

# Effect of Breastfeeding and Formula Feeding on Transmission of HIV-1

## A Randomized Clinical Trial

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**M**OTHER-TO-CHILD TRANSMISSION of human immunodeficiency virus type 1 (HIV-1) may occur in utero, at time of delivery, or through breastfeeding, but transmission frequency during each period has been difficult to determine. Breast milk HIV-1 transmission was first described in women newly infected after delivery through blood transfusion or heterosexual exposure.<sup>1,2</sup> Based on meta-analysis, frequency of breast milk transmission during acute maternal infection was estimated to be 29% (95% confidence interval [CI] 16%-42%).<sup>3</sup> For women with established infection, the additional risk of HIV-1 in breastfed infants was estimated at 14% (95% CI, 7%-22%).<sup>3</sup>

Most observational cohort studies are characterized by feeding practice ho-

**Context** Transmission of human immunodeficiency virus type 1 (HIV-1) is known to occur through breastfeeding, but the magnitude of risk has not been precisely defined. Whether breast milk HIV-1 transmission risk exceeds the potential risk of formula-associated diarrheal mortality in developing countries is unknown.

**Objectives** To determine the frequency of breast milk transmission of HIV-1 and to compare mortality rates and HIV-1-free survival in breastfed and formula-fed infants.

**Design and Setting** Randomized clinical trial conducted from November 1992 to July 1998 in antenatal clinics in Nairobi, Kenya, with a median follow-up period of 24 months.

**Participants** Of 425 HIV-1-seropositive, antiretroviral-naive pregnant women enrolled, 401 mother-infant pairs were included in the analysis of trial end points.

**Interventions** Mother-infant pairs were randomized to breastfeeding (n = 212) vs formula feeding arms (n = 213).

**Main Outcome Measures** Infant HIV-1 infection and death during the first 2 years of life, compared between the 2 intervention groups.

**Results** Compliance with the assigned feeding modality was 96% in the breastfeeding arm and 70% in the formula arm ( $P < .001$ ). Median duration of breastfeeding was 17 months. Of the 401 infants included in the analysis, 94% were followed up to HIV-1 infection or mortality end points: 83% for the HIV-1 infection end point and 93% to the mortality end point. The cumulative probability of HIV-1 infection at 24 months was 36.7% (95% confidence interval [CI], 29.4%-44.0%) in the breastfeeding arm and 20.5% (95% CI, 14.0%-27.0%) in the formula arm ( $P = .001$ ). The estimated rate of breast milk transmission was 16.2% (95% CI, 6.5%-25.9%). Forty-four percent of HIV-1 infection in the breastfeeding arm was attributable to breast milk. Most breast milk transmission occurred early, with 75% of the risk difference between the 2 arms occurring by 6 months, although transmission continued throughout the duration of exposure. The 2-year mortality rates in both arms were similar (breastfeeding arm, 24.4% [95% CI, 18.2%-30.7%] vs formula feeding arm, 20.0% [95% CI, 14.4%-25.6%];  $P = .30$ ). The rate of HIV-1-free survival at 2 years was significantly lower in the breastfeeding arm than in the formula feeding arm (58.0% vs 70.0%, respectively;  $P = .02$ ).

**Conclusions** The frequency of breast milk transmission of HIV-1 was 16.2% in this randomized clinical trial, and the majority of infections occurred early during breastfeeding. The use of breast milk substitutes prevented 44% of infant infections and was associated with significantly improved HIV-1-free survival.

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mogeneity, with women in developed countries using formula and those in developing countries breastfeeding. More recent observational studies in South Africa and Brazil had a more equal distribution of feeding practice.<sup>4,5</sup> Although these have provided valuable estimates of transmission risk, choice of feeding modality may be associated with other factors that influence transmission likelihood. Also, attempts to measure breast milk transmission rate have been hampered by the difficulty of distinguishing late in utero, intrapartum, and early breast milk transmission.

Following the first report of breast milk HIV-1 transmission, the Centers for Disease Control and Prevention issued guidelines recommending that infants of HIV-1-infected women should not be breastfed, which became the standard of care in the industrialized world.<sup>6</sup> Unfortunately, most at-risk infants are in the developing world where breastfeeding is a pillar of child survival, associated with reduced morbidity and mortality from infectious disease and providing inexpensive nutrition.<sup>7</sup> Thus, the World Health Organization's (WHO's) Global Programme on AIDS (acquired immunodeficiency syndrome) recommended in 1987 and 1992 that in regions where infectious disease and malnutrition are primary causes of infant mortality, women should breastfeed irrespective of HIV-1 status.<sup>8,9</sup> In 1996, the Joint United Nations Programme on HIV/AIDS (UNAIDS) recommended that HIV-1-seropositive women in resource-poor areas be encouraged to make an informed choice about infant feeding, ie, that consideration of risks and benefits of feeding practices be individualized for each woman.<sup>10</sup> To make this informed choice, a reasonably accurate estimate of risk and timing of breast milk HIV-1 transmission is required.

In 1992, we initiated a randomized clinical trial of breastfed and formula-fed infants of HIV-1-infected women to determine frequency of breast milk HIV-1 transmission and to compare mortality rates in the 2 arms.

## METHODS

### Study Population

Pregnant women attending 4 Nairobi City Council antenatal clinics were offered counseling and serologic testing for HIV-1 by the research team. Seropositive women were invited to attend the research clinic at Kenyatta National Hospital. Women were eligible if they resided in Nairobi and had access to municipal-treated water. Of Nairobi residents, 92% had access to municipal water during the study.<sup>11</sup>

All eligible women received extensive counseling by study physicians (R.N., G.J., and D.M.) on mother-to-child HIV-1 transmission, risks and benefits of breastfeeding and formula feeding, and the nature of randomized clinical trials. Policy recommendations for breastfeeding by HIV-1-infected women issued by the WHO or UNAIDS were discussed.<sup>8-10</sup> Women agreeing to adhere to the mode of infant feeding determined by the randomization process were enrolled.

### Enrollment and Randomization

At enrollment, the women had a standardized interview and a physical examination. Interim history and physical examination were obtained at each prenatal visit. A pelvic examination, which included screening for genital tract infections and collection of cervical and vaginal secretions for HIV-1 DNA-polymerase chain reaction (PCR) analysis was conducted at about 32 weeks' gestational age.<sup>12</sup> To determine CD4 and CD8 cell counts, HIV-1 RNA viral loads, and vitamin A levels, 15 mL of blood was collected. Antiretroviral therapy was not used by participants.

Women were randomized to breastfeed or formula feed at about 32 weeks using computer-generated block randomization. The formula group was given free dried milk formula and safe preparation was demonstrated. Women were told to boil water for mixing formula and to feed the infant with a cup instead of a bottle to minimize bacterial contamination. At the next visit, women were asked to demonstrate formula preparation. We purchased for-

mula made in Kenya and packaged in 500-g tins with a standardized measuring scoop, costing 400 Kenya shillings (US \$7) per tin. Six months of formula costs about US \$300.

### Delivery and Follow-up

At delivery, 15 mL of cord blood was collected for lymphocyte separation. Mother-infant pairs were followed up monthly in the first year and quarterly in the second year of an infant's life. At each follow-up visit, an interim history was obtained, including feeding history, and a physical examination of infant and mother was performed. At 3-month intervals, 25 mL of breast milk was collected. At birth, 6 weeks, 14 weeks, and every 3 months until 24 months of age, 5 mL of anticoagulated infant blood was collected in EDTA tubes, along with 5 drops of blood on filter paper (Schleicher & Schuell, Keene, NH) for HIV-1 DNA PCR testing. Study infants received standard childhood immunizations and general medical care in the research clinic.

After delivery, staff again observed formula preparation and cup feeding. A nurse visited women in their homes within 2 weeks of delivery to counsel them about infant feeding, with repeat visits on an as-needed basis or following a failed clinic appointment. Women who had left Nairobi were traced to their rural homes.

The study was approved by the universities of Nairobi's and Washington's institutional review boards and all women provided informed consent. Women were encouraged to discuss study participation with husbands and key persons, and many had several discussions with study physicians before deciding. The Kenyan Ministry of Health gave permission to conduct the study.

### Laboratory Methods

For HIV-1 serologic testing an HIV enzyme-linked immunosorbent assay (ELISA) (Behring, Ausgaba, Germany) was used for screening and a second ELISA (Cambridge Biotech, Rockville, Md) for confirmation. The CD4 and CD8 cell counts were determined

using monoclonal antibodies (Becton Dickinson, Erembodegem-Aalst, Belgium) and flow cytometry. Vitamin A levels were measured using high-performance liquid chromatography on maternal serum or plasma samples.

Processing of peripheral blood mononuclear cell (PBMC), filter paper, breast milk, cervical, and vaginal specimens has been described.<sup>12-14</sup> Nested PCR that detects a single HIV-1 provirus copy was used to detect HIV-1 in all specimens. Primers that recognized highly conserved gag gene sequences were used.<sup>12-14</sup>

All PBMC samples from all children at all time points were tested using HIV-1 PCR, allowing us to evaluate consistency of PCR results for each infant and frequency of false-positive and false-negative test results. Of infants classified as HIV-1 infected, 157 (95%) of 166 PBMC PCR test results, taken after infant diagnosis of HIV-1 infection, were positive. Of infants classified as HIV-1 uninfected, 1293 (98%) of 1318 noncord blood PBMC PCR results were negative.

Maternal HIV-1 *env* subtype was determined using heteroduplex mobility assays and/or sequence analysis.<sup>15</sup> Maternal plasma viral load was measured using Gen-Probe HIV-1 RNA assay (Gen-Probe, La Jolla, Calif).<sup>15</sup> The lower limit of detection is 50 copies/mL.

A child was determined to be HIV-1 infected if PBMC or filter paper blood samples from 2 consecutive dates had positive test results for HIV-1 DNA by PCR, if a single blood sample had a positive test result for HIV-1 DNA if the sample was obtained at last visit seen, or if a serum sample had a positive HIV-1 ELISA test result if the sample was obtained at the last visit of a child 15 months or older with no sample from that date available for PCR testing. A child was determined to be HIV-1 uninfected if no HIV-1 infection criteria were met, and if a blood sample obtained at the child's last visit had a negative HIV-1 DNA test result, or if a serum sample obtained at the last visit had a negative ELISA test result from a child 15 months or older with no sample from that date available for PCR testing. For a child determined to be HIV-1 infected because of 2 consecutive

positive PCR test results, time of infection was defined by the first test result.

### Data Analysis

The study was designed to have a sample size of 400 for 90% power to detect a 1.6-fold difference in HIV-1 infection rates between breastfed and formula-fed infants using a 2-sided test with  $\alpha = .05$ , and allowing for 25% loss to follow-up, based on the 48% transmission rate seen in a prior Kenyan observational study.<sup>16</sup>

All analyses were intent-to-treat. For routine analyses, SPSS Version 8.0 (SPSS Inc, Chicago, Ill) was used. Differences between the 2 groups were tested using the Mann-Whitney U test for continuous variables and Pearson  $\chi^2$  test or Fisher exact test for binary variables. All tests were 2-tailed.

Only live-born singletons and first-born (more similar to singleton delivery) twins were analyzed. In follow-up analyses of the HIV-1 infection end point, an infant was defined as lost to follow-up at birth if not tested for HIV-1. An infant was defined as lost to follow-up at time of the infant's last HIV-1 test if the test preceded the end of the study or the infant's death by more than 3 months. Follow-up duration was defined as age at last HIV-1 test, or 24 months, whichever was less.

Mortality rates in the 2 arms were compared using Kaplan-Meier survival analysis. However, we chose not to use Kaplan-Meier techniques for analysis of the HIV-1 infection end point comparisons because we were concerned that our data violated 2 key assumptions. One necessary assumption in Kaplan-Meier analysis is that censored subjects are at the same risk as those remaining in the analysis, ie, censoring is noninformative. Infants testing HIV-1 negative several months prior to death would be censored at the time of last negative HIV-1 test result in a standard Kaplan-Meier analysis. Since death and HIV-1 infection are highly correlated, death status provides information about infection status. Thus, in these data, we had informative censoring. Another assumption in Kaplan-Meier analysis is that event times are known exactly. In perinatal

HIV-1 studies, it is only known that infant infection occurred between the last negative and the first positive HIV-1 test result; ie, the data are interval censored. Due to informative censoring (death) and interval censoring, standard survival analysis methods were inappropriate for use in estimating timing of HIV-1 transmission and HIV-1-free survival. Also, as seen in other perinatal HIV-1 studies, use of cord blood to assess HIV-1 infection at birth was less than 100% specific, due to possible contamination by maternal blood.<sup>17</sup> In our study, the HIV-1 PCR assay had a 90% (124/138) specificity when applied to cord blood samples vs 100% for infant venous birth samples, and 98% for all infant venous samples. Of the 233 available birth blood samples, 181 were cord blood and 52 were infant venous blood draws. Thus, a method for estimating joint distribution of death and HIV-1 infection, which accounts for informative censoring, interval censoring, and imperfect diagnostic test specificity at birth, was developed to estimate timing of transmission and HIV-1-free survival.<sup>18</sup> For the following equation, let  $s$  denote the time of HIV-1 infection and  $t$  denote time of death. Then  $w_{s,t} = f_t g_{s|t}$  where  $w$  is the joint density of death and HIV-1 infection time,  $f$  is the marginal density of death time, and  $g$  is the conditional density of HIV-1 infection time given the death time. Since all death times were known or right censored (we assume noninformatively),  $f_t$  was modeled nonparametrically. Conditional on time of death,  $t$ , the density  $g$  was assumed to have the form of a Weibull distribution with point mass at time 0 (representing proportion of infants infected at birth), and point mass for infants uninfected at 24 months or death (whichever came first). Thus,

$$\begin{aligned} g_{s|t} &= 1 - P_t \\ &= P_t \frac{C_t S_t^{C_t-1}}{b_t^{C_t}} \exp \left[ - \left( \frac{S}{b_t} \right)^{C_t} \right] \\ &= P_t \exp \left[ - \left( \frac{t}{b_t} \right)^{C_t} \right] \end{aligned}$$

in the equation on the previous page, the first line is the value of  $g_{s|t}$  when  $s = 0$ , the second line is the value of  $g_{s|t}$  when  $0 \leq s \leq t$ , and the third line is the value of  $g_{s|t}$  when  $s = NI$ , where NI equals not infected. The probability of escaping in utero infection,  $p_t$ , and the Weibull parameters,  $b_t$ , and  $c_t$  were assumed to vary slowly in  $t$  using natural splines with 0 or 1 knot to accomplish this. The overall model was fit by alternately maximizing the likelihood of  $f_t$  conditional on  $g_{s|t}$  and then maximizing the likelihood of  $g_{s|t}$  conditional on  $f_t$  until convergence. The marginal distribution of time of HIV-1 infection and estimates of HIV-1-free survival were derived by appropriate summations or integration of the joint distribution  $w_{s,t}$ . Tests for differences at fixed ages between the 2 groups were based on standard  $z$  tests using jackknife SEs.<sup>19</sup> A permutation test was used to test for a difference in the

distribution of HIV-1 infection time.<sup>19</sup> More details are included in a technical report.<sup>18</sup> All conclusions about timing of HIV-1 transmission and HIV-1-free survival were robust to different assumptions and analytic approaches, including Kaplan-Meier survival analysis.

### Data and Safety Monitoring Board

The data and safety monitoring board (DSMB) reviewed study progress and interim analyses of primary end point data every 6 months; it made recommendations for study continuation or cessation based on O'Brien-Fleming criteria for group sequential testing.<sup>20</sup> No outcome data analyses were available to physician investigators during the study to ensure masking to interim results.

The trial was originally designed to continue until 24 months following the last birth. In July 1997, the DSMB recommended the study continue only until 6 months after the last birth, by which time 95% of the primary infant outcome results would be available. In April 1998, it recommended that breastfeeding women be advised to stop breastfeeding and be given formula, and that the study continue until July 31, 1998 (6 months after the last delivery) to complete data collection. In April 1998, 13 women still being followed up had reported breastfeeding at their last visit (including 12 randomized to the breastfeeding arm). These women were advised to stop breastfeeding.

## RESULTS

### Study Population

From November 6, 1992, to October 7, 1997, 16 529 women attending 4 antenatal clinics were screened for HIV-1 antibodies. Of these, 2315 (14%) were HIV-1 seropositive. Of the HIV-1-seropositive women, 1708 (74%) returned for results. Of those, 425 (25%) were enrolled. Thus, 18% of all seropositive pregnant women identified were enrolled in the trial. This was a highly selected subgroup, including women willing to have the feeding modality for their infant be randomly assigned and meeting eligibility criteria that would limit morbidity due to for-

mula use and maximize follow-up and compliance likelihood. The most common reasons for nonparticipation were participants' unwillingness to be randomized to formula feeding and their plans to leave Nairobi after delivery.

Median age of enrolled women was 23 years. Subjects were largely of lower socioeconomic status, with 61% of women living in a 1-room home, 74% sharing a toilet with other households, and 5% owning a refrigerator. All women had access to clean water, and 76% had access to flush sanitation.

The HIV-1-related immunosuppression at about 32 weeks gestation was moderate in the group overall, with only 47 (12%) of 381 women tested having absolute CD4 cell counts of less than  $200 \times 10^6/L$ . The median plasma viral load at this same visit was 42 360 (range, 112-2 483 750) copies/mL.

Of the 425 women enrolled, 212 were randomly assigned to breastfeed and 213 to use formula (FIGURE). The 2 groups had similar enrollment characteristics (TABLE 1).

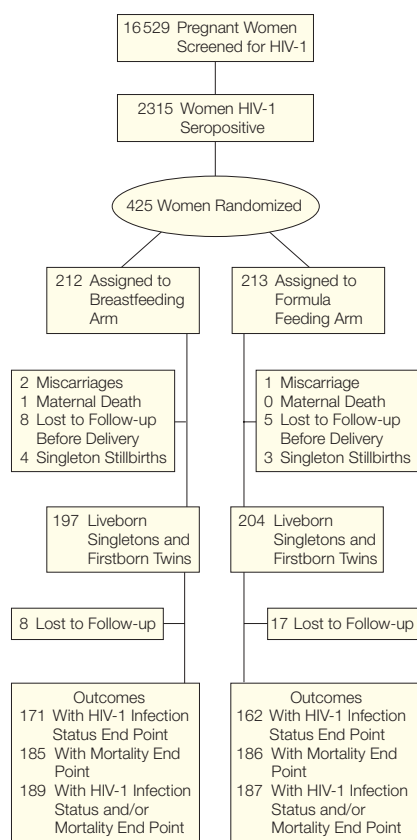
At the time of delivery, 408 women remained in the study (Figure). After excluding stillbirths and second-born twins, 401 infants remained. Women randomized to the breastfeeding and formula groups had similar pregnancy, labor, delivery, and neonatal characteristics (TABLE 2).

### Follow-up

Of the 401 mother-infant pairs included in the analysis data set for trial end points, 197 were in the breastfeeding arm and 204 in the formula arm. The median follow-up time was 24 months for both groups ( $P = .88$ ). Of the 401 infants, 68 (17%) were lost to follow-up before determining their HIV-1 status at age 2 years, death, or study completion. Women with infants lost to follow-up were not significantly different from women with followed up infants in age, years of education, absolute CD4 cell count, plasma RNA viral load, and infant birth weight.

The HIV-1 status outcome at study end was available for 333 infants (83%), including 171 (87%) in the breastfeed-

**Figure.** Trial Flow Diagram of Participants



HIV-1 indicates human immunodeficiency virus type 1.

ing arm and 162 (79%) in the formula arm (Figure). Mortality data at study end were available for 371 infants (93%), and HIV-1 infection status and/or mortality data at study end for 376 infants (94%), including 189 (96%) in the breastfeeding arm and 187 (92%) in the formula arm. Children at study end ranged in age from 6 to 24 months due to early trial termination. At study end, 66% (263) of children had been enrolled at least 24 months previously, 82% (330) 12 months or more, and 100% (401) 6 months or more.

### Compliance With the Intervention

Compliance with the formula-feeding intervention was defined by complete avoidance of breast milk. Compliance with the breastfeeding intervention was defined by any use of breast milk. Compliance determinations were based on self-reported feeding practices. Overall, 331 (83%) of the 401 participants reported that they had complied with the assigned feeding modality, but the percentages differed significantly by group. Of the 197 in the breastfeeding arm 189 (96%) complied, but only 142 (70%) of the 204 women in the formula arm ( $P < .001$ ) complied.

Among compliant women in the breastfeeding arm, 95% were breastfeeding at 3 months, 90% at 6 months, 80% at 12 months, 47% at 18 months, and 23% at 24 months. Median breastfeeding duration was 17 months (range, <1 wk to >24 mo). Median time to introduction of weaning foods was 3.8 months. In the breastfeeding arm, 83% of women were exclusively breastfeeding at 6 weeks, 62% at 3 months, and 9% at 6 months after delivery (exclusive breastfeeding defined as no intake other than breast milk).

### HIV-1 Infection

Of the 401 infants in the study, 92 were HIV-1 infected, including 61 in the breastfeeding group and 31 in the formula group. Cumulative probability of HIV-1 infection was significantly higher for infants randomized to breastfeeding than for those randomized to formula ( $P < .001$ ). At 24 months, the cu-

mulative probability of HIV-1 infection was 36.7% (95% CI, 29.4%-44.0%) in the breastfeeding arm and 20.5% (95% CI, 14.0%-27.0%) in the formula arm ( $P = .001$ ) (TABLE 3). Thus, the excess transmission occurring in the breastfeeding arm of the trial was 16.2% (95% CI, 6.5%-25.9%). In this arm, 44.1% of all transmission was attributable to breastfeeding.

To assess timing of HIV-1 infection through breastfeeding, we compared cumulative infection probabilities in the 2 groups at 6 specific ages at which blood sampling was scheduled (Table 3). There was a significant difference between the 2 groups at all ages after birth, with most difference occurring early. Even by the 6-week point, the risk difference was already 10.2% (95% CI, 3.1%-17.3%).

**Table 1.** Comparison of Enrollment Characteristics of the Two Infant Feeding Groups\*

Variables	Breastfeeders	Formula Feeders	P value
<b>Demographics</b>			
Age, y	23 (16-39)	23 (15-40)	.79
Currently married, No. (%)	158/212 (75)	167/213 (78)	.35
No. of years education	8 (0-17)	8 (0-16)	.62
Rooms in household	1 (1-6)	1 (1-5)	.21
No. (%) with refrigerators	9/212 (4)	12/213 (6)	.51
<b>Obstetric and sexual history</b>			
Gravidity	2 (1-8)	2 (1-7)	.85
Age at first sexual intercourse, y	17 (10-24)	17 (12-24)	.59
Lifetime No. of sex partners	3 (1-50)	3 (1-100)	.95
Gestational age of fetus at enrollment, wk	29 (8-39)	29 (11-39)	.28
<b>Laboratory values†</b>			
Hemoglobin, g/L	110 (6-18)	108 (6-16)	.12
No. (%) with anemia‡	93/200 (47)	114/207 (55)	.08
CD4, %	25 (2-47)	25 (4-49)	.77
Absolute CD4 cell count, $\times 10^6/L$	399 (10-1165)	415 (15-1207)	.35
Absolute CD4 cell count, $\times 10^6/L$ , No. (%)			
<200	22/187 (12)	25/194 (13)	.74
200-499	109/187 (58)	109/194 (56)	.68
$\geq 500$	56/187 (30)	60/194 (31)	.84
Plasma HIV-1 RNA viral load, $\times 10^5$ §	47 (0.1-2484)	38 (0.5-1228)	.34
<b>HIV-1 viral subtype, No. (%)</b>			
A	108/158 (68)	117/162 (72)	.42
C	11/158 (7)	11/162 (7)	.98
D	36/158 (23)	29/162 (18)	.24
G	0/158 (0)	1/162 (1)	.99
Recombinant	3/158 (2)	4/162 (3)	.99
Vitamin A level, $\mu\text{mol/L}$	0.86 (0.18-1.87)	0.88 (0.27-2.05)	.25
No. (%) with vitamin A level of $<0.70 \mu\text{mol/L}$	48/146 (33)	40/145 (28)	.33
<b>STDs and genital conditions, No. (%)†</b>			
Genital ulcer disease	32/203 (16)	30/204 (15)	.77
Syphilis (RPR)	19/202 (9)	13/207 (6)	.24
Trichomoniasis	20/101 (20)	20/106 (19)	.87
Candidiasis	51/190 (27)	53/196 (27)	.97
Gonorrhea	12/193 (6)	9/200 (5)	.45
Chlamydia	17/193 (9)	16/192 (8)	.87
Bacterial vaginosis	87/184 (47)	92/198 (47)	.87
Cervical HIV-1 DNA	66/182 (36)	67/190 (35)	.84
Vaginal HIV-1 DNA	40/186 (22)	29/187 (16)	.14

\*Data are presented as median (range) unless otherwise indicated; STD indicates sexually transmitted disease; HIV-1, human immunodeficiency virus type 1; and RPR, rapid plasma reagin.

†At about 32 weeks' gestation.

‡Anemia is defined as hemoglobin of less than 11 g/dL.

§182 were in each group.

||To convert from micromoles per liter to milligrams per deciliter divide by 0.0349.

Thus, 63% of overall risk difference had occurred by 6 weeks, 75% by 6 months, and 87% by 12 months of age. These data suggest that most breast milk HIV-1 transmission occurs early during breastfeeding. However, the transmission risk difference between the 2 arms continued to increase throughout the 24 months of the study, consistent with ongoing breast milk HIV-1 transmission throughout the duration of exposure. It should be noted that there was a non-statistically significant 3.9% risk differ-

ence between the 2 arms at birth. If this difference was subtracted from the 24-month risk difference, excess transmission in the breastfeeding arm at 2 years was still statistically significant ( $P = .01$ ).

**Mortality**

Eighty-four children died during the study, 45 in the breastfeeding arm and 39 in the formula arm. The 24-month mortality rate was 24.4% (95% CI, 18.2%-30.7%) in the breastfeeding arm and 20.0% (95% CI, 14.4%-25.6%) in

the formula feeding arm ( $P = .30$ ) (TABLE 4). There was no significant difference in mortality curves overall ( $P = .41$ ) nor at 5 specific time points (Table 4), although there was a trend for formula-fed infants regarding increased mortality during the first 6 weeks of life (1.0% breastfed vs 3.9% formula-fed,  $P = .06$ ). An ongoing analysis of morbidity and mortality will be published separately.

**HIV-1-Free Survival**

Eighty infants in the breastfeeding arm and 58 in the formula arm were either dead or HIV-1 infected by 24 months of age. The percentage of those who were dead or infected at 24 months was significantly higher in the breastfeeding arm than in the formula arm (42.0% vs 30.0%,  $P = .02$ ). The risk difference at 24 months was 12.0% (95% CI, 2.4%-21.6%). Expressed conversely, HIV-1-free survival rates in the 2 arms were 58.0% and 70.0%, respectively.

**COMMENT**

In this trial, the estimated rate of breast milk HIV-1 transmission was 16.2% during the first 2 years of life. Given an HIV-1 infection rate of 36.7% in the breastfeeding arm, breast milk transmission accounted for 44% of all infant infections among those exposed to breast milk. Because more than one quarter of women in the formula arm admitted to noncompliance with feeding modality, our estimated breast milk transmission rate is an underestimate. If we assumed

**Table 2.** Comparison of Labor, Delivery, and Neonatal Characteristics of Breastfeeding and Formula Feeding Arms\*

	Breastfeeders	Formula Feeders	P Value
<b>Labor Characteristics</b>			
Labor, median (range), h	9 (0-36)	10 (0-48)	.44
>4 Hours from rupture of membranes to delivery	59/189 (31)	51/187 (27)	.40
Laceration	27/193 (14)	32/191 (17)	.45
Episiotomy	43/193 (22)	40/191 (21)	.75
Vacuum extraction	2/193 (1)	3/192 (2)	.65
Forceps delivery	0/193 (0)	2/192 (1)	.16
Induced labor	13/193 (7)	13/193 (7)	.99
Cesarean delivery	17/193 (9)	15/193 (8)	.71
<b>Outcome of delivery</b>			
<b>Singletons</b>			
Live birth	192/201 (96)	197/207 (95)	.87
Stillbirth	4/201 (2)	3/207 (1)	.72
<b>Twins</b>			
Both live births	3/201 (1)	6/207 (3)	.50
1 Live birth and 1 stillbirth	2/201 (1)	1/207 (1)	.62
<b>Neonatal characteristics</b>			
Birth weight, median (range), kg	3.2 (1.1-4.4)	3.2 (1.0-4.2)	.35
Low birth weight, <2500 g	15/183 (8)	12/178 (7)	.60
Premature by dates, <37 weeks	37/194 (19)	36/202 (18)	.75
Premature by Dubowitz, <37 weeks	8/118 (7)	9/139 (7)	.92

\*Data are presented as No. (%) unless otherwise indicated.

**Table 3.** Cumulative Human Immunodeficiency Virus Type 1 (HIV-1) Infection for Infants in the Breastfeeding and Formula Feeding Arms\*

Infant Age	No. of Infants With HIV-1 Infection†		No. of Infants Whose HIV-1 Status Is Known‡		Cumulative HIV-1 Infection Rate (95% CI)§		Difference in Cumulative HIV-1 Infection Rates (95% CI)	P Value
	Breastfeeders	Formula Feeders	Breastfeeders	Formula Feeders	Breastfeeders	Formula Feeders		
Birth	15	7	191	193	7.0 (2.3 to 11.7)	3.1 (-2.4 to 8.6)	3.9 (-3.3 to 11.1)	.35
6 Weeks	43	20	186	180	19.9 (14.2 to 25.6)	9.7 (5.4 to 14.0)	10.2 (3.1 to 17.3)	.005
14 Weeks	47	28	182	173	24.5 (18.4 to 30.6)	13.2 (7.9 to 18.5)	11.3 (3.2 to 19.4)	.007
6 Months	53	32	175	169	28.0 (21.7 to 34.3)	15.9 (9.6 to 22.2)	12.1 (3.2 to 21.0)	.009
12 Months	63	36	165	161	32.3 (25.6 to 39.0)	18.2 (11.9 to 24.5)	14.1 (4.9 to 23.3)	.003
24 Months	71	41	142	128	36.7 (29.4 to 44.0)	20.5 (14.0 to 27.0)	16.2 (6.5 to 25.9)	.001

\*CI indicates confidence interval.

†Data represent the number of estimated HIV-1 infections that would have occurred by this age based on the model. The observed numbers of infections at each of the 6 ages were 9, 28, 37, 42, 50, and 61 in breastfed infants and 2, 11, 16, 22, 24, and 31 in formula-fed infants.

‡Data represent the number of infants known to be uninfected at or after each age and the total number of infants known to be infected.

§Estimated using statistical methods given in the "Data Analysis" section.

that all transmissions in the formula arm that occurred after 14 weeks were due to noncompliance, our estimated breast milk transmission rate would be 23.5% (36.7% – 13.2%, Table 3).

The second important finding of our trial concerns the timing of breast milk HIV-1 transmission. Because cumulative HIV-1 infection rates in the 2 arms were significantly different as early as 6 weeks of life, our data suggest that substantial transmission occurs early during breastfeeding. By 6 months, an estimated 75% of all breast milk transmission had occurred, despite ongoing exposure for an average of 1 additional year. Thus, transmission risk is nonlinear during breast milk exposure duration. This could be due to variation in breast milk infectivity over time. In a prior study of breast milk samples from birth to older than 9 months, we found the highest prevalence of HIV-1 DNA in breast milk cells in samples collected between 1 week and 3 months.<sup>13</sup> Also, exposure diminishes as weaning foods are introduced and milk intake declines. Infant susceptibility may vary over time and may be affected by the developing immune system or gastrointestinal tract maturity. Finally, within a cohort, as infections occur, the remaining pool of children may be relatively resistant to infection and/or the pool of mothers less likely to transmit.

The third major finding was that infants assigned to the breastfeeding and formula arms had comparable mortality rates through 24 months. There was a nonstatistically significant increased mortality in the formula arm during the

first 6 weeks, when newborns may be at particular risk of serious infectious disease morbidity. The mortality rates we observed were much higher than those for the Nairobi population as a whole; the infant mortality rate was reported to be 4.1% in 1998.<sup>11</sup> However, mortality rates in our trial were comparable to those seen in other cohorts of children of HIV-1–infected women in Africa.<sup>21</sup>

The goal of any intervention to prevent mother-to-child HIV-1 transmission is to have an uninfected infant who survives. The HIV-1–free survival at age 2 years best captures the cumulative hazards of breast milk and formula since most breastfed infants are completely weaned by this age (no longer exposed to HIV-1) and most formula-fed infants should no longer be at risk of diarrheal disease mortality. We found that HIV-1–free survival rates at 2 years were significantly lower in the breastfeeding arm than in the formula arm. Only 58% of women in the breastfeeding arm had an infant at 2 years who was alive and HIV-1–infection free.

The major strength of this study was its randomized clinical trial design. This approach avoided the limitations of observational cohort data as outlined above. Because of randomization, women in both arms were comparable in all measured baseline variables. Our results should thus give an unbiased estimate of breast milk-transmission risk, since any differences in infant infection rates in the 2 arms should be attributable to breastfeeding. Also, the clinical trial design sidesteps difficulties in determining route of infection for

an individual child by comparing overall transmission rates in the 2 arms.

Although the randomized clinical trial design was the major strength of our study, the unique nature of the intervention contributed to its main weakness, compliance. Breastfeeding is the norm in Kenya, and women assigned to the formula arm often experienced community, family, or spousal pressure to breastfeed and were sometimes concerned about maintaining confidentiality of their HIV-1 status. Also, formula-feeding logistics are more difficult than breastfeeding, particularly in resource-poor areas. Despite knowledge about the breast milk HIV-1 transmission risk, more than one quarter of women randomized to use formula admitted to noncompliance. The true rate may have been higher. Thus, our results are biased toward the null hypothesis, and the breast milk transmission rate is an underestimate.

We conducted a randomized clinical trial, and we used the standard intent-to-treat approach for all analyses, as is recommended for this study design.<sup>22</sup> We included data on compliance rates, time of introduction of weaning foods, and breastfeeding duration to aid in interpretation of results or in considering generalizability to other populations. We did not present secondary analyses of self-reported feeding behavior and HIV-1 transmission or mortality, because feeding practices may be associated with confounding variables that affect primary study outcomes. For example, compliance with formula feeding was associated with significantly higher ma-

**Table 4.** Cumulative Mortality Rates for Infants in the Breastfeeding and Formula Feeding Arms\*

Infant Age	No. of Cumulative Infant Deaths		No. of Infants at Risk†		Cumulative Mortality Rate, (95% CI)‡		Difference in Cumulative Mortality Rates (95% CI)	P Value for Difference
	Breastfeeders	Formula Feeders	Breastfeeders	Formula Feeders	Breastfeeders	Formula Feeders		
6 Weeks	2	8	194	196	1.0 (0.0 to 2.4)	3.9 (1.3 to 6.6)	-2.9 (-5.9 to 0.1)	.06
14 Weeks	8	13	186	190	4.1 (1.3 to 6.9)	6.4 (3.0 to 9.7)	-2.3 (-6.6 to 2.1)	.30
6 Months	17	22	175	180	8.8 (4.8 to 12.7)	10.8 (6.6 to 15.1)	-2.1 (-7.9 to 3.7)	.48
12 Months	32	31	151	162	16.7 (11.4 to 22.0)	15.4 (10.4 to 20.4)	1.4 (-5.9 to 8.6)	.71
24 Months	45	39	114	121	24.4 (18.2 to 30.7)	20.0 (14.4 to 25.6)	4.4 (-4.0 to 12.9)	.30

\*CI indicates confidence interval.

†Number of infants known to be alive at this age.

‡Estimated using Kaplan-Meier analysis.

ternal plasma viral load, ie, the decision to breastfeed despite randomization to formula was related to maternal disease status.

Similarly, our trial was not designed to assess the impact of weaning practices or duration of breastfeeding on HIV-1 transmission risk, and a secondary analysis may well be misleading. The most common reasons for early introduction of weaning foods in our trial were maternal illness, insufficient milk production (often due to maternal illness), or poor infant feeding, all of which may be linked to infant HIV-1 infection. If an association between weaning practices and HIV-1 transmission were observed (as reported from an observational study),<sup>23</sup> it would be difficult to adjust for all confounders and to establish direction of causality.<sup>23</sup>

The results of this trial have significant public health implications. Breastmilk avoidance could potentially decrease overall mother-to-child transmission by 44%, a similar magnitude of reduction as that found at 3 to 6 months with the short-course zidovudine regimens assessed for perinatal transmission prevention in Côte d'Ivoire and Burkina Faso.<sup>24,25</sup> Risks of HIV-1 transmission via breast milk may be fairly comparable across populations, given similar exposure, but risks associated with formula are community specific. In our trial, mortality rates in the formula and breastfeeding arms were similar, but participants had access to clean water and extensive instruction in safe use of formula. In developing country communities in which clean water and formula-feeding knowledge are limited, the balance of risks and benefits could be shifted. Finally, our results suggest that children continue to acquire HIV-1 infection throughout their exposure to breast milk but most transmission occurs during the first few months. Early cessation of breastfeeding would prevent some infections but complete avoidance would be necessary to markedly reduce transmission.

Our trial demonstrated that formula feeding results in a substantial de-

crease in HIV-1 transmission risk, but formula is unaffordable for most HIV-1-infected women in sub-Saharan Africa. In addition, this intervention requires antenatal HIV-1 testing and a health care infrastructure to provide education on formula feeding. Given the high HIV-1 seroprevalence in pregnant women in sub-Saharan Africa, the current priority is to find ways to make interventions to prevent infant HIV-1 infection widely available.

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