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A review of HIV transmission through breastfeeding

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Explanation of terms

AZT (also known as Zidovudine (ZDV)) is an antiretroviral drug which inhibits HIV replication. It is used in the prevention of mother-to-child transmission.

Breast-milk substitute means any food being marketed or otherwise represented as a partial or total replacement for breast milk, whether or not suitable for that purpose.

CD4 cells means main target cells for HIV. CD4 lymphocytes (a type of white blood cells) are key in both humoral and cell-mediated immune responses. Their number decreases during HIV infection.

CD8 cells means lymphocytes which play an important role in fighting infections. Their number may be increased during HIV infection.

Cell-associated virus means HIV which lives inside the cell, measured as HIV-DNA.

Cell-free virus means parts of the virus (virions) not associated with a cell, measured as HIV-RNA.

Cervical ectopy means a turning outward of the edges of the endocervix; it may result from chronic inflammation of the cervix.

Cessation of breastfeeding means stopping breastfeeding.

Chorioamnionitis means inflammation of the fetal membranes, associated with a bacterial or parasitic infection (e.g. malaria).

Colostrum is the thick yellow milk secreted by the breasts during the first few days after delivery, that gradually evolves into mature milk at 3-14 days postpartum. It contains more antibodies and white blood cells than mature breast milk.

Commercial infant formula means a breast-milk substitute formulated industrially in accordance with applicable Codex Alimentarius standards to satisfy the nutritional requirements of infants up to four to six months of age.

Complementary food means any food, whether manufactured or locally prepared, suitable as a complement to breast milk or to infant formula, when either becomes insufficient to satisfy the nutritional requirements of the infant.

DNA, an abbreviation for deoxyribonucleic acid, is the carrier of genetic information found in cell nuclei.

Early breastfeeding means breastfeeding in the first 3 weeks of life.

Early postpartum means the first 3-6 weeks after delivery.

Enterocyte is the cell of the lining of the intestinal wall.

Epithelial means the surface layer of cells covering cutaneous, mucosal and serous surfaces.

Exclusive breastfeeding means giving an infant no other food or drink, not even water, apart from breast milk (including expressed breast milk), with the exception of drops or syrups consisting of vitamins, mineral supplements or medicines.

Glycosaminoglycans means linear polysaccharides composed of repeating disaccharides, usually more than 20 per chain, that attach to a protein core forming a proteoglycan. They are macromolecules that may inhibit the binding of HIV to the CD4 receptor.

Human immunodeficiency virus (HIV) refers to HIV-1 in this document, since cases of mother-to-child transmission of HIV-2 are rare.

Immunoglobulins means any of the five distinct antibodies present in the serum and external secretions of the body (IgA, IgD, IgE, IgG and IgM).

Infant means a child from birth to 12 months of age.

Intestinal lumen means the space within the tubular part of the bowel.

Intrapartum means the period during labour or delivery.

Lactoferrin means an iron-binding protein found in human milk.

Late postnatal transmission means a breastfed child who becomes infected with HIV only after 3-6 months of age. Definitions of late postnatal transmission vary between studies.

Lipase means any fat-splitting enzyme.

Lipid means any one of a widely varying group of fats and fat-like organic substances.

Macrophage means a large “wandering” phagocytic cell that ingests foreign matter, and plays an important role in resisting infection.

Mature breast milk means milk produced from about 14 days postpartum to the cessation of breastfeeding.

Meta-analysis means the statistical method of combining the results of similar, but separate, studies.

M-cells means specialised epithelial cells found on the intestinal mucosa.

Mixed feeding means partial breastfeeding and giving some other milk, often bottles of infant formula.

Mother-to-child transmission (MTCT) means transmission of HIV to a child from an HIV-infected woman during pregnancy, delivery or breastfeeding. The term “vertical transmission” is commonly used interchangeably with MTCT.

Mucosa means mucous membranes.

Neonatal describes the period immediately following birth and continuing through the first month of life.

P24 antigen means a protein part of the virus membrane that can stimulate the production of specific antibodies

PCR means polymerase chain reaction, a laboratory method in which the genetic material (DNA or RNA) of the virus is detected and amplified. It can be both qualitative or quantitative.

Partial breastfeeding means some breastfeeding while giving other forms of food.

Replacement feeding means the process of feeding a child, who is not receiving any breast milk, with a diet that provides all the nutrients the child needs. During the first six months this should be with a suitable breast-milk substitute – commercial infant formula, or home-prepared formula with micronutrient supplements. After six months this should preferably be with a suitable breast-milk substitute, and complementary foods made from appropriately prepared and nutrient-enriched family foods that are given three times a day. If breast-milk substitutes are not available, appropriately prepared family foods should be further enriched and given five times a day.

RNA means ribonucleic acid, a substance found in the nucleus of all living cells and in many viruses. It is an intermediate of DNA and the medium by which genetic instructions from the nucleus are transmitted to the rest of the cell.

Supernatant means the upper layer of material, liquid or lighter solid, that remains after the precipitation of a solid part of a mixture.

Synctium-forming virus means a virus that has the ability to form a network, thus involving cells that are not strictly infected.

Tropism means a predilection for a specific tissue.

Viral culture means growth of virus on artificial media under ideal conditions for growth.

Wet-nursing means the breastfeeding of an infant by someone other than the infant’s mother.

A REVIEW OF HIV TRANSMISSION THROUGH BREASTFEEDING

Introduction

In the past three decades, strategies to reduce child mortality and to promote family health have resulted in considerable improvements in child health (World Development Report, 1993). Promotion of breastfeeding has played an important role since breastfeeding contributes to reduced mortality by providing optimum nutrition, by protecting against common childhood infections, and by its child-spacing effects (American Academy of Pediatrics, 1997; Golding *et al.*, 1997; Goldman, 1993; De Soyza *et al.*, 1991; Akré, 1990; Monteiro *et al.*, 1990; Thapa *et al.*, 1989; Habicht *et al.*,1988; Victora *et al.*, 1987).

However, the emergence of HIV threatens to reverse gains in child health, since children are at risk of acquiring HIV infection through transmission from an HIV-infected mother. It is recognized that breastfeeding by an HIV-infected mother increases the risk of HIV transmission to her infant.

Since the beginning of the HIV pandemic, approximately three million children under 15 years of age worldwide have been infected with HIV and current estimates suggest that 600 000 children are newly infected annually (UNAIDS/WHO, 1998). The majority of these children live in sub-Saharan Africa, where between 25-40% of HIV-infected children die before their fifth birthday, and HIV is already contributing to increased childhood mortality (UNAIDS/WHO, 1998; Ryder *et al.*, 1994; Nesheim *et al.*, 1994). Although HIV transmission through breastfeeding is only partially responsible for this increase, HIV and infant feeding is an important public health issue, particularly in regions where HIV prevalence is high, and infectious diseases and malnutrition are the leading causes of childhood death. Countries need to develop sound policies regarding the prevention of HIV transmission through breastfeeding while continuing to protect, promote and support breastfeeding for infants of HIV-negative women and women of unknown serostatus.

This document reviews current scientific knowledge about breast-milk transmission of HIV, and serves as the foundation for two complementary documents:

HIV and infant feeding: Guidelines for decision-makers[1]

HIV and infant feeding: A guide for health care managers and supervisors[2]

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[1] Guidelines for decision-makers, 1998, 36 pages [E]; WHO/FRH/NUT/CHD 98.1

[2] A Guide for Health Care Managers and Supervisors, 1998, 36 pages [E]; WHO/FRH/NUT/CHD 98.2

Mother-to-child transmission

Mother-to-child transmission (MTCT) of HIV, which can occur during pregnancy, delivery or breastfeeding, is responsible for more than 90% of HIV infection in children worldwide (UNAIDS/WHO, 1998). The present review focuses on HIV-1. Both HIV type 1 (HIV-1) and HIV type 2 (HIV-2) can be transmitted from mother to child, but HIV-2 is transmitted much less frequently, as it is less pathogenic than HIV-1 (Adjorlolo-Johnson *et al.*, 1994; Andreasson *et al.*, 1993; Morgan *et al.*, 1990).

The remaining 10% of paediatric infections are attributed to transfusion with contaminated blood and blood products, use of contaminated medical equipment, other practices that cut or pierce the skin, or sexual contact (MAP, 1998; UNAIDS/WHO, 1998; Tovo *et al.*, 1988).

HIV infection in women

Most children acquire the virus through transmission from an HIV-infected mother, therefore, the incidence of paediatric HIV reflects that of HIV infection in women of childbearing age. In areas of high seroprevalence, a significant number of children are at risk.

Mother-to-child transmission (MTCT) of HIV focuses attention on women, but the use of the term MTCT is not to imply blame, whether or not a woman is aware of her own infection status. A woman can acquire HIV through unprotected sex with an infected partner, by receiving contaminated blood, or through exposure to unsterile instruments or medical procedures. HIV is often introduced into the family through the woman's sexual partner, often the father of her child.

The prevalence of HIV varies considerably from region to region. Women and children in sub-Saharan Africa are disproportionately affected, with eight in every 10 HIV-infected women worldwide, and nine in every 10 newly infected children living in this region (MAP, 1998; UNAIDS/WHO, 1998). In West and Central Africa, HIV prevalence in pregnant women currently reaches 10-15% in some urban areas and 1-5% in others. Prevalences in East Africa are higher at 15-25% in urban areas and 5-10% in rural areas, while in Southern Africa antenatal seroprevalences of 20-30%, and in some places even as high as 40%, have been reported (MAP, 1998; UNAIDS/WHO, 1998). In the Caribbean, Central America and South America, HIV-1 seroprevalence rates currently range from 0.1% - 5.0%. Asia is experiencing a rapidly growing epidemic with seroprevalence rates in big cities of Cambodia, India and Thailand currently ranging from 1-5% (UNAIDS/WHO, 1998).

Rates of mother-to-child transmission

Estimates of the rate of mother-to-child transmission of HIV in cohorts of women who have not received any preventive treatment (such as antiretrovirals) range from 15-25% in industrialized countries to 25-45% in developing countries (Msellati *et al.*, 1995). The highest rates of MTCT have been found in women in Africa (Kind *et al.*, 1998; Maguire *et al.*, 1997; Ometto *et al.*, 1995; Lallemant, Le Coeur *et al.*, 1994; Roques *et al.*, 1993; European Collaborative Study, 1992; Blanche *et al.*, 1989).

Differences in study methods, the composition of the populations studied, and the prevalence of co-factors of transmission may explain some of these differences. However, it is likely that much of the increased rate of transmission seen in women in sub-Saharan Africa is associated with breastfeeding,[1] where many women breastfeed for about 2 years (The Working Group on Mother-to-Child Transmission, 1995; Ryder and Behets, 1994; Dabis *et al.*, 1993).

In an attempt to quantify the relative contribution of intrauterine and intrapartum transmission of HIV in *non-breastfed infants*, a working definition of timing has been proposed (Bryson *et al.*, 1992).

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[1] Many women who breastfeed do not breastfeed exclusively. Other fluids (juices, milks, teas) and foods may also be given to the infant. In many studies looking at HIV transmission and breastfeeding no differentiation is made between women who "exclusively" or "partially" breastfeed. In this document, unless otherwise stated, "breastfeeding women" will often include both women who "exclusively" or "partially" breastfeed.

In utero infection. In this, a child is classified as having been infected during pregnancy (in utero) if HIV-1 genome is detected within 48 hours of delivery by polymerase chain-reaction test (DNA-PCR) or viral culture.

Intrapartum infection. Acquisition of infection is assumed to have occurred during delivery (intrapartum) if these diagnostic tests were negative in a sample taken during the first 48 hours after delivery, but became positive in subsequent samples taken within 7-90 days of delivery.

Following this classification, a French study estimated that of the infants infected with HIV, 35% of the non-breastfed infants studied were infected before birth and 65% were infected late in pregnancy or during delivery (Rouzioux *et al.*, 1995). A recent review indicated that in women who did not breastfeed their infants, about one-third of MTCT infection was acquired during the intrauterine period. In women who did breastfeed their infants, less than a quarter of all MTCT was acquired during the intrauterine period (Newell, 1998).

Table 1. Percentage HIV infection acquired by different routes *

	Partially breastfed/breastfed infants	Non-breastfed infants
During intrauterine period	20%	33%
During delivery	45-50%	67%
Postpartum, by breastfeeding	30-35%	0

*These rates are observed in the absence of interventions to reduce MTCT

Evidence for breast-milk transmission

Breast-milk transmission of HIV has been well documented. The first reports indicating the possibility of HIV-1 transmission through breast milk were in breastfed infants of women who were infected postnatally through blood transfusion or through heterosexual exposure (Palasanthiran *et al.*, 1993; Van de Perre *et al.*, 1991; Stiehm and Vink, 1991; Hira *et al.*, 1990; Colebunders *et al.*, 1988; Lepage *et al.*, 1987; Ziegler *et al.*, 1985;). There were also reports of infants, with no other known exposure to HIV, who were infected through wet-nursing and through pooled breast milk (Nduati *et al.*, 1994; Colebunders *et al.*, 1988;).

Generally, higher rates of mother-to-child transmission of HIV are observed where most infants are breastfed rather than where fewer infants are breastfed. However, other reasons for variations in transmission rates, such as maternal nutritional status, stage of HIV disease and possible differences in transmission of HIV subtypes cannot be excluded. Additional evidence is provided by results from prospective studies which indicate that among infants born to HIV-infected mothers, those who are breastfed are more likely to be infected than those who are formula-fed, even allowing for other factors known to be associated with mother-to-child transmission of HIV (European Collaborative Study, 1992; Ryder, 1991; Blanche *et al.*, 1989; Tovo *et al.*, 1988; Tess *et al.*, 1998a).

Mechanisms of breast-milk transmission

Although HIV has been detected in breast milk, (Nduati, 1995; Ruff, 1994; Van de Perre *et al.*, 1993) mechanisms of breast-milk transmission are not yet fully understood. The respective roles of cell-free and cell-associated virus in breast-milk transmission are not known, nor is the association between plasma and milk virus levels understood. The portal of entry for the virus via the infant mucosa also merits further investigation.

Animal models have been used to explore potential mechanisms of transmission. It is possible to infect neonatal rhesus monkeys with simian immunodeficiency virus (Baba *et al.*, 1994) and kittens with feline immunodeficiency virus (Sellon *et al.*, 1994) by applying cell-free virus on the mucosa. This suggests that cell-free HIV in breast milk could infect cells of the intestinal mucosa. M-cells, which are specialized epithelial cells found in the Peyer's patches of the intestinal mucosa, may be a mechanism allowing infectious agents such as HIV to cross the intact mucosa. M-cells engulf and transport the pathogen and present it to macrophages that indent the serosal surface of the M-cell (Featherstone, 1997). Results from *in vitro* studies on rabbit M cells suggest that HIV-1 particles could use M cells to cross the intestinal barrier (Amerongen *et al.*, 1991). A recent *in vitro* study indicated that HIV-infected cells themselves may also play an important role by stimulating ordinary enterocytes to engulf HIV particles presented by HIV-infected cells in the intestinal lumen (Bomsel, 1997). Moreover, HIV RNA has been detected in the oropharyngeal and gastric aspirates of a substantial proportion of infants born to HIV-infected mothers (Nielsen *et al.*, 1996, Ait-Khaled *et al.*, 1998).

Quantifying the risk of breast-milk transmission

Early studies investigating the frequency of breast-milk transmission and associated factors were limited by small numbers as well as by the predominance of one method of infant feeding in any one cohort (European Collaborative Study, 1992; Ryder, 1991; Blanche *et al.*, 1989; Tovo *et al.*, 1988).

In 1992, a meta-analysis was carried out using data from four studies reporting on 42 recently infected women and six studies reporting on 1772 women with established infection. The majority of the women had breastfed for 2-4 weeks, and 106 women had breastfed for longer than six months. The estimated *additional risk* of transmission from breast milk, above that occurring during pregnancy and delivery, among women with *established HIV infection*, was approximately 15% (95% Confidence Interval 7-22%) (Dunn *et al.*, 1992). However, 15% may be an under-estimation among women who breastfeed for longer periods of time.

The risk of transmission through breast milk among women with *recent infection* (HIV infection acquired in the postpartum period) was nearly twice as high (29% (95% CI 16-42%)).

Insufficient information is available to estimate the exact association between duration of breastfeeding and the risk of transmission. However, there is strong evidence for a gradual and continued increase in transmission risk as long as the child is breastfed (Taha *et al.*, 1998, Leroy *et al.*, 1998).

Timing of HIV transmission during breastfeeding

Transmission of HIV through breast milk can take place at any point during lactation. **The persistence of maternal antibodies and the presence of a "window period" during which infection is undetectable using currently available technology, make it impossible to determine whether an infant has been infected during delivery (intrapartum) or through breastfeeding in the period following birth.** Therefore, when seropositive women breastfeed their infants, it is not possible to differentiate between HIV transmission attributable to delivery and that resulting from breastfeeding from birth. (Newell, 1998; Bobat *et al.*, 1997; Mandelbrot *et al.*, 1996; Bertolli *et al.*, 1996; Simonon *et al.*, 1994; Datta *et al.*, 1994).

Later postnatal transmission through breastfeeding can be determined using currently available diagnostic tools. Studies of infants found to be negative by PCR testing at 2-6 months of age, but who subsequently showed evidence of infection, have provided estimates of the *risk of late postnatal transmission* (after 3-6 months of age) ranging from 4-12% (Ekpini *et al.*, 1997; Karlsson *et al.*, 1997; Bertolli *et al.*, 1996; Simonon *et al.*, 1994).

Table 2. Studies of the risk of late postnatal transmissions

Study	Time of negative PCR	Median length of breastfeeding	Risk of HIV infection through late postnatal breastfeeding	Number of infants in study
Leroy *et al.*, 1998 (meta-analysis)	2.5-15.7 months	15 months	9.2%	429
Taha *et al.*, 1998	7 weeks	Not available	9.6%	621
Ekipini *et al.*, 1997	3-6 months	20 months	12%	45
Bertolli *et al.*, 1996	3-5 months	12 months	4%	189
Simonon *et al.*, 1994	3 months	19 months	4.9%	180

Colostrum and mature milk

Cell-free and cell-associated HIV-1 have been detected in both colostrum and mature breast milk of women with established HIV infection. In a study in Haiti, HIV DNA (cell-associated virus) was detected in 70% of 47 colostrum samples and about 50% of breast-milk samples obtained at 6 (n=30) and 12 (n=15) months postpartum (Ruff *et al.*, 1994). HIV DNA was detected in 47% of 129 samples of breast milk collected 15 days after delivery, and in 20% of 96 samples collected six months after delivery (Van de Perre *et al.*, 1993). Both studies suggest a higher level of cell associated HIV in early milk compared to later, which would reflect the relatively high level of cells in colostrum compared to mature milk.

Somewhat differently, in a study in Kenya (Nduati et al., 1995) a higher proportion of samples of milk collected between seven days and six months had HIV DNA (65% of 108 breast milk samples) than did colostrum (51% of 77 samples) (p=0. 05). Among positive samples, the proportion of infected cells ranged from less than 1 in 10 000 cells to 1 in 3. High concentrations of HIV-infected cells were more common during the period 8-90 days after delivery than in samples taken either earlier or later. A second study quantified HIV-1 RNA (measuring cell-free virus) from breast-milk supernatants collected from the same group of women at the same times (Lewis et al., 1998). The range of viral concentration in the breast-milk supernatants was very wide and, although the difference was not statistically significant, viral load tended to be higher in milk collected more than 8 days after delivery than in milk samples taken earlier (p=0.10).

The potential effect of various factors makes it difficult to draw any conclusions about the relative risk of transmission through colostrum and mature breast milk. First, colostrum and mature breast milk contain different types of cells and different levels of immune modulating components (e.g. vitamin A, immunoglobulins and lactoferrin). Second, the total volume of colostrum ingested by the infant is much smaller than that of mature breast milk. Third, the infant's immune system is less well developed during the first few days of lactation than in later lactation, while younger infants have an increased blood concentration of maternal antibodies. In the study by Tess *et al.* (1998b), vertical transmission was not associated with a history of colostrum intake in 148 breastfed children.

Factors associated with the risk of mother-to-child transmission

The overall risk of mother-to-child transmission is increased by a range of factors related to HIV disease, the mother, and the infant (for a comprehensive review of these factors see Newell *et al.*, 1997). Some of these factors may also affect the risk of transmission through breast milk. Maternal risk factors include indicators of disease progression, such as high viral load, low CD4 count, and viral characteristics. The observation

that the risk of transmission through breastfeeding is higher if the mother is infected postnatally (Dunn *et al.*, 1992) suggests that the higher **viral load** associated with recent infection may also increase the risk of breastfeeding transmission. However, it is not clear whether viral load in blood and in breast milk are correlated. Viral load in the breast milk of postnatally infected women is an area that requires further study. Low **CD4 counts** have been associated with detection of HIV DNA in breast milk. A Kenyan study (Nduati *et al.*, 1995) found a strong correlation between maternal immunosuppression (low CD4 counts) and the prevalence and concentration of breast milk HIV-1 DNA. However, knowledge of the role of maternal immunosuppression and advanced HIV disease in breast-milk transmission remains limited.

In a Malawi study of 338 women with HIV, 196 (58%) of whom were deficient in vitamin A, HIV transmission was significantly associated with vitamin A status, independent of maternal CD4 status (Semba *et al.*, 1994). Vitamin A deficiency may increase the risk of mother-to-child HIV transmission by impairing T and B cell function, resulting in increased maternal viral load and reduced antibody concentrations. Alternatively, vitamin A deficiency could be a marker of advanced HIV disease, which may be the cause of the higher observed mother-to-child transmission rate. In a study of 72 women with CD4 counts of less than 400/ml in Nairobi (Nduati *et al.*, 1995), **vitamin A deficiency** was associated with a linear increase in the prevalence of HIV-1 DNA in breast-milk cells. All six women with vitamin-A levels < 20 □g/dl had detectable HIV-1 DNA in their breast milk, compared to only three of eight women with vitamin-A levels at or above 40 □g/dl. Although the association between vitamin A and HIV in breast milk has been documented, no studies have been published concerning the role of vitamin A deficiency in breast-milk transmission. Vitamin A deficiency in HIV-infected women has been reported to be associated with **fissured nipples** (Nduati *et al.*, 1997), which may facilitate transmission of HIV through breastfeeding. Poor breastfeeding techniques, especially poor attachment of the infant to the breast, may result in fissured nipples and hence HIV transmission may be prevented through breastfeeding counselling, and skilled help with positioning and attachment (Tess *et al.*, 1998b; Van de Perre, 1992; Ekpini *et al.*, 1997).

Table 3 Risk factors associated with increased overall risk of mother-to-child transmission

Strong evidence	*Limited evidence*
Maternal	
High viral load	Vitamin A deficiency
Viral characteristics	Anaemia
Advanced disease	Sexually transmitted disease
Immune deficiency	Chorioamnionitis
HIV infection acquired during pregnancy or breastfeeding period	Frequent unprotected sexual intercourse*
	Multiple sex partners*
	Smoking
	Injecting drug use
Obstetric	
Vaginal delivery (compared with caesarean)	Invasive procedures
Prolonged rupture of membranes	Episiotomy
Infant	
Prematurity	Lesions of skin and/or mucous membranes (oral thrush)
Breastfeeding	

*Probably due to acquisition of further virus or minor trauma

See: *Review* Newell *et al.*, 1997; *and* Kind *et al.*, 1998; Mandelbrot *et al.*, 1998; Read *et al.*, 1998; Simonds *et al.*, 1998; Bulterys *et al.* 1997, Burns *et al.*,1997; Coll *et al.*, 1997; Ekpini *et al.*1997; Greenberg *et al.*, 1997; Kuhn *et al.*, 1997; Maguire *et al.*, 1997; Matheson *et al.*, 1997; Mayaux *et al.*, 1997; Pitt *et al.*, 1997; Shearer *et al.*, 1997; Thea *et al.*, 1997; Zollner *et al.*, 1997; Dickover *et al.*, 1996; Guay *et al.*, 1996; Landesman *et al.*, 1996; Lapointe *et al.*, 1996; Lutz-Friedrich *et al.*, 1996; Mandelbrot *et al.*, 1996; Rodriguez *et al.*, 1996; Shaffer *et al.*, 1996; Wabire-Mangen *et al.*, 1996; Harmsen *et al.*, 1995; Matheson *et*

al., 1995; Mayaux *et al.*, 1995; Ometto *et al.*, 1995; Temmerman *et al.*, 1995; Borkowsky *et al.*, 1994; Boyer *et al.*, 1994; Burns *et al.*, 1994; Dunn *et al.*, 1994; Kliks *et al.*, 1994; Lallemant *et al.*, 1994; Nduati *et al.*, 1994; Semba *et al.*, 1994; Thomas *et al.*, 1994; Clerici *et al.*, 1993; Galli *et al.*, 1993; Jackson *et al.*, 1993; Lepage *et al.*, 1993; Nair *et al.*, 1993; Roques *et al.*, 1993; Scarlatti *et al.*, 1993; Scarlatti, Hodara *et al.*, 1993; St Louis *et al.*, 1993; Van de Perre *et al.*, 1993; Villari *et al.*, 1993; Dunn *et al.*, 1992; European Collaborative Study, 1992; Goedert *et al.*, 1991; Hutto *et al.*, 1991; Lindgren *et al.*, 1991; Monforte *et al.*, 1991; Hira *et al.*, 1990; Van de Perre *et al.*, 1991; Tovo *et al.*, 1988.

HIV has been recovered from vaginal and cervical secretions of pregnant women (Henin *et al.*, 1993; John *et al.*, 1997; Loussert-Ajaka *et al.*, 1997) and from gastric secretions of infants born to HIV-seropositive women (Ait-Khaled *et al.*, 1997; Nielsen *et al.*, 1996). **Delivery factors** that increase contact between the infant and HIV-infected maternal body fluids (cervico-vaginal secretions and blood) may therefore be the mechanism for increased risk of transmission (Read *et al.*, 1998; European Collaborative Study, 1994). Vitamin A deficiency may also be a co-factor for increased risk associated with delivery, through impaired integrity of epithelial surfaces (Bridbord and Willoughby, 1994) and increased vaginal viral shedding (John *et al.*, 1997, Mostad *et al.*, 1997).

Neonatal skin and mucous membranes are ineffective barriers against infective organisms. Direct invasion of the skin and oral and gastric mucosa by HIV may play a role in transmission from mother to child, including through breast milk. Traumatic or inflammatory disruption of the skin or mucous membranes may further increase the risk of transmission (Ekpini *et al.*, 1997; Clerici *et al.*, 1993; European Collaborative Study, 1992; Hutto *et al.*, 1991; Goedert *et al.*, 1989). Disruption of the **epithelial integrity of the mucous membranes** of the intestine or mouth, caused by nutritional factors or infection, may increase the risk of HIV transmission through breast milk.

Factors resulting in disruption of the integrity of infants' mucous membranes, such as **oral thrush**, may be associated with an increased risk of breast-milk transmission (Ekpini *et al.*, 1997; Njenga *et al.*, 1997; European Collaborative Study, 1992).

Feeding with cow's milk, allergic reactions to complementary foods, and infectious illness can all result in intestinal damage. Because damage to the epithelial integrity of the intestine may facilitate entry of HIV, **mixed feeding** might be more risky for HIV transmission than exclusive breastfeeding. Infants could thus be doubly disadvantaged by being at risk of HIV transmission through simultaneous exposure to HIV through breastfeeding, and the risks related to replacement feeding. Only three studies have compared the rate of transmission in exclusively breastfed, partially breastfed and formula-fed infants (Tess *et al.*, 1998b; Bobat *et al.*, 1997; Ryder *et al.*, 1991;). Although the highest transmission rate was found in exclusively breastfed infants, the lowest rate in formula-fed infants, and intermediate rates in the mixed-feeding groups, the number of exclusively formula-fed or breastfed infants in these studies was small and the differences in rates of transmission were not statistically significant.

Anti-infective properties of breast milk in women with HIV

General infections

One of the most important benefits of breast milk is its ability to protect against common childhood infections such as diarrhoea, pneumonia, neonatal sepsis and acute otitis media (Golding, 1998; Duncan *et al.*,1993; Goldman, 1993; Ashraf *et al.*, 1991; Huffman *et al.*, 1990; Lucas A., 1990; Habicht *et al.*, 1986 & 1988; Victora *et al.*, 1987; Hanson *et al.*, 1985). It has been assumed, but not proven, that the breast milk of HIV-infected women also protects infants against these infections.

In a study in Kinshasa of 19 infected children, development of clinical AIDS was not associated with two particular types of infant feeding practice (Ryder *et al.*, 1991). However, morbidity was significantly higher

in 237 non-HIV-infected children (of both infected and uninfected mothers) who were not exclusively breastfed, compared with 81 uninfected infants who were exclusively breastfed during the first six months of life (Ryder *et al.*, 1991). In Durban, South Africa, exclusively breastfed infected children had a slower rate of progression to AIDS than those on mixed feeds (Bobat *et al.*, 1997).

Two recent studies from South Africa compared partially breastfed and exclusively formula-fed HIV-infected infants (Bobat et al., 1997; Gray et al., 1996). In these studies, both groups had similar frequencies of failure to thrive, diarrhoea, and pneumonia. Uninfected infants of HIV-positive mothers also had a comparable frequency of these conditions, whether they were partially breastfed or exclusively formula-fed. However, these results should be interpreted with great caution since the failure to detect a difference in health outcomes between breastfed and formula-fed infants may reflect factors specific to these studies. These include: short duration of exclusive breastfeeding and the inclusion of infants that had stopped breastfeeding in the breastfeeding group; a relatively safe environment (water, electricity, sanitation etc.) that minimized the risks of formula feeding; and a relatively literate, urban study population with access to continual health care, as part of a research study design. It is unlikely that these findings would be replicated in studies from other settings in sub-Saharan Africa without additional support being given to women who choose not to breastfeed.

HIV infection

Breast milk contains maternal antibodies. All basic forms of immunoglobulins IgG, IgM, IgA, IgD, and IgE are present in breast milk. The most abundant is usually secretory IgA (Lawrence, 1994). The role of HIV-specific antibodies in breast milk in inhibiting HIV transmission through breastfeeding has been investigated. Breast milk in women with established HIV infection has been found to have HIV-specific IgG, with its wide spectrum of activity against HIV proteins, comparable to HIV-specific IgG in serum. The spectrum of activity of serum IgA against HIV has been found to be similar to that of serum IgG, but the spectrum of activity of HIV-specific secretory IgA (sIgA) in breast milk is directed against only a limited number of viral proteins (env protein, gp 160, core proteins).

In a study of breast-milk samples from 215 HIV-infected women in Rwanda (Van de Perre *et al.*, 1993), the most frequently identified HIV-specific antibody in breast milk was IgG (in >95% of samples), the next was IgM (in 41-78% of samples) and the least frequent was IgA (in 23-41% of samples). Lack of persistence of HIV-specific IgM in breast milk collected at 18 months was associated with a high risk of transmission of HIV. Of 20 children receiving breast milk with detectable HIV DNA in samples collected at day 15, but without detectable IgM in later samples, 47% were infected with HIV. In those with detectable DNA in breast milk samples at day 15, and with IgM in later samples only 30% became infected. This suggests that IgM may protect against breast-milk transmission of HIV. The rate of transmission was 18% in infants of mothers whose breast-milk sample at day 15 had undetectable HIV DNA, regardless of IgM levels (Van de Perre *et al.*, 1993).

Other components of breast milk are protective against viral infections. Human lactoferrin has been shown *in vitro* to have an inhibitory activity against HIV (Harmsen *et al.*, 1995), and lipid-dependent antiviral activity directed at HIV and other enveloped viruses and bacteria has also been described (Orloff *et al.*, 1993; Isaacs and Thormar, 1990). An additional factor that has also been identified in breast milk, possibly a sulphated protein, glycoprotein mucin or glycosaminoglycan, appears to inhibit the binding of HIV to CD4 receptors (Newburg *et al.*, 1992).

Strategies to reduce breast-milk transmission

Primary prevention

The best way to prevent HIV infection of children through mother-to-child transmission, including transmission through breast milk, is to prevent HIV infection of young girls and women of childbearing age. In sub-Saharan Africa, Asia and the Caribbean the main mode of HIV transmission is heterosexual contact. In industrialized countries, although most women with HIV have a history of injecting drug use (IDU), or

sexual partners with a history of IDU or bi-sexuality, heterosexual transmission is becoming an increasingly important route of infection (Wortley and Fleming, 1997; Gabiano *et al.*, 1992; Holmes, 1991).

The risk of HIV infection in women is increased by such factors as immaturity of the genital tract, cervical ectopy, sexually transmitted diseases, and poor nutritional status (Mostad and Kreiss, 1996; Leroy *et al.*, 1994; Plummer *et al.*, 1994;). Cultural, social and economic factors also contribute to HIV transmission by increasing the vulnerability of girls and women (Ankrah *et al.*, 1994; UNDP, 1994).

Strategies to prevent all mother-to-child transmission of HIV, including through breast-milk, should be linked to primary prevention programmes that provide education about safer sex, condoms, and diagnosis and treatment of sexually transmitted diseases, and that ensure the safety of medical procedures. HIV prevention should be emphasized for women who test seronegative in pregnancy because of the particularly high risk of MTCT if mothers are infected with HIV during pregnancy and breastfeeding.

Replacement feeding

For an HIV-infected woman to eliminate completely the risk of HIV transmission through breastfeeding she needs to feed her infant from birth with suitable replacements for breast milk (such as commercial infant formula or home-prepared formula made from modified animal milks). The range of replacement feeding options is described in *HIV and infant feeding: A guide for health managers and supervisors*. Currently there is little information on the safety and feasibility of using breast-milk substitutes in developing countries.

Several investigators have attempted to use mathematical models to offer guidance to policy-makers in different settings for weighing the relative risks and benefits of breastfeeding and other infant feeding methods in view of the HIV epidemic (Kuhn and Stein, 1997; Hancock *et al.*, 1996; Nagelkerke *et al.*, 1995; Nicoll *et al.*, 1995; Del Fante *et al.*, 1993; Hu *et al.*, 1992; Heyman, 1990). These models are limited by the available data regarding the risks associated with various methods of infant feeding and their inability to consider all the factors that influence decision-making about infant feeding. In particular, although there is much evidence of the benefits of breastfeeding in reducing morbidity and mortality in infants whose mothers are *not infected* with HIV, currently there is little information regarding the effect of replacement feeding on infant morbidity and mortality for infants whose mothers are HIV-infected.

Where adequate replacement feeding is not possible, mothers may choose among three other strategies to reduce the risk of breast-milk transmission:

- Exclusive breastfeeding followed by early cessation of breastfeeding. Early cessation of breastfeeding may reduce exposure and hence the risk of breast milk transmission, while not eliminating the risk entirely, as the infant remains exposed for the first few months.
- Heat treatment of expressed breast milk
- Wet-nursing by a tested HIV-negative women

Early cessation of breastfeeding

Early cessation of breastfeeding reduces the risk of HIV transmission by limiting the length of time that an infant is exposed to HIV through breast milk. Women who are not able to provide adequate and hygienic replacement feeding to their infants from birth may consider this option in order to reduce the cumulative risk of longer breastfeeding duration (Leroy *et al.*, 1998; Epkini *et al.*, 1997; Van de Perre, 1997). It is not yet possible to specify the optimum time for cessation of breastfeeding.

Treatment of breast milk

In vitro studies have demonstrated that heat treatment of breast milk to which a known quantity of HIV had been added, using the Holder pasteurization method (at 62.5^{o}C for 30 minutes), reduces the infectious titre of cell-free and cell-associated virus by more than five logs and six logs, respectively (Orloff *et al.*, 1993).

As discussed earlier, breast milk contains substances that inhibit infectious agents (Goldman, 1993). Several studies have reported that HIV is inactivated when milk is left to stand at room temperature for half an hour (Orloff *et al.*, 1993; Newburg *et al.*, 1992; Isaacs and Thormar, 1990). In the first two of these studies, the inhibitory effects of breast milk were attributed to a milk-lipase-activated factor that released fatty acids which were thought to dissolve or disrupt the viral envelope. Newburg *et al.* demonstrated that human milk glycosaminoglycans inhibit binding of HIV glycoprotein gp120 to host cell CD4 receptors. There is a need to evaluate alternatives for treating breast milk, which utilize or enhance the action of naturally occurring anti-HIV factors to prevent breast-milk transmission of HIV.

However, all strategies to modify or treat breast milk to render it non-infectious would involve expressing milk, and some women may find it difficult to sustain this process for long periods of time. This should not prevent the option being offered, and professional support should be provided when women choose it. Expression and heat treatment may also be a temporary solution during periods of increased transmission risk, as in cases of cracked nipples or breast abscess, and for low-birth-weight or sick infants for whom the risk of artificial feeding is greater.

Heat treatment of breast milk is recommended for all milk banks, which should also screen milk donors for HIV.

Wet-nursing by a tested HIV-negative woman

In communities where wet-nursing by a family member is practised this option can be considered. It will be necessary for the wet-nurse to agree to and understand the implications of voluntary HIV counselling and testing (VCT). She would also have to be counselled about HIV and be able to avoid becoming infected during breastfeeding.

Antiretroviral therapy

The use of AZT (zidovudine) during the second and third trimester in pregnant women and in infants during the first six weeks of life, in the absence of breastfeeding, can reduce mother-to-child transmission of HIV by two-thirds (Connor *et al.*, 1994). A “short course” regimen of AZT (after 36 weeks gestation and without the neonatal component), combined with formula feeding, has recently been shown in Thailand to reduce mother-to-child HIV transmission by half (Centers for Disease Control, 1998). The latter approach may be more feasible where women present late for prenatal care, or where health service resources are limited. Further reductions in mother-to-child HIV transmission may be possible with the use of a combination of antiretroviral drugs (Bryson, 1996), which are currently being evaluated in clinical trials in both breastfeeding and non-breastfeeding populations (Fowler, 1997).

The effectiveness of AZT in reducing mother-to-child transmission has been demonstrated only in non-breastfed infants. It is currently not known to what extent infants who have escaped infection during pregnancy and delivery, following prophylactic therapy in their mothers with AZT, are at risk of becoming infected subsequently through breastfeeding. However, it is likely that antiretroviral therapy around the time of delivery will not be as effective if the infant is then exposed through breastfeeding. Since many HIV-infected mothers may face obstacles to replacement feeding - for example stigma, affordability, risk to the infant of other infections and malnutrition - the effectiveness of antiretroviral treatment of breastfeeding mothers/or breastfed infants, with and without a postnatal treatment component, is an important research question. Several trials are under way, in populations where breastfeeding is the norm, to evaluate various AZT regimens, combination therapy using two antiretrovirals (AZT with 3TC), and Nevirapine (Fowler, 1997). Results of these and other trials will be available by mid-1999. It is important for this information to be available before policies are adopted which introduce antiretroviral therapy to reduce the risk of mother-to-child transmission in situations where infant feeding choices are limited.

Summary and Conclusion

Current scientific evidence provides the basis for the following statements and suggests areas where additional research is required.

Mother-to-child transmission of HIV

- The overall risk of mother-to-child transmission of HIV is about 15-25% among seropositive women who do not breastfeed (in the absence of interventions to reduce the likelihood of transmission), and between 25-45% among women who breastfeed.

HIV can be transmitted through breast milk

- The virus has been detected in components of breast milk.
- HIV infection has been found in infants of mothers who became infected with HIV during the breastfeeding period.
- Infants of HIV-negative mothers have been infected through exposure to HIV in unpasteurized breast milk from unscreened donors and HIV-infected wet-nurses.
- Infants diagnosed as HIV-negative at three months of age or later have been infected subsequently, with breastfeeding being the only risk factor.

Breastfeeding can be an important mode of mother-to-child transmission of HIV

- Where the mother has established HIV infection, the overall *additional risk* of HIV transmission during breastfeeding is at least 15%.
- In populations where breastfeeding is the main method of infant feeding, approximately one third of paediatric HIV is due to breast-milk transmission.

The mechanisms of breast-milk transmission are not yet fully understood

- The respective roles of cell-free and cell-associated HIV in breast-milk transmission are not known, nor is the association between plasma and milk virus levels understood.
- The portal of entry for the virus via infant mucosa requires further investigation.

Certain factors may increase the risk of HIV transmission through breast milk

- When a mother has been *recently* infected with HIV, the risk of transmission through breastfeeding may be twice as high as that of a women whose infection is already established (29% compared with 15%). This is probably due to high viral load occurring with recent infection. However, it is not clear whether a high serum viral load is correlated with a high viral load in breast milk. Further research is required.
- Increased risk of mother-to-child transmission is associated with markers of advanced HIV infection and maternal immunosuppression, including plasma viral load, clinical symptoms, and low CD4 and high CD8 cell counts. However, current knowledge about the role of maternal immunosuppression and advanced HIV disease in breast-milk transmission is limited and requires further investigation.
- Vitamin A deficiency is associated with an increased risk of overall mother-to-child transmission and with HIV in breast milk, but no studies have confirmed the role of vitamin A deficiency in increasing the risk of transmission through breastfeeding. Vitamin A supplementation has not been proved to be effective in reducing MTCT.

- Disruption of the epithelial integrity of the mucous membranes of the infant mouth or intestine (caused by nutritional or infectious factors such as mixed feeding and oral thrush), and nipple fissures may play a role in increasing the risk of transmission through breastfeeding. Research in this area continues.

- The effect on HIV transmission due to breastfeeding of giving AZT during pregnancy and delivery is not known, nor is the effect of postnatal treatment of breastfed infants with ARVs. Research is being carried out and results will be available in 1999.

Transmission can take place at any point during breastfeeding

- The risk of breast-milk transmission of HIV appears to be cumulative. The longer the duration of breastfeeding, the greater the additional risk of HIV transmission through breast milk.

- Because it is not known whether the risk of transmission differs at different times during lactation, the degree of efficacy resulting from early cessation of breastfeeding cannot be predicted.

- HIV has been detected in colostrum and mature breast milk; however, based on current evidence, it is not possible to establish the relative risks of transmission through colostrum and breast milk.

- Currently available diagnostic tools are inadequate for estimating risk associated with breastfeeding in the first few months of life. *The risk of late postnatal transmission* through breastfeeding is estimated to be 4-12%. This may possibly account for about half of transmission through breastfeeding.

The anti-infective properties of breast milk in the context of HIV

- HIV-positive women who breastfeed infants who are already infected with HIV may provide some protection against common childhood infections. Further research is required.

- Anti-infective substances in the breast milk of HIV-infected women, including immunoglobulins, lactoferin, and mucins, may target HIV, but further studies are needed to investigate the correlation between risk of transmission and the presence or absence of these substances.

The safety of different methods of infant feeding

- There is very little information on the safety and feasibility of infant-feeding alternatives for seropositive mothers and these aspects need to be studied (including commercial infant formula, home-made infant formula, heat-treated expressed breast milk, and wet-nursing). It is also important to identify approaches to treating expressed breast milk to eliminate the risk of transmission while preserving the milk's nutritional content.

- It is important to determine the efficacy of antiretroviral therapy given to the mother or the child during the breastfeeding period.

- Little is known about the effect of different feeding methods, including mixed feeding, on the course of HIV infection and other health outcomes in HIV-infected children.

Finally, research is needed on the effect of breastfeeding on the nutritional and immune status of the mother. The benefits of breastfeeding may be different for women infected with HIV.

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