. C. Chantry participated in study conception, study design, data interpretation, and manuscript preparation. A. Coutsoudis participated in study conception, study design, recruitment, data collection, data interpretation, and manuscript preparation. H. Sheppard participated in study design, virology analysis, data interpretation, and manuscript preparation. L. Sibeko participated in study design, recruitment, data collection, data interpretation, and manuscript preparation. B. Abrams participated in study conception, study design, data interpretation, and manuscript preparation.

REFERENCES

1. United Nations Program on HIV/AIDS, World Health Organization. *AIDS Epidemic Update*. Geneva, Switzerland: UNAIDS; 2005.
2. Kourtis AP, Lee FK, Abrams EJ, et al. Mother-to-child transmission of HIV-1: timing and implications for prevention. *Lancet Infect Dis*. 2006; 2006:726–732.
3. Creek T, Lao C, Quick T. HIV-exposed children highly affected by deadly outbreak of diarrhea and severe acute malnutrition—Botswana. 2006 [late breaker abstract 1]. Presented at: 2006 HIV/AIDS Implementers Meeting of the President's Emergency Plan for AIDS Relief; 2006; Durban.
4. Bahl R, Frost C, Kirkwood BR, et al. Infant feeding patterns and risks of death and hospitalization in the first half of infancy: multicentre cohort study. *Bull World Health Organ*. 2005;83:418–426.
5. Fowler MG, Newell ML. Breast-feeding and HIV-1 transmission in resource-limited settings. *J Acquir Immune Defic Syndr*. 2002;30:230–239.
6. Coutsoudis A, Goga AE, Rollins N, et al. Free formula milk for infants of HIV-infected women: blessing or curse? *Health Policy Plan*. 2002;17: 154–160.
7. WHO/UNICEF/UNAIDS/UNFPA. *HIV and Infant Feeding: A Guide for Health-Care Managers and Supervisors*. Geneva, Switzerland: WHO; 2004.
8. World Health Organization. Consensus statement: held on behalf of the Inter-Agency Task Team (IATT) on Prevention of HIV Infections in Pregnant Women, Mothers, and Their Infants. Presented at: WHO HIV and Infant Feeding Technical Consultation; 2006; Geneva.
9. Coutsoudis A, Pillay K, Kuhn L, et al. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. *AIDS*. 2001;15:379–387.
10. Iliff PJ, Piwoz EG, Tavengwa NV, et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. *AIDS*. 2005;19:699–708.
11. Welsh JK, May JT. Anti-infective properties of breast milk. *J Pediatr*. 1979;94:1–9.
12. Odoff SL, Wallingford JC, McDougal JS. Inactivation of human immunodeficiency virus type 1 in human milk: effects of intrinsic factors in human milk and of pasteurization. *J Hum Lact*. 1993;9: 13–17.
13. Eglin RP, Wilkinson AR. HIV infection and pasteurisation of breast milk. *Lancet*. 1987;1:1093.
14. McDougal JS, Martin LS, Cort SP, et al. Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotropic virus-III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. *J Clin Invest*. 1985;76:875–877.
15. Lawrence R, Lawrence R. *Breastfeeding: A Guide for the Medical Profession*. 6th ed. Philadelphia: Elsevier Mosby; 2005.
16. Israel-Ballard KA, Maternowska MC, Abrams BF, et al. Acceptability of heat treating breast milk to prevent mother-to-child transmission of human immunodeficiency virus in Zimbabwe: a qualitative study. *J Hum Lact*. 2006;22:48–60.
17. Israel-Ballard K, Chantry C, Dewey K, et al. Viral, nutritional, and bacterial safety of flash-heated and Pretoria-pasteurized breast milk to prevent mother-to-child transmission of HIV in resource-poor countries: a pilot study. *J Acquir Immune Defic Syndr*. 2005;40:175–181.
18. Israel-Ballard K, Coutsoudis A, Chantry CJ, et al. Bacterial safety of flash-heated and unheated expressed breastmilk during storage. *J Trop Pediatr*. 2006;52:399–405.
19. Yun Z, Fredriksson E, Sonnerborg A. Quantification of human immunodeficiency virus type 1 proviral DNA by the TaqMan real-time PCR assay. *J Clin Microbiol*. 2002;40:3883–3884.
20. Seyoum E, Wolday D, Girma M, et al. Reverse transcriptase activity for quantitation of HIV-1 subtype C in plasma: relation to RNA copy number and CD4 T-cell count. *J Med Virol*. 2006;78:161–168.
21. Sivapalasingam S, Essajee S, Nyambi PN, et al. Human immunodeficiency virus (HIV) reverse transcriptase activity correlates with HIV RNA load: implications for resource-limited settings. *J Clin Microbiol*. 2005; 43:3793–3796.
22. Richardson BA, John-Stewart GC, Hughes JP, et al. Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. *J Infect Dis*. 2003;187:736–740.
23. Nuanualsuwan S, Cliver DO. Infectivity of RNA from inactivated poliovirus. *Appl Environ Microbiol*. 2003;69:1629–1632.
24. Piatak M Jr, Saag MS, Yang LC, et al. Determination of plasma viral load in HIV-1 infection by quantitative competitive polymerase chain reaction. *AIDS*. 1993;7(Suppl 2):S65–S71.
25. Flint SJ, Enquist LW, Racaniello VR, et al. *Principles of Virology*. 2nd ed. Washington, DC: ASM Press; 2004.
26. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med*. 2004;351:23–32.
27. Israel-Ballard K, Coutsoudis A, Sturm W, et al. Bacterial safety of flash-heated and unheated breast milk samples during storage. Presented at: Academy of Breastfeeding Medicine Meeting; 2005; Denver.
28. Rousseau CM, Nduati RW, Richardson BA, et al. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. *J Infect Dis*. 2004;190:1880–1888.
29. Rollins N, Meda N, Becquet R, et al. Preventing postnatal transmission of HIV-1 through breast-feeding: modifying infant feeding practices. *J Acquir Immune Defic Syndr*. 2004;35:188–195.
30. Becquet R, Leroy V, Ekouevi DK, et al. Complementary feeding adequacy in relation to nutritional status among early weaned breastfed children who are born to HIV-infected mothers: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. *Pediatrics*. 2006;117:e701–e710.
31. Johnson W, Alons C, Fidalgo L, et al. The challenge of providing adequate infant nutrition following early breastfeeding cessation by HIV-positive, food-insecure Mozambican mothers. Presented at: XVI International AIDS Conference; 2006; Toronto.
32. Thea DM, Aldrovandi G, Kankasa C, et al. Post-weaning breast milk HIV-1 viral load, blood prolactin levels and breast milk volume. *AIDS*. 2006; 20:1539–1547.

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Bacterial Safety of Flash-heated and Unheated Expressed Breastmilk during Storage

by K. Israel-Ballard,[a] A. Coutsoudis,[b] C. J. Chantry,[c] A. W. Sturm,[d] F. Karim,[d] L. Sibeko,[e] *and* B. Abrams[a]

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Summary

Heat-treated breastmilk is one infant-feeding option recommended by the WHO to reduce mother-to-child transmission of HIV in developing countries. Flash-heat, a simple pasteurization method that a mother could perform in her home, has been shown to inactivate cell-free HIV-1. Since heating may affect the naturally occurring antimicrobial properties found in breastmilk, storing heated breastmilk may present a safety issue in resource-poor settings due to lack of refrigeration and potential contamination. To address this, we investigated the ability of flash-heat to eliminate bacteria and to prevent growth over time compared with unheated breastmilk. We collected breastmilk samples from 38 HIV positive mothers in South Africa and aliquoted them to flash-heated and unheated controls. Samples were stored at room temperature for 0, 2, 6 and 8 h and then plated and incubated for 24 h at 37°C in CO_2. We performed total colony counts and identified *Escherichia coli*, *Staphylococcus aureus* and Group A and Group B streptococci. Unheated samples had a significantly higher number of samples positive for bacterial growth at each time point ($p < 0.0001$), as well as mean colony-forming units (CFU)/ml in those samples that were positive at each time point ($p < 0.0001$). In addition, unheated samples had a significantly higher rate of bacterial propagation over time than flash-heated samples when comparing log values of CFU/ml across 0–8 h ($p < 0.005$). No pathogenic growth was observed in the flash-heated samples, while the unheated samples showed growth of *E. coli* ($n = 1$) and *S. aureus* ($n = 6$). Our data suggest that storage of flash-heated breastmilk is safe at room temperature for up to 8 h.

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Introduction

Mother-to-child transmission (MTCT) is responsible for ~90% of the 725000 HIV infections that occur each year among the children of the world, of which 90% are in sub-Saharan Africa [1]. Breastfeeding for extended periods is widespread and is responsible for one-third to one-half of paediatric HIV infections in sub-Saharan Africa. Even the low-cost, two-dose nevirapine prophylaxis does not substantially decrease the transmission from prolonged breast-feeding [2, 3]. For HIV positive mothers in developing countries, complete avoidance of breastfeeding may not be a safe option due to cost, lack of safe water, unsanitary conditions and socio-cultural factors. In addition, formula-fed infants who lack the immune protection conferred by breastmilk experience increased rates of morbidity and mortality due to diarrhoeal, respiratory and other infections [4–8].

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In light of this, the current World Health Organization (WHO) guidelines stipulate that HIV positive women avoid breastfeeding when replacement feeding options are acceptable, feasible, affordable, safe and sustainable. If these conditions are not in place, the WHO recommends that mothers exclusively breastfeed for the first months of life, then abruptly wean [9, 10]. Modifications to breastmilk are also a recommended alternative. Use of manually expressed, heat-treated breastmilk is one such modification recommended by WHO, UNICEF and UNAIDS [9, 10]. We previously reported that flash-heat, a simple in-home pasteurization method for mothers in developing countries, is capable of inactivating cell-free HIV in HIV-spiked breastmilk samples, while retaining the milk's nutritional value [11]. In addition, our ongoing research suggests that flash-heat can also destroy HIV in naturally infected breastmilk from HIV positive mothers [12]. However, safe storage of manually expressed and heated breastmilk is of concern in countries that lack refrigeration as bacterial contamination could result in infant morbidity, such as diarrhoeal illness.

Previous studies have shown a wide range of bacterial levels in donated expressed breastmilk (EBM), from no growth to 10^6 colony-forming units (CFU)/ml [13–15]. This variation may be due to the mode of breastmilk collection and storage, and differences in personal hygiene practices [14]. Breastmilk obtained by manual expression has been reported to have less risk of contamination than milk obtained with breast pumps, although manual expressing at home resulted in higher bacterial contamination than that performed in a hospital [15–17]. Commercial heat treatment methods, such as Holder Pasteurization (62.5°C for 30 min), are used by human milk banks to eliminate potential pathogens in donated EBM [18]. However, appropriate low-technology methods are needed for use in resource-poor countries. Jeffery *et al.* [19] reported that one simple method, Pretoria Pasteurization, eliminated clinically significant bacteria in 93% of the EBM samples tested. Similarly, we previously reported pilot results that the flash-heat method eliminated spiked *Escherichia coli and Staphylococcus aureus* in breastmilk from healthy mothers in the United States [11]. The objectives of this study were to determine if flash-heat could eliminate naturally occurring bacteria in EBM and to assess if flash-heated EBM could be safely stored at room temperature for up to 8 h.

Materials and Methods

HIV positive breastfeeding mothers, not currently receiving antiretrovirals or antibiotics, were recruited during postnatal clinic visits at an informal settlement in Durban, South Africa between October and December 2004. Approximately 80% of the mothers in this community of 120 000 are unemployed. Within the settlement, 50% of the homes have no running water, electricity or sanitation. Thirty-eight mothers agreed to participate in this study. Following the washing of their hands with soap and water, each of them manually expressed 75–150 ml of breastmilk into a sterile glass jar. Breastmilk samples were covered and stored immediately in an ice water bath, then transported within 2 h to the laboratory where the same sterile glass jar was used for flash-heating. Fifty millilitres of each EBM sample were aliquoted to be flash-heated and the remaining volume was aliquoted to be used as an unheated control.

The flash-heat method has been described in detail elsewhere [11]. Briefly, 50 ml of EBM in an uncovered sterile 16 oz commercial glass food jar was placed in 450 ml of water in a 1 : 1 Hart brand 1 quart aluminium pan. Water and milk were heated together over a single burner butane stove, used to imitate the intense heat of a fire, until the water reached 100°C and was at a rolling boil. The breastmilk was immediately removed from the water bath and allowed to cool to 37.0°C. Temperature data were collected at 15 s intervals using thermometer probes (Cole-Palmer Digi-Sense® DuaLogR® Thermocouple Thermometers). Flash-heat typically reached temperatures above 56.0°C for 6 min and 15 sec, and peaked at 72.9°C.

Flash-heated and unheated samples were stored at 2–8°C overnight to be processed for microbiology assays the next morning, ~18–24 h after collection. At this time, both flash-heated and unheated aliquots were placed at room temperature (~23°C) and allowed to stand, in capped vials, for up to 8 h. For both the flash-heated and unheated aliquots, at 0, 2, 4, 6 and 8 h, 100 µl of undiluted EBM and 100 µl of a 1 : 100 dilution of EBM were plated with sterile streaking loops on cysteine lactose electrolyte deficient (CLED) medium, colistine nalidixic acid blood agar (CNA) and mannitol salt agar (MSA). Plates were incubated for 24 h at 37°C in CO_2. The dilution with a number of colonies between 20 and 200 at time zero, or baseline, was used to determine the number of CFU/ml at each subsequent time point irrespective of the number of colonies at the subsequent time. CFU/ml were determined using 33/38 and 5/38 undiluted and 1 : 100 diluted samples, respectively. Growth on the CLED agar was used to determine the total count, while growth of *E. coli*, *S. aureus* and β-haemolytic streptococci was quantified on CLED, MSA and CNA, respectively. If >200 colonies were observed, this was considered too numerous to count (TNTC). For all cases where colony counts yielded values below the set minimum (20) and above the set maximum (200) number of colonies at the dilution used, we substituted a proxy value. We calculated the geometric mean between the

CFU/ml obtained from the highest number of colonies countable before designating TNTC, 2000 CFU/ml (>200 colonies in 100 µl aliquot), and the CFU/ml value obtained from the count just below the minimum acceptable for 1:100 dilutions, which would be 19 000 CFU/ml (<20 colonies in 100 µl aliquot of 1:100 dilution). The geometric mean of these observed values at baseline, 2000 CFU/ml and 19 000 CFU/ml, was calculated to be 6166 CFU/ml (log value = 3.79 CFU/ml). This value was then used for all time points with TNTC values.

All statistical analyses were performed using Stata, version 8.0, Stata Corporation, College Station, Texas.

This study was approved by the Committees for the Protection of Human Subjects at the University of California campuses at Berkeley and Davis and the University of KwaZulu-Natal.

Results

Thirty-eight EBM samples were flash-heated and compared with unheated controls for bacterial growth over 8 h at room temperature. At baseline, immediately after heating, 16% (6/38) of the flash-heated samples showed some bacterial growth, compared with 100% (38/38) of the unheated samples. No growth to very-limited growth (<99 CFU/ml) was observed overall time points for the majority of the flash-heated samples (89–92%) compared with unheated controls (3–5%) ($p < 0.0001$), while substantial growth (>1000 CFU/ml) was observed in very few flash-heated samples (0–3%) compared with the majority of unheated controls (61–66%) ($p < 0.0001$). Similarly, the majority of unheated samples (61%) had $>1 \times 10^3$ CFU/ml starting at baseline and continued up to 8 h. Eleven percent (4/38) of the unheated samples, including one 1:100 dilution, at baseline and 42% (16/38), including two 1:100 dilutions, at 8 h had unreadable plates, and were considered TNTC. Additionally, after 8 h incubation, zero bacterial growth was observed in the majority, 84% (32/38), of the flash-heated samples, compared with 0% (0/38) of the unheated samples. These differences between flash-heated and unheated samples were found to be statistically significant when comparing the number of samples positive for bacterial growth at each time point (Table 1) as well as the mean log values of CFU/ml at each time point (Fig. 1, Table 2).

We observed a decline in CFU/ml among breastmilk samples positive for bacterial growth in at least one time point over the 8 h in 83% (5/6) of flash-heated samples and 82% (31/38) of the unheated samples, although this decrease was not statistically significant. Unheated samples had significantly greater bacterial propagation over time than flash-heated samples when comparing log values of CFU/ml across 0–8 h ($p < 0.005$, Wilcoxon signed-rank test).

TABLE 1
Comparison of number of flash-heated and unheated samples with bacterial growth at each time point

Time point	Flash-heated (n=38)	Unheated (n=38)
0	6	38
2	11	38
4	6	37
6	5	38
8	5	38

$p < 0.0001$, paired Student's t-test.

None of the flash-heated samples were considered TNTC at any time point. Among unheated samples, four were considered TNTC at baseline, five at 2 h, seven at 4 h, thirteen at 6 h, and sixteen at 8 h. These samples were assigned the imputed value of 6166 CFU/ml (log value = 3.79 CFU/ml).

Among the flash-heated samples, 0/38 showed pathogenic growth at any time point (Table 3, Fig. 2). Among the unheated samples, 20 CFU/ml of *E. coli* were observed in one sample at 6 h and *S. aureus* was observed at $\leq 1 \times 10^3$ CFU/ml in 8% (3/38) of samples. Similar to the total bacterial growth described above, we observed a decline in CFU/ml in at least one time point for pathogens in 100% (7/7) of unheated samples, although this decrease also was not statistically significant. Neither the flash-heated nor the unheated samples had Group A or B streptococcus growth at any time point.

Discussion

Flash-heat was successful in completely eliminating bacteria in the majority of samples, and prevented substantial growth for up to 8 h when stored at room temperature. We observed significantly less bacterial growth in the flash-heated samples compared with unheated ones at each time point. Although the majority of unheated samples had $>1 \times 10^3$ CFU/ml starting at baseline through 8 h, unfortunately, because of the dilutions used, we were not able to ascertain the upper limits of growth for those considered TNTC. The interpolated value we used was derived only from time point 0. This suggests that by 8 h, our samples of unpasteurized EBM stored at room temperature (23°C) had substantial bacterial growth. Current recommendations by the Human Milk Bank Association of North America state that storage of EBM at room temperature is safe for up to 6–8 h. Based on our results and previous findings [20], however, we would urge that further research is needed to evaluate the safe

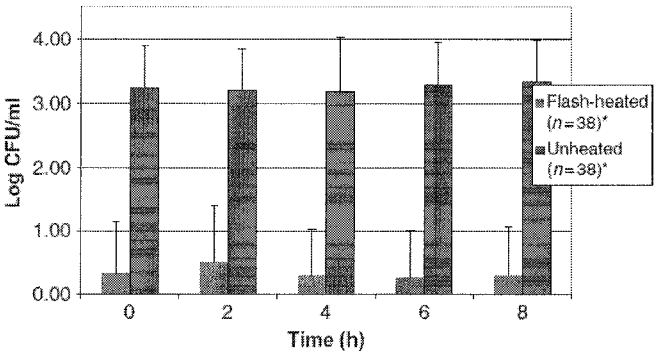


FIG. 1. Mean log comparison of non-pathogenic growth in flash-heated or unheated positive breastmilk samples at 0–8 h storage ($p < 0.0001$). Samples labelled TNTC were set to 6166 CFU/ml (log value = 3.79), which may underestimate the actual bacterial growth in these samples. (No flash-heated samples were considered TNTC at any time point. Among unheated samples, 4,5,7,13 and 16 samples were considered TNTC at 0,2,4,6 and 8 h, respectively.)

TABLE 2
Comparison of mean log values of CFU/ml for flash-heated and unheated samples at each time point

Time point	Flash-heated		Unheated	
	Mean log (S.D)	Median	Mean log (S.D)	Median
0	0.328 (0.817)	0	3.239 (0.672)	3.251
2	0.504 (0.898)	0	3.213 (0.632)	3.111
4	0.294 (0.745)	0	3.182 (0.853)	3.127
6	0.269 (0.741)	0	3.292 (0.667)	3.161
8	0.292 (0.779)	0	3.339 (0.653)	3.159

$p < 0.0001$, paired Student *t*-Test.
Samples labelled TNTC were set to 6166 CFU/ml (log value = 3.79), which may underestimate the actual bacterial growth in these samples. (No flash-heated samples were considered TNTC at any time point. Among unheated samples, 4,5,7,13 and 16 samples were considered TNTC at 0,2,4,6 and 8 h, respectively.)

TABLE 3
Flash-heated and unheated breastmilk samples with non-pathogen and pathogen growths at any time point over 0–8 h

	Flash-heated (n=38) No. of samples positive (%)	Unheated (n=38) No. of samples positive (%)
Pathogens		
E. coli	0 (0)	1 (2.6)
S. aureus	0 (0)	6 (15.8)
Group B strep	0 (0)	0 (0)
Non-pathogens	13 (34.2)	38 (100)

duration for storing EBM at room temperature—for consumption of either raw or pasteurized EBM.

We observed decreases in bacterial growth at several time points in some flash-heated and unheated breastmilk samples. Although these decreases were not statistically significant, previous studies have suggested similar decreases and fluctuations in bacterial growth over time in breastmilk due to its naturally occurring antimicrobial activity [21–24]. We find it interesting that data from several of our samples agree with these previous findings that an initial increase in bacterial growth was followed by a decrease and then subsequent increase again. This fluctuation in bacterial growth suggests a delay in anti microbial activity and is hypothesized to be due to possible activation and involvement of complement and to a progressive increase in free fatty acids by milk lipases in stored milk, which are known to have cytotoxic effects on pathogenic organisms [25–28].

Breastmilk is not a sterile bodily fluid and can play an important role in promoting the infant immune response if the bacterial concentrations are at acceptable levels. Common bacteria found in donated EBM include non-pathogens such as *Staphylococcus epidermidis*, α-haemolytic streptococcus, *Bacillaceae* species, as well as pathogens such as *S. aureus* and *E. coli*. The criteria for safe donor milk, as specified by the Human Milk Banking Association of North America, are

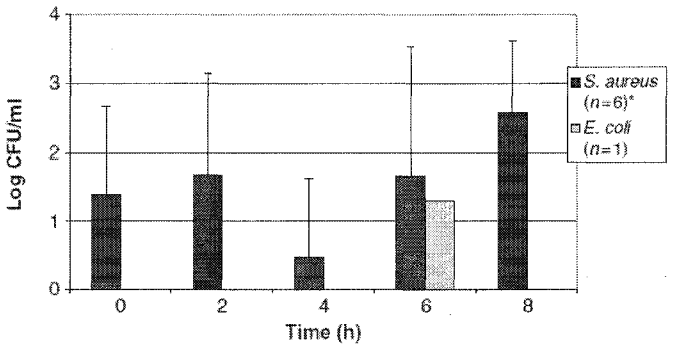


FIG. 2. Mean log of pathogenic growth in unheated breastmilk samples positive for *S. aureus* ($n=6$) and *E. coli* ($n=1$) at 0–8 h storage. Samples labelled TNTC were set to 6166 CFU/ml (log value = 3.79), which may underestimate the actual bacterial growth in these samples. (No flash-heated samples were considered TNTC at any time point. Among unheated samples 4,5,7,13 and 16 samples were considered TNTC at 0,2,4,6 and 8 h, respectively.)

counts of $<1\times10^5$ CFU/ml for non-pathogens, $<1\times10^3$ CFU/ml of *S. aureus* and no *E. coli* in pre-pasteurized samples and no growth of any species in post-pasteurized samples after 48 h stored at 4°C [29].

In resource-poor settings where infants may continually be exposed to potential pathogens, it is important that the immuno-protective elements of breastmilk remain after heating. Examples of important vertically transferred anti-infective components include oligosaccharides, leukocytes, secretory IgA, lactoferrin and lysozyme, which are protective against enteric pathogens. This biochemical protection manifests itself in a dose-responsive inverse correlation between lower morbidity and mortality rates and milk volume consumption and duration of breastfeeding among breastfed infants. Oligosaccharides are simple sugars that bind bacteria and form complexes that are then safely excreted in the infant's urine [30, 31]. Leukocytes, including neutrophils, macrophages and lymphocytes, actively respond to the presence of enteric pathogens [32]. Secretory IgA, which is the primary immunoglobulin in human milk, is an important immune factor for epithelial surfaces [33–36]. Lactoferrin, in addition to its antiviral, antioxidant, anti-inflammatory, immune-modulating and anticancer activities, causes breastmilk to become bacteriostatic for some bacteria, including *E. coli, Stretococcus mutans*, and *Vibrio cholerae* [37–44]. Human lysozyme kills most Gram-positive bacteria by damaging their surface peptidoglycan and is also active against Gram-negative organisms. Other studies have found that storage of breastmilk in refrigeration or deep freeze has been associated with increased anti microbial properties, thought to be due to an increase in levels of free fatty acids [21, 28, 45]. Moreover, the presence of non-pathogenic bacteria in EBM are thought to inhibit pathogenic growth [46].

Low-temperature, long-time (LTLT) heat treatment methods, such as Holder Pasteurization at 62.5°C for 30 min, are reported to maintain the majority of such immunological components and macronutrients of breastmilk, although research shows a substantial reduction in lactoferrin, vitamins and immunoglobulins [47–50]. Thus, the flash-heat method was designed to imitate high-temperature, short-time (HTST) heat treatments used commercially, which typically heat to 72°C for 15 s. HTST methods are considered to be superior since they can kill bacteria and cytomegalovirus, with no decrease in vitamins, lactoferrin, total IgA concentrations or secretory IgA activity [51–53].

This study had several limitations. Since samples were refrigerated overnight prior to processing, this delay may have allowed lipolysis of fatty acids resulting in enhanced antimicrobial ability of both flash-heated and unheated samples. Additionally, in order to have quantifiable data, samples identified as TNTC were set as 6166 CFU/ml, based on the geometric mean between the observable baseline values of 2000 and 19 000 CFU/ml. This may underestimate our values after time zero since potential bacterial growth after the baseline reading of these samples was not captured. In light of this, although we found the difference to be significant, the actual magnitude of this difference between bacterial growth in flash-heated vs unheated samples may have been greater than that presented here.

The purpose of this study was to determine if flash-heat was capable of eliminating naturally

occurring bacteria in EBM and in preventing contamination. We acknowledge that our study design does not allow us to accurately assess the antimicrobial activity remaining in flash-heat breastmilk since additional contaminants were not introduced post-heating to specifically test this. It may be safest to flash-heat the breastmilk immediately after expressing and prior to storage to avoid potential replication of pathogens over time, such as *S. aureus* whose toxin may remain post-heat [54]. Further research is needed in this area.

In summary, this study suggests that flash-heat is capable of eliminating pathogenic and non-pathogenic bacteria and that an 8 h storage period outside the refrigerator does not result in a significant increase of bacteria. This is an important finding since HIV is not the only microbe of concern that must be eliminated in EBM for safe infant consumption. Flash-heat is a simple EBM pasteurization method that could be a safe infant-feeding option for mothers in need of breastmilk modifications, such as HIV positive mothers in developing countries where resources such as refrigeration are lacking.

References

1. UNAIDS/WHO. AIDS epidemic update. UNAIDS/05.19E. Geneva: UNAIDS, 2005.
2. De Cock KM, Fowler MG, Mercier E, *et al.* Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. JAMA 2000;283:1175–82.
3. Guay LA, Musoke P, Fleming T, *et al.* Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 1999;354:795–802.
4. Bahl R, Frost C, Kirkwood BR, *et al.* Infant feeding patterns and risks of death and hospitalization in the first half of infancy: multicentre cohort study. Bull World Health Organ 2005;83:418–26.
5. Jones G, Steketee RW, Black RE, *et al.* And the Bellagio Child Survival Study Group. How many child deaths can we prevent this year? Lancet 2003;362:65–71.
6. Coutsoudis A. Breastfeeding and the HIV positive mother: the debate continues. Early Hum Dev 2005;81:87–93.
7. Coutsoudis A, Goga AE, Rollins N, *et al.* Free formula milk for infants of HIV-infected women: blessing or curse? Health Policy Plan 2002;17:154–60.
8. Victora CG, Smith PG, Vaughan JP, *et al.* Evidence for protection by breast-feeding against infant deaths from infectious diseases in Brazil. Lancet 1987;2:319–22.
9. WHO, UNICEF, UNAIDS, UNFPA. HIV and infant feeding: a guide for health-care managers and supervisors. Geneva, Switzerland: WHO, 2004.
10. WHO, UNICEF, USAID *et al.* HIV and infant feeding: a question and answer guide for counselors, 2005.
11. Israel-Ballard K, Chantry C, Dewey K, *et al.* Viral, nutritional, and bacterial safety of Flash-heated and Pretoria-Pasteurized breast milk to prevent mother-to-child transmission of HIV in resource-poor countries: a pilot study. J Acquir Immune Defic Syndr 2005;40:175–81.
12. Israel-Ballard K, Donovan R, Chantry C, *et al.* Viral safety of flash-heated breast milk as an infant feeding option for HIV positive mothers in developing countries. Oral Presentation at XVI International Aids Conference 2006, Toronto, Canada.
13. Ajusi JD, Onyango FE, Mutanda LN, *et al.* Bacteriology of unheated expressed breast milk stored at room temperature. East Afr Med J 1989;66:381–7.
14. Ng DK, Lee SY, Leung LC, *et al.* Bacteriological screening of expressed breast milk revealed a high rate of bacterial contamination in Chinese women. J Hosp Infect 2004;58:146–50.
15. Narayanan I, Prakash K, Gujral VV. Management of expressed human milk in a developing country-experiences and practical guidelines. J Trop Pediatr 1982;28:25–8.
16. Liebhaber M, Lewiston NJ, Asquith MT, *et al.* Comparison of bacterial contamination with two methods of human milk collection. J Pediatr 1978;92:236–7.
17. Boo NY, Nordiah AJ, Alfizah H, *et al.* Contamination of breast milk obtained by manual expression and breast pumps in mothers of very low birthweight infants. J Hosp Infect 2001;49:274–81.
18. Lawrence R, Lawrence R. Breastfeeding: A Guide for the Medical Profession 5th edn. St Louis: Mosby, Inc., 1999.
19. Jeffery BS, Soma-Pillay P, Moolman G. The effect of Pretoria pasteurization on bacterial contamination of hand-expressed human breastmilk. J Trop Pediatr 2003;49:240–4.
20. Nwankwo MU, Offor E, Okolo AA, *et al.* Bacterial growth in expressed breast-milk. Ann Trop Paediatr 1988;8:92–5.
21. Ogundele MO. Techniques for the storage of human breast milk: implications for anti-microbial functions and safety of stored milk. Eur J Pediatr 2000;159:793–7.
22. Chen HY, Allen JC. Human milk antibacterial factors: the effect of temperature on defense systems. Adv Exp Med Biol 2001;501:341–8.
23. Pardou A, Serruys E, Mascart-Lemone F, *et al.* Human milk banking: influence of storage processes and of bacterial contamination on some milk constituents. Biol Neonate 1994;65:302–9.
24. Jones CL, Jennison RF, D'Souza SW. Bacterial contamination of expressed breast milk. Br Med J 1979;2:1320–2.
25. Sakaguchi M, Tomomasa T, Kuroume T. Cytolytic action of stored human milk on blood cells in vitro. J Perinat Med 1995;23:293–300.
26. Ogundele MO. Complement-mediated bactericidal activity of human milk to a serum-susceptible strain of E. coli 0111. J Appl Microbiol 1999;87:689–96.
27. Ogundele MO. Cytotoxicity by stored human breast-milk: possible contribution of complement system. Cell Biol Int 1999;23:585–8.
28. Lavine M, Clark RM. Changing patterns of free fatty acids in breast milk during storage. J Pediatr Gastroenterol Nutr 1987;6:769–74.
29. U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration. Grade "A" pasteurized milk ordinance. Public Health Bulletin 220; 2003.

30. Lonnerdal B. Biochemistry and physiological function of human milk proteins. Am J Clin Nutr 1985;42:1299–317.
31. Coppa GV, Zampini L, Galeazzi T, *et al.* Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: Escherichia coli, Vibrio cholerae, and Salmonella fyris. Pediatr Res 2006;59:377–82.
32. Georgeson JC, Filteau SM. Physiology, immunology, and disease transmission in human breast milk. AIDS Patient Care STDS 2000;14:533–9.
33. Noguera-Obenza M, Ochoa TJ, Gomez HF, *et al.* Human milk secretory antibodies against attaching and effacing Escherichia coli antigens. Emerg Infect Dis 2003;9:545–51.
34. Van de Perre P. Transfer of antibody via mother's milk. Vaccine 2003;21:3374–6.
35. Boesman-Finkelstein M, Finkelstein RA. Sequential purification of lactoferrin, lysozyme and secretory immunoglobulin A from human milk. FEBS Lett 1982;144:1–5.
36. Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. Pediatr Infect Dis J 1993;12:664–71.
37. Rainard P. Bacteriostatic activity of bovine milk lactoferrin against mastitic bacteria. Vet Microbiol 1986;11:387–92.
38. Ellison RT, 3rd, Giehl TJ. Killing of gram-negative bacteria by lactoferrin and lysozyme. J Clin Invest 1991;88:1080–91.
39. Spik G, Cheron A, Montreuil J, *et al.* Bacteriostasis of a milk-sensitive strain of Escherichia coli by immunoglobulins and iron-binding proteins in association. Immunology 1978;35:663–71.
40. Tomita M, Bellamy W, Takase M, *et al.* Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. J Dairy Sci 1991;74:4137–42.
41. Edde L, Hipolito RB, Hwang FF, *et al.* Lactoferrin protects neonatal rats from gut-related systemic infection. Am J Physiol Gastrointest Liver Physiol 2001;281:G1140–50.
42. Steijns JM, van Hooijdonk AC. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. Br J Nutr 2000;84(Suppl 1):S11–7.
43. Harmsen MC, Swart PJ, de Bethune MP, *et al.* Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro. J Infect Dis 1995;172:380–8.
44. Andersson Y, Lindquist S, Lagerqvist C, *et al.* Lactoferrin is responsible for the fungistatic effect of human milk. Early Hum Dev 2000;59:95–105.
45. Hernandez J, Lemons P, Lemons J, *et al.* Effect of storage processes on the bacterial growth-inhibiting activity of human breast milk. Pediatrics 1979;63:597–601.
46. Heikkila MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk. J Appl Microbiol 2003;95:471–8.
47. Hernell O, Olivecrona T. Human milk lipases. II. Bile salt-stimulated lipase. Biochim Biophys Acta 1974;369:234–44.
48. Ford JE, Law BA, Marshall VM, *et al.* Influence of the heat treatment of human milk on some of its protective constituents. J Pediatr 1977;90:29–35.
49. Van Zoeren-Grobben D, Schrijver J, Van den Berg H, *et al.* Human milk vitamin content after pasteurisation, storage, or tube feeding. Arch Dis Child 1987;62:161–5.
50. Bjorksten B, Burman LG, De Chateau P, *et al.* Collecting and banking human milk: to heat or not to heat? Br Med J 1980;281:765–9.
51. Goldblum RM, Dill CW, Albrecht TB, *et al.* Rapid high-temperature treatment of human milk. J Pediatr 1984;104:380–5.
52. Morgan JN, Lin FJ, Eitenmiller RR, *et al.* Thermal destruction of Escherichia coli and Klebsiella pneumoniae in human milk. J Food Pro 1988;51:132–6.
53. Dhar J, Fichtali J, Skura BJ. Pasteurization Efficiency of a HTST System for Human Milk. J Food Sci 1996;61:569–73.
54. Guidelines for the establishment and operation of human milk banks in the UK. 3rd edn. United Kingdom Association for Milk Banking, London, England, 2003.

APPENDIX V

BREASTFEEDING COUNSELORS TRAINING COURSE

16 hour course

Course is interactive including role playing (minor didactic format)

Developed and training conducted by Lindiwe Sibeko, M.Sc., IBCLC.

Some material for this course has been modified from The Training manual: Breastfeeding Counseling for Community Health Workers.

Learning objectives:

Overall, the aim of this training course is to build the knowledge base of lay breastfeeding counselors on lactation and the process of breastfeeding. More specifically to:

a) provide information and improve knowledge on breastfeeding for general population.

b) provide information and improve knowledge on breastfeeding and HIV.

c) provide information and improve knowledge on flash-heating of expressed breastmilk.

d) discuss and teach skills on building mother's confidence to breastfeed successfully.

Training is in the form of 9 sessions with final evaluation (written and oral).

SESSIONS

BEING A BREASTFEEDING COUNSELLOR

1. Examining own attitudes and beliefs, why train breastfeeding counselors?
2. What is counseling? Sending messages and listening skills
3. Development of non-judgemental environment
4. Building confidence
5. Assessing mother's support network
6. Assess mother's infant feeding beliefs and practices

BENEFITS OF BREASTFEEDING

1. To infant
2. To mother
3. To family

4. To community

WHY BREAST MILK IS SUPERIOR MILK FOR A GROWING BABY

1. Nutrient composition
2. Immune factors
3. Safety
4. Bonding

BREAST MILK PRODUCTION

1. How breast makes milk
2. Colostrum, foremilk, mature milk and hind milk
3. Increasing milk production: supply and demand

ASSESSING THE INFANT AT THE BREAST

1. Assess Infant
2. Assess Mother
3. Assessing good latch: positioning and attachment using more of 'hands-off' approach where mother takes an active role for adjustments and counselor is a facilitator and provides encouragement.
4. Role playing: practicing of latch and assessment
5. Assess infant is feeding effectively: number of bowel movements and urine output.
6. Knowing when to refer mother to the clinic to be seen or baby has to be seen.

PATTERNS OF BREASTFEEDING

1. South African breastfeeding guidelines
2. Child survival, common childhood illnesses
3. Exclusive breastfeeding, mixed breastfeeding-risks and benefits
4. Dangers of formula feeding within South African setting

BREASTFEEDING CHALLENGES:

1. Breast problems and solutions: engorgement, mastitis
2. Infant thrush
3. Infant feeding constantly
4. Concern of milk supply
5. Challenges mothers can have with partners, family and friends; ways to address some of these problems
6. Recognize infant failing to thrive

BREASTFEEDING AND HIV

1. mother-to-child transmission, extent of problem in SA
2. International guidelines: Infant feeding options
3. What is AFASS
4. Risk of mixed feeding
5. Exclusive breastfeeding: minimizing risk of infection, promote child health
6. Safe breastfeeding
7. How does mother make decision: using AFASS tool
8. Breastfeeding cessation, concerns and possible solutions
9. Expressing breast milk
10. Heat-treatment of expressed breast milk (HTEBM)
11. Cup and spoon feeding

BREASTFEEDING CESSATION AND COMPLEMENTARY FEEDING

1. Complementary foods, active feeding
2. Replacement milk: use of HTEBM, use of formula benefits and limitations
3. Case studies

Evaluation of trainees: Mock oral test with peer assessment, feedback and interaction. Written (short answer questions) and oral test (2 scenarios).Individual feedback and review of the tests to clarify issues. Certification presentation.

APPENDIX VI

BREASTFEEDING AND HIV INFORMATION SESSION

Information session presented to hospital staff from: Labour & Delivery, Nursery, Obstetrics, Antenatal Care and Pediatrics

20 minute information session and question period.

Learning objectives:

1. To provide hospital staff with most current information on infant feeding and HIV guidelines and background evidence for basis of recommendations.

2. To encourage and facilitate a collaborative approach to supporting mothers wanting to EBF to initiate breastfeeding soon after delivery.

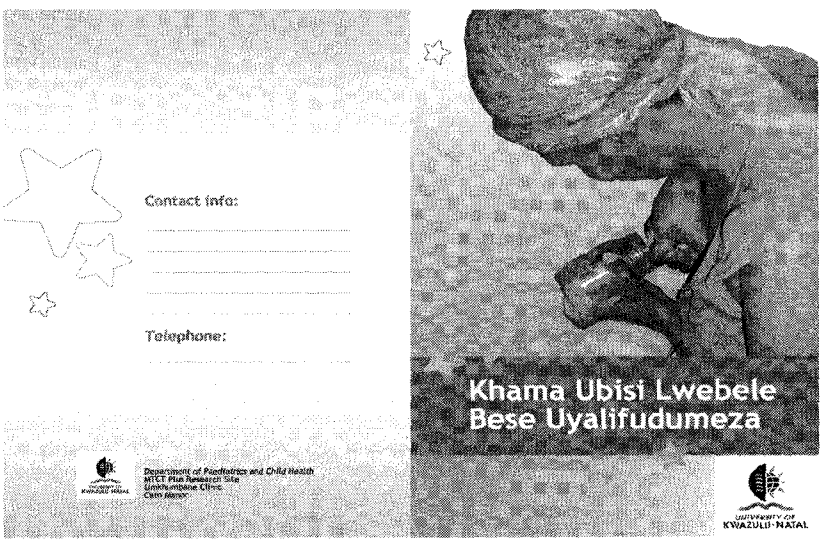
Content

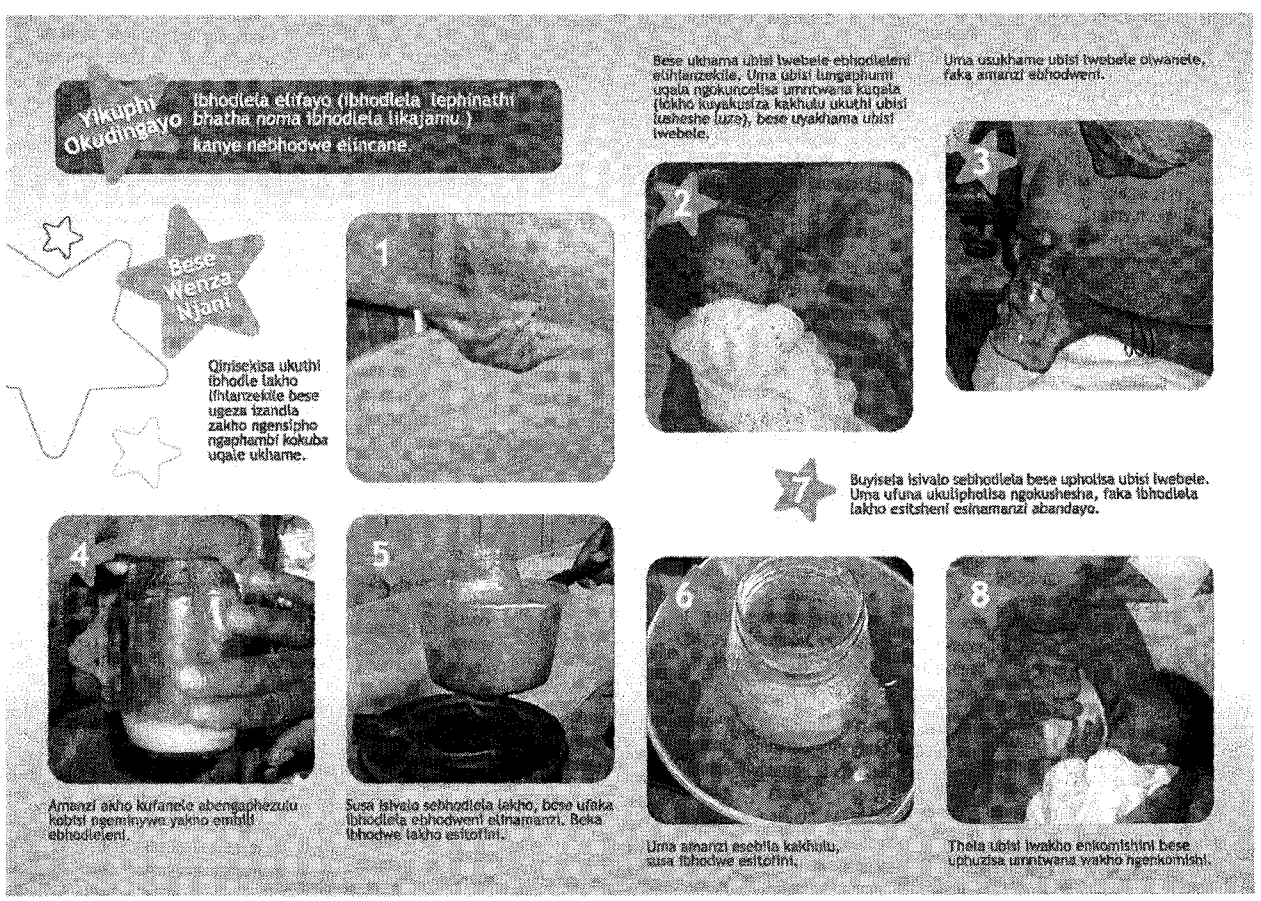
1. Optimal feeding for infant feeding to promote infant survival
2. Breastfeeding and HIV:
    - i) What is EBF, evidence for low HIV transmission risk and EBF practice
    - ii) International infant feeding guidelines, what facilitates EBF for 6 months
    - iii) AFASS tool used for counseling women on feeding options
    - iv) Dangers of mixed beastfeeding
3. Supporting the mother who has chosen to breastfeed.

    How to support mother who is breastfeeding, why it is important for her and her infant.

Questions and concerns.

APPENDIX VII Heat-treatment counseling resource developed in collaboration with mothers from the community





Appendix VIII

INTAKE FORM

EXCLUSIVE BREASTFEEDING and HEAT TREATMENT OF BREAST MILK STUDY:

Date:

Name: **I.D. #:**_____

Address:

Phone:

D.O.B (mother dd/mo/yr):

Age:

Estimated date of delivery (dd/mo/yr): **Gestational age (wks):**

Number of pregnancies: **Number of live births:**

Living with partner or married: **Number of children living with you:**

Ht (m): **Wt (Kg):** **TSF (mm):**

Have you disclosed your HIV status to anyone:

If yes, who:

Do you feel you have support, comment:

Employed outside the home: **YES** **NO**

If yes, what type of work: **Work schedule:**

Last day of work before delivery: **returning to work on:**

Education: none ☐ **some primary** ☐ **Std 8** ☐ **matric** ☐ **tertiary**

House:

Water source:

Electricity: **Y** **N**

Fuel source:

Sanitation (toilet type):

Date of next visit:

Questions/concerns/comments:

APPENDIX IX

EBF +HTBM STUDY: PROGRESS NOTES

DATE OF SESSION: _____

PATIENT NAME: _____

NUMBER: _____

PURPOSE OF VISIT SESSION: _____

_____

CONTENT OF VISIT:

_____

_____

_____

ASSESSMENT:

_____

_____

_____

PLANNING:

_____

_____

_____

NEXT VISIT: _____ SIGNED: _____

APPENDIX X

Checklist: preparation of cessation of breastfeeding, Use of HTBM and complementary foods.

Practice of expressing breastmilk

Practice of HTEBM

Discuss complementary foods

Discuss some of the common issues and coping strategies of early weaning

Discuss mother's/family members concerns

APPENDIX XI

Exclusive Breastfeeding and Heat-Treatment of Expressed Breastmilk Study: Cessation of breastfeeding

Form to be completed at first visit once weaning begins

Name: Study I.D. #:

1. Date mum started to discontinue BF:

2. Date completely stopped BF:

3. How did mother stop BF:

4. Any difficulty with stopping BF:
   physical difficulty baby: (refusing food, illness)

   mother (breast pain, engorgement, mastitis)

   emotional difficulty baby:

   mother:

5. How did mother manage the problems: mother's issues

   baby's issues:

6. How is expressing and heating of breast milk progressing:

7. Family response to HTEBM

8. Other comments

APPENDIX XII

ENGLISH TRANSLATION OF SAMPLE CONVERSATION FROM EBF AND HTEBM VIDEO

Counsellor: Hi! How are you? We are going to talk about exclusive breastfeeding and its meaning. Exclusive breastfeeding is giving the baby only breastmilk until the baby is 6 months without giving any other foods like purity, cereals, yoghurt and other foods that you can think of or any other liquids like water, juice, tea and commercial formula. It is because the baby's tummy is not ready for other foods or drinks.

Breastmilk has all the nutrients that the baby needs. That is why we encourage mothers to exclusively breastfeed their babies for 6 months.

Mum 1: It is very important to give the baby breastmilk because it helps mother and baby to bond. It also saves time and money and helps the baby to grow very well. As I am a mother who is not working and not schooling, it was a good opportunity for me to breastfeed my baby. As you can see I give her love, I have enough time to spend with her and I am comfortable with what I am doing. I think it is important for you to breastfeed your child, even if you are working. You can express breastmilk and leave it with those who will look after your child. Like me when I am going to town, I wake up early at about 6 am and start expressing. By about 7 am I am finished and leave the milk with the baby's granny to give to the baby. Sometimes when I come back I find that she didn't finish the milk that I left with her because there was so much!

Counsellor: Working and schooling mothers are sometimes worried about how they will give their babies breastmilk only for 6 months, but it is possible. You can breastfeed your babies exclusively for six months by expressing breastmilk and leaving it with those who will look after the baby so that they can cup feed the baby while you are away.

You can heat-treat the breastmilk if you want to. Heat-treating breastmilk helps kill viruses and other germs and even if it is heat treated it still has all the nutrients. It also helps the milk last longer without needing refrigeration.

Let's hear from the mother who is expressing for her baby when she is away.

Mum 2: I express breastmilk and heat-treat it. Would you like me to show you?

Mum 1: How? Is that true? I will be happy to learn.

Mum 2: Okay, I will show you.

Mum 1: That will be great.

Mum 2: This is how you should do it: You express your milk into a clean glass jar, then you take a pot and put water in it. Place the jar (without the lid) in the water, making sure that the level of the water is above the level of milk by two fingers. Then place the pot containing the glass jar onto the stove, and bring the water to the boil. When it is boiling remove the pot from the stove, take the jar out of the pot and put the lid on the jar. If you want to feed the child at that time you must close the jar and put it in cold water to cool. Pour the milk into a cup and feed the baby.

Mum 1: Now I know how to express and heat-treat breastmilk and I think it is easy to get glasss bottles/ jars because you can use peanut butter or jam bottles. The bottles can be washed easily. It is important to make sure that the bottles you use have a lid. It is so easy to express and heat treat!

It also helps to listen at the clinic when they are doing health education.
Some other people are scared to breastfeed in the taxis but I don't see a problem because that is the right thing to do for the baby.

Mum 2: I also don't see the problem. It's the best thing for my baby.