

# Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa

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**Objective:** To determine the risk of HIV transmission by infant feeding modality.

**Design and setting:** A prospective study in two hospitals in Durban, South Africa.

**Participants:** A total of 551 HIV-infected pregnant women enrolled in a randomized trial of vitamin A.

**Interventions:** Women self-selected to breastfeed or formula feed after being counselled. Breastfeeders were encouraged to practice exclusive breastfeeding for 3–6 months.

**Main outcome measures:** Cumulative probabilities of detecting HIV over time were estimated using Kaplan–Meier methods and were compared in three groups: 157 formula-fed (never breastfed); 118 exclusively breastfed for 3 months or more; and 276 mixed breastfed.

**Results:** The three feeding groups did not differ in any risk factors for transmission, and the probability of detecting HIV at birth was similar. Cumulative probabilities of HIV detection remained similar among never and exclusive breastfeeders up to 6 months: 0.194 (95% CI 0.136–0.260) and 0.194 (95% CI 0.125–0.274), respectively, whereas the probabilities among mixed breastfeeders soon surpassed both groups reaching 0.261 (95% CI 0.205–0.319) by 6 months. By 15 months, the cumulative probability of HIV infection remained lower among those who exclusively breastfed for 3 months or more than among other breastfeeders (0.247 versus 0.359).

**Conclusion:** Infants exclusively breastfed for 3 months or more had no excess risk of HIV infection over 6 months than those never breastfed. These findings, if confirmed elsewhere, can influence public health policies on feeding choices available to HIV-infected mothers in developing countries.

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Contributors: A. Coutsooudis was the principal investigator, wrote the protocol, supervised the study, and wrote the manuscript. K. Pillay and E. Spooner assisted with the study design, were responsible for the clinical management of the mothers and children and edited the final manuscript. L. Kuhn assisted by W.-Y. Tsai were responsible for all the statistical analysis and contributed to the writing of the manuscript. H.M. Coovadia assisted with the study design and writing of the manuscript.

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## Introduction

Antiretroviral agents have had such a positive impact on mother-to-child-transmission (MTCT) in the industrialized world that the likelihood of eliminating this problem has been seriously entertained [1]. Short [2-4] and very short [5] courses of antiretroviral agents, which are effective, simple, safe and affordable, are available for developing countries, and are being implemented in a number of these states. The major effect of these drugs is on intrapartum transmission. The transmission of HIV-1 through breastfeeding, which accounts for approximately 44% of the total MTCT rate over 24 months [6], remains a pivotal issue among poor communities in Africa and south Asia. The avoidance of breastfeeding is not a realistic option for the vast majority of women in these regions; recent trials in Africa [3-7] have shown that even after counselling on feeding choices, a large proportion of HIV-infected pregnant women breastfed their babies. It is unclear whether this choice is made through freedom or necessity. Moreover, the DITRAME study of breastfeeding women in Cote d'Ivoire and Burkina Faso [8] has shown that the 35% efficacy in reducing MTCT at 6 months is 30% at 15 months; similarly, the Cote d'Ivoire data [9] show a fall off from 37% efficacy at 3 months to 23% at 24 months. Therefore, the initial benefits of the prevention of vertical HIV infection are partly reversed through breastfeeding for many women (although differences between treated and untreated groups remain). Furthermore, the risks of not breastfeeding are great among disadvantaged populations. The benefits of breastfeeding are widely recognized and have been reinforced by a recent meta-analysis [10]. These arguments suggest that in order to reduce MTCT of HIV-1 in developing countries, the provision of affordable and effective antiretroviral regimens should be combined with efforts to reduce the risk of transmission of the virus through breastfeeding.

In an early report, we observed a significantly lower risk of HIV infection by 3 months of age among children exclusively breastfed to this age than among other breastfed children. The risk of HIV infection by 3 months among exclusively breastfed children was no different from that among never breastfed children [11]. Here we present the completed results from this study. We describe HIV transmission by feeding patterns through to 15 months, and examine in more detail the dynamics of transmission over time.

## Methods

Details of the methodology have been described previously [11]. Briefly, the mother-infant pairs in the study were participating in a vitamin A intervention

trial to reduce MTCT of HIV-1. The study was conducted at antenatal clinics at two hospitals in Durban, South Africa: King Edward V111th Hospital and McCords Hospital. Women were recruited between July 1995 and April 1998, and were randomly assigned to receive vitamin A or placebo. No women on the study received any antiretroviral therapy, which was not available at the time the study was conducted.

During antenatal visits all women were counselled about the risks of transmission of virus through breast-milk and of the other health benefits of breastfeeding. Women were asked to make an informed choice on whether to breastfeed or formula feed in accordance with the recent recommendations from UNAIDS, WHO and UNICEF. Women who chose to breastfeed were counselled to consider exclusive breastfeeding because of all its benefits for infant health and development and to avoid gut wall damage possibly relevant to HIV transmission. All staff were careful not to influence women's choices, and once a choice on feeding was made to support the woman in her choice. However, women who chose to formula feed were not supplied with free formula; instead they were given the option to purchase it from the hospital at a subsidized rate.

Mothers were asked to attend a follow-up clinic when their infants were 1 week, 6 weeks and 3 months of age and thereafter every 3 months. At each pediatric follow-up visit mothers were asked about feeding of the infant and breastfeeding practices. Specific questions were asked about feeding of any fluid or solid, and the responses were recorded on a datasheet that allowed for a record of the date at which each item was introduced to the infant.

Infant venous blood was drawn on the first day after birth and again at 1 week, 6 weeks and 3 months of age and 3 monthly intervals thereafter until 15 months of age. If children were breastfed beyond 15 months, an additional sample was drawn at least 3 months after the complete cessation of breastfeeding. Plasma samples collected from children before 9 months of age were tested using a quantitative assay of HIV RNA using polymerase chain reaction (Roche Molecular Systems, Branchburg, NJ, USA). For a few samples collected on the day of birth, an insufficient volume of plasma was obtained and blood spot samples were tested. Samples collected from children after 9 months of age were tested for HIV antibodies (Abbott Laboratories, Chicago, IL, USA). Samples collected for HIV-RNA testing were stored and were sent for testing towards the end of the study. Samples from children confirmed to be uninfected (two negative enzyme-linked immunosorbent assay results at 9 months or older among those either not breastfed or who had stopped breastfeeding more than 3 months before their last sample) were not

sent for HIV-RNA testing. Among those sent for HIV-RNA testing, the last available sample was tested first, and if this sample was negative, an earlier sample was tested. If the last available sample was positive, the first available sample was tested as well as each sequential sample until two positive results were obtained, or until all available samples had been tested.

The study was approved by the Ethics Committee of the University of Natal. Written informed consent was obtained from all women who participated.

### Statistical methods

The data have been updated since the earlier analysis [11]. We restricted all analyses to singleton infants with at least one HIV test result. As the duration of follow-up varied for all mother-child pairs (because requirements for determining HIV status vary by infant feeding practices, and because of infant deaths and loss to follow-up) all analyses were based on Kaplan-Meier life tables or Cox proportional hazards models.

In the first analysis, we described the cumulative probability of detecting HIV infection over time among 'ever' and 'never' breastfed mother-child pairs based on the failure-time distribution from Kaplan-Meier life tables. The child's age at first positive HIV test was defined as the event. Children who tested only negative were considered uninfected up to the age of their last negative test, at which time they were censored. No child obtained a negative HIV test result after an earlier positive one. The censoring times for children who never tested positive and who were breastfed were truncated if their last test was performed more than 6 weeks after all breastfeeding had ended. For these children, they were considered at risk of HIV transmission up to 6 weeks after the cessation of all breastfeeding and were censored at this time. Failure to truncate artificially lowers the estimate of transmission attributable to breastfeeding and is noticeable in this study population, which included a relatively large proportion of women who breastfed for short periods only. The cumulative probabilities [and 95% confidence intervals (CI) using the log minus log transformation] of detecting HIV infection at birth, and by 6 weeks (defined as up to 48 days), 3 months (100 days), 6 months (190 days), 12 months (365 days) and 15 months (450 days) were calculated and tested using the Z statistic. These ages were selected to correspond with the protocol-scheduled HIV tests. We chose to analyse the age of first positive HIV test rather than the mid-point between the last negative and the first positive test because it is the simplest and most readily interpretable end-point because testing was on a regular scheduled protocol. Mid-point estimates are generally poor approximations of the underlying 'true' infection time.

We repeated the Kaplan-Meier analysis after dividing the 'ever' breastfeeders into 'exclusive' and 'mixed' breastfeeders. 'Exclusive' breastfeeders were required to maintain exclusive breastfeeding for 3 months or longer, or up to the time of loss to follow-up or child death. 'Mixed' breastfeeders were all other breastfeeders who had either never exclusively breastfed, or who had exclusively breastfed for periods of less than 3 months. Other maternal and child characteristics between the three feeding groups were tested using the chi-square test for categorical variables and the Mann-Whitney U test for continuous variables.

We performed a second, complementary analysis using a Cox proportional hazards model with a time-dependent variable for feeding modality. Children with positive HIV-RNA tests on the day of birth were excluded. Age at first positive HIV test was defined as the event, and children who tested only negative were censored at their last negative test. A time-dependent variable  $Z(t)$  was defined as 0 if the child was exclusively breastfed at time  $t$  and 1 if the child was mixed breastfed at time  $t$ . An additional indicator variable coded as 1 if ever breastfed and 0 if never breastfed was also included. The coefficient for the time-dependent variable  $Z(t)$  estimates the hazard ratio (HR) of HIV detection among mixed versus exclusive breastfeeders over all ages up to the maximum duration of exclusive breastfeeding (6 months). The coefficient for the indicator variable estimates the HR of HIV detection among exclusive versus never breastfeeders. A multivariate model was run including the time-dependent and indicator feeding practice variables, and variables for each factor possibly associated with HIV transmission or with feeding practice to investigate confounding.

### Results

Of 728 HIV-seropositive women recruited into the study, 57 withdrew before delivery and 10 experienced fetal deaths. Of 661 who had a live-birth, 30 had multiple births and were excluded. Of 631 singletons, 75 (12%) were lost to follow-up before their feeding practices could be established, three had no HIV tests available, and for two the duration of exclusive breastfeeding was unknown. The analysis thus included 551 mother-child pairs. Four out of 80 (5%) excluded were known to have died, compared with 38 out of 551 (6.9%) included in the analysis. There were no differences in maternal, socioeconomic, or neonatal characteristics between the included and excluded, except for a significant increase in cesarean deliveries (45% among the excluded versus 27% among the included).

### Feeding practices over time

At least some breastfeeding was initiated among 394 (71.5%) mother-child pairs. The other 157 (28.5%) pairs were formula-fed from birth. The median duration of all breastfeeding was 6 months (95% CI 4.75-7.5). The Kaplan-Meier probability of still breastfeeding at one month was 0.78, at 3 months 0.59, at 6 months 0.49, at 12 months 0.27 and at 15 months was 0.18. The median duration of exclusive breastfeeding was 3 weeks (95% CI 2 weeks to 1 month). The probability of still exclusively breastfeeding at one month was 0.49, at 3 months was 0.29, and at 6 months was 0.04, after which time all exclusive breastfeeding ceased. As soon as solids or liquids were introduced to a breastfeeding child, exclusive breastfeeding was considered to have permanently ended. The child could not transition back to become an exclusive breastfeeder once other solids or liquids had been introduced.

Among the 394 mother-child pairs who ever breastfed, 103 were exclusively breastfed for 3 months or longer and 15 were exclusively breastfed when they were lost, together constituting the 'exclusive' breastfeeding group. A total of 121 infants were never breastfed exclusively, and 155 were breastfed exclusively for periods of less than 3 months (median duration of exclusive breastfeeding 3 weeks), together constituting the 'mixed' breastfeeding group. All but 10 out of 103 mothers exclusively breastfeeding for 3 months were known to have continued breastfeeding in conjunction with the provision of other foods after exclusive breastfeeding ended (median duration of mixed breastfeeding after the end of exclusive breast feeding was 9.75 months, 95% CI 7.5-11 months), four mothers reported abruptly weaning their children, and six were still exclusively breastfeeding when last seen. Eighty-eight out of 155 in the mixed group who exclusively breastfed for periods of less than 3 months continued

with breastfeeding after exclusive breastfeeding had ended.

The total duration of all breastfeeding was longer among those who maintained exclusive breastfeeding for 3 months (median 13 months 95% CI 11-15 months) than among those still breastfeeding at 3 months but not exclusively (median 9.25 months, 95% CI 7.5-10 months). For children in the mixed breastfeeding group still breastfeeding at 3 months, 66% had introduced milk formula to the child's diet (27% formula only, 23% with other solids, 9% with other liquids, 7% with other liquids and solids), 25% had introduced no formula only other liquids or solids (14% other liquids alone, 11% liquids and solids), and 9% had introduced only solids. Cereals were the most commonly introduced solids (82%), the remainder introduced fruit-vegetable preparations.

### HIV detection over time

Among 157 children who were never breastfed, the probability of HIV detection was 0.076 (95% CI 0.042-0.125) at birth, 0.180 (95% CI 0.124-0.245) by 6 weeks, and 0.194 (95% CI 0.136-0.260) by 128 days, after which no new infections were observed. Among 394 children who were ever breastfed, the estimates of the cumulative probabilities of detecting HIV over time was similar to the never breastfed at birth, but surpassed the never breastfed group by 6 weeks, and continued to diverge over time intervals to reach 0.316 (95% CI 0.253-0.381) by 15 months (Table 1).

At birth, transmission rates in the never, exclusive and mixed breastfeeding groups were similar. At 6 weeks, never breastfeeders and exclusive breastfeeders still had highly similar cumulative probabilities of infection (0.180; 95% CI 0.124-0.245 and 0.150; 95% CI 0.091-0.222, respectively) but mixed breastfeeders

**Table 1.** Cumulative probability of HIV detection by age among 157 never breastfed children and 394 ever breastfed children.

	Birth	6 weeks	3 months	6 months	12 months	15 months
Never breastfed						
Cumulative probability of HIV detection by age (95% CI)	0.076	0.180	0.187	0.194		
Cumulative no. of HIV infections	12	28	29	30		
Number left	145	125	123	116		
Ever breastfed						
Cumulative probability of HIV detection by age (95% CI)	0.069	0.199	0.218	0.242	0.291*	0.316**
Cumulative no. of HIV infections	27	76	81	86	93	95
Number left 'at risk' i.e. still breastfeeding within 6 weeks	367	278	205	147	65	54

CI, Confidence interval.

\* $P = 0.02$  comparison of probability of HIV infection by 365 days among ever breastfed compared with never breastfed infants.

\*\* $P = 0.007$  comparison of probability of HIV infection by 450 days among ever breastfed compared with never breastfed infants.

$P$  values for all other comparisons in this table are  $> 0.05$ .

were starting to surpass both groups (0.219; 95% CI 0.172–0.271). Over the period up to 6 months, only very small increases in the cumulative probabilities of HIV detection occurred in the never breastfeeders and the exclusive breastfeeders (by 6 months, 0.194 in both groups). In contrast, the cumulative probability of HIV detection increased steadily over this time period among the mixed breastfeeders, reaching 0.261 (95% CI 0.205–0.319) by 6 months. After 6 months (i.e. after the age at which no further exclusive breastfeeding continued), new infections started to occur among the previously exclusive breastfeeders, and the cumulative probability in this group rose to 0.247 (95% CI 0.160–0.344) by 15 months of age. This rate remained lower than that observed by 15 months in the mixed breastfeeders (0.359; 95% CI 0.267–0.451) (Fig. 1, Table 2).

If the categories are revised so that the 67 who exclusively breastfed for periods of less than 3 months with no reported subsequent mixed breastfeeding are allocated to the 'exclusive' breastfeeding group, the results are essentially unchanged. The probability of HIV detection at birth was similar in the two groups, but in the revised mixed group surpassed that in the revised exclusive group at all ages, e.g. at 3 months 0.24 (95% CI 0.180–0.300) in the revised mixed and

0.19 (95% CI 0.131–0.249) in the revised exclusive category.

Deaths before 3 months of age occurred in seven out of 157 (4.5%) of the never and 15 out of 394 (3.8%) of the ever breastfed. Five out of seven children who died among the never and 10 out of 15 among the ever breastfed had at least one positive HIV-RNA test before their death. Seven out of 15 children who died among the ever breastfed were never exclusively breastfed, eight were exclusively breastfed for periods of less than 3 months, and none was exclusively breastfeeding up to their age of death.

### Comparability of the feeding groups

The three feeding groups did not differ significantly in any other risk factors previously identified to be associated with MTCT: preterm delivery, mode of delivery, duration of membrane rupture, maternal CD4 : CD8 cell ratio, CD4 cell count, or serum retinol level (Table 3). The groups also did not differ with regard to the maternal plasma HIV-RNA quantity in those with this information ( $n = 31$  in the never, 30 in the exclusive and 80 in the mixed feeding groups). Socioeconomic differences between never and exclusive breastfeeders were observed, but differences be-

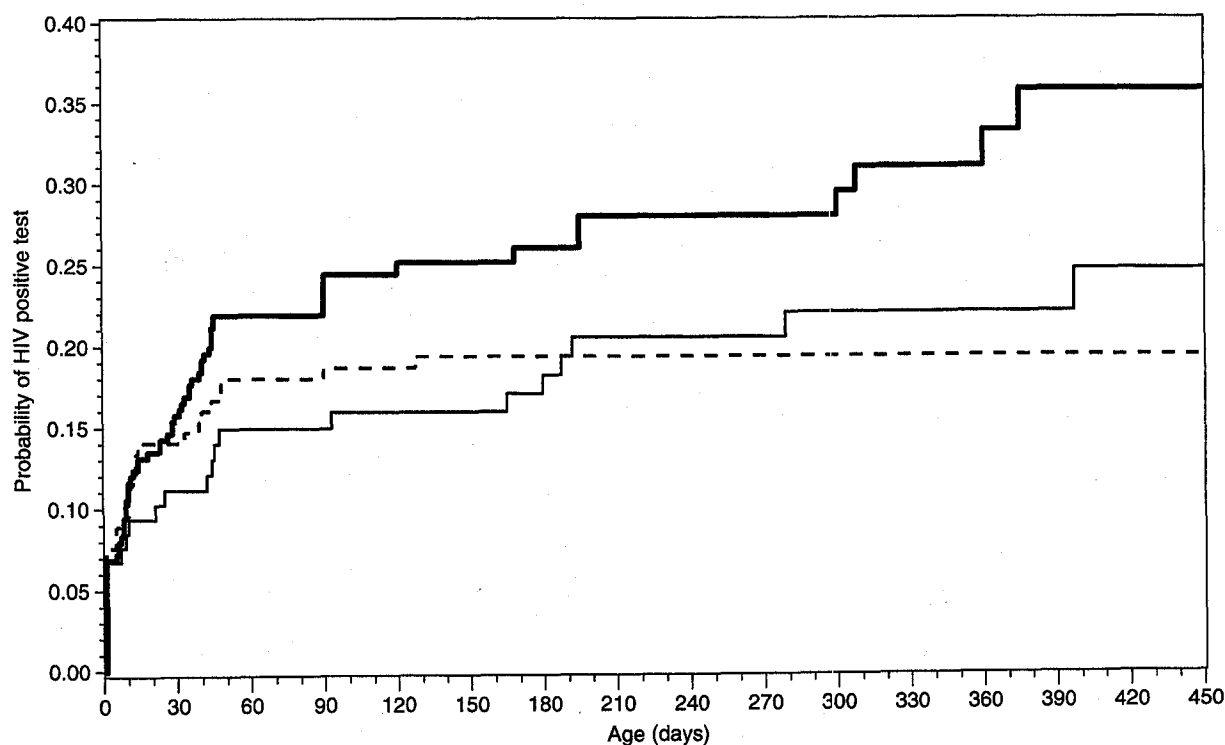


Fig. 1. Cumulative probability of detecting HIV infection over time among 157 children who were never breastfed (---), 118 exclusive breastfeeders (—), and 276 mixed breastfeeders (—).

**Table 2.** Cumulative probability of detecting HIV infection over time among 157 children who were never breastfed, 118 exclusive breastfeeders and 276 mixed breastfeeders.

	Birth	6 weeks	3 months	6 months	12 months	15 months
<b>Never breastfed</b>						
Cumulative probability of HIV detection by age	0.076	0.180	0.187	0.194		
(95% CI)	0.042-0.125	0.124-0.245	0.130-0.252	0.136-0.260		
Cumulative no. of HIV infections	12	28	29	30		
Number left	145	125	123	116		
<b>Exclusively breastfed</b>						
Cumulative probability of HIV detection by age	0.068	0.150	0.160	0.194	0.221	0.247
(95% CI)	0.032-0.123	0.091-0.222	0.099-0.233	0.125-0.274	0.145-0.307	0.160-0.344
Cumulative no. of HIV infections	8	17	18	21	23	24
Number left 'at risk' i.e. still breastfeeding within 6 weeks	110	88	85	69	35	29
<b>Mixed breastfed</b>						
Cumulative probability of HIV detection by age	0.069	0.219	0.244	0.261	0.333	0.359
(95% CI)	0.043-0.103	0.172-0.271	0.193-0.299	0.205-0.319	0.253-0.415	0.267-0.451
Cumulative no. of HIV infections	19	59	63	65	70	71
Number left 'at risk' i.e. still breastfeeding within 6 weeks	257	190	120	78	30	25

CI, Confidence interval.

**Table 3.** Comparability of never, exclusive, and mixed breastfeeders by known risk factors for mother-to-child transmission of HIV and other sociodemographic characteristics.

	Never breastfed	Exclusive breastfed	Mixed breastfed
Number (%) each characteristic <sup>a</sup>			
Assigned to vitamin A treatment	81/157 (51.6)	61/118 (51.7)	137/276 (49.6)
CD4 cell count < 200 × 10 <sup>6</sup> /l	15/142 (10.6)	10/102 (9.8)	22/253 (8.7)
CD4 : CD8 cell ratio < 0.5	53/139 (38.1)	57/102 (44.1)	108/253 (42.7)
Serum retinol < 20 µg/dl	31/140 (22.1)	32/104 (30.8)	80/247 (32.4)
HIV RNA > 100 000 copies/ml	11/31 (35.5)	7/37 (18.9)	25/73 (34.3)
Hemoglobin < 10 mg/dl	34/144 (23.6)	30/106 (28.3)	68/254 (26.8)
Positive syphilis test	9/127 (7.1) *	18/105 (17.1)	37/213 (17.4)
Completed high school	77/153 (50.3)*	30/117 (25.6)	97/273 (35.5)
Employed	68/155 (43.9)*	37/118 (31.4)	100/275 (36.4)
Electricity in home	121/154 (78.6)*	67/117 (57.3)	195/274 (71.2)*
Piped water inside home	77/155 (49.7)*	43/118 (29.6)	126/271 (46.5)
Gestational age < 37 weeks	19/149 (12.8)	10/114 (8.8)	25/254 (9.8)
Cesarean delivery	43/154 (27.9)	35/118 (29.7)	67/274 (24.5)
Membrane rupture < 4 h	39/132 (29.6)	26/88 (29.6)	63/196 (32.1)
Male	79/153 (51.6)	56/115 (48.7)	130/269 (48.3)
Mean (standard deviation)			
CD4 cell count × 10 <sup>6</sup> /l	434 (196)	487 (228)	479 (223)
Serum retinol (µg/dl)	29.1 (11.4)	26.6 (9.8)	27.3 (12.1)
Log <sub>10</sub> HIV-RNA copies/ml	4.52 (0.81)	4.25 (0.8)	4.60 (1.02)
Age (years)	27.0 (4.5)*	25.8 (4.7)	25.9 (5.0)
Parity	2.4 (1.2)	2.2 (1.1)	2.3 (1.3)
Birth weight (g)	3141 (562)	3118 (484)	3123 (525)

<sup>a</sup>As a result of missing data for some characteristics denominators do not always equal totals.

Exclusive breastfeeders were compared with never breastfeeders and mixed breastfeeders. All significant differences ( $P < 0.05$ ) are marked with an asterisk.

tween exclusive and mixed breastfeeders were less consistent.

There were no differences in diagnosis with mastitis or breast abscesses during follow-up among five out of 118 (4.2%) exclusive breastfeeders compared with five

out of 276 (1.8%) mixed breastfeeders. Mastitis or breast abscesses were slightly, but not significantly, more common among mothers of breastfed children who acquired HIV infection: five out of 99 (5.1%) compared with those who did not, five out of 295 (1.7%)  $P = 0.06$ .

To investigate whether morbidity in the child before the detection of HIV infection may have resulted in mothers switching away from exclusive breastfeeding, we compared morbidity in children at risk of postnatal HIV infection (those not diagnosed with HIV infection before 6 weeks of age, i.e. uninfected children and those with infections detected after 6 weeks combined) by feeding practices. In the first 6 week interval, reported symptoms and clinical signs between children who were exclusively breastfed for 6 weeks were compared with children started on exclusive breastfeeding but switched to mixed feeding before 6 weeks. There were no differences in reported diarrhea: three out of 119 (2.5%) versus two out of 73 (2.7%); lower respiratory tract infections: six out of 119 (5.0%) versus one out of 73 (1.4%); or candida: 15 out of 119 (12.6%) versus 10 out of 73 (13.7%) in those maintaining or switching away from exclusive breastfeeding by 6 weeks. Similarly in the interval between 6 weeks and 3 months, there were no differences in reported diarrhea: two out of 43 (4.7%) versus one out of 47 (2.1%), lower respiratory tract infections: three out of 43 (7.0%) versus two out of 47 (4.3%); or candida: five out of 43 (11.6%) versus five out of 47 (10.6%) between those maintaining or switching away from exclusive breastfeeding by 3 months in those with morbidity data for this interval.

#### Exclusive breastfeeding and HIV: time-dependent Cox model

Exclusive breastfeeding, defined as a time-dependent variable in a Cox model, was associated with a significantly lower risk of HIV infection (HR 0.56, 95% CI 0.32–0.98,  $P=0.04$ ) than mixed breastfeeding, and had a similar risk to never breastfeeding (HR 1.19, 95% CI 0.63–2.22,  $P=0.59$ ). After adjusting for treatment assignment, CD4 cell count, CD4 : CD8 cell ratio, serum retinol, hemoglobin, mode of delivery, duration of membrane rupture, syphilis, preterm delivery, parity, maternal age, education, employment, water source and electricity, the magnitude of the time-dependent coefficient comparing exclusive to mixed breastfeeding (HR 0.56, 95% CI 0.22–1.42) and exclusive to never breastfeeding (HR 0.87, 95% CI 0.33–2.33) remained unchanged to that observed in the unadjusted analysis (above), but standard errors were larger with the inclusion of many additional covariates.

#### Discussion

We have confirmed, in this 15 month study, the main finding from the early report at 3 months on this cohort, that the pattern of breastfeeding influences the rate of postnatal transmission of HIV-1. In particular, the results demonstrate that infants on exclusive breastfeeding had no excess risk of MTCT of HIV-1 over 6 months when compared with infants who were not breastfed at all but given formula and other foods.

Those at greatest risk were infants fed by HIV-infected mothers on a mixture of breastmilk and other foods and liquids. The association remained after adjusting for many variables previously reported as influencing HIV transmission in the perinatal period.

We have included much new information on the duration and type of breastfeeding, and on the dynamics of breastfeeding transmission of HIV-1. Despite counselling that the optimal period of exclusive breastfeeding should be 6 months, the duration of exclusive breastfeeding was unfortunately very brief (median 3 weeks). Overall, breastfeeding continued for a median duration of 6 months, and by 12 months 27% of all infants were still on breast milk. This profile of breastfeeding is consistent with that generally observed in South Africa. Cereals, fruit and vegetables were the common solids introduced into the infant's diets, formula was given often and other liquids included water, weak tea and glucose-water solution.

Throughout the world, the dominant form of breastfeeding is mixed breastfeeding despite recommendations for women to practise exclusive breastfeeding for approximately 6 months [12]. There is considerable evidence that exclusive breastfeeding has many advantages over mixed. Exclusive compared with mixed breastfeeding has been shown to be associated with a reduced incidence of diarrhoea [13,14], respiratory illness [15,16], and allergy [17]. Even replacing colostrum with prelacteal feeds was observed to result in a threefold increase in neonatal mortality in a study from the Gambia [18]. Accordingly, there have been a number of attempts in different regions to promote exclusive breastfeeding as a desired norm. In Bangladesh [19], a community-based intervention showed that peer counselling increased the rate of exclusive breastfeeding at 5 months of age from 6 to 70% in the intervention group. Our study, which achieved less than 30% exclusive breastfeeding at 3 months, highlights the importance of interventions to improve rates of exclusive breastfeeding in the general population if we hope to improve practices in HIV-infected women.

In this study, in which women were not randomly assigned to feeding groups but self-selected formula (and other foods), mixed or exclusive breastfeeding, there is always the hazard of differences between the groups in characteristics that may influence MTCT. Antenatal factors that might determine intrauterine infection were probably similarly distributed between the groups as the transmission rates at birth were no different. The most significant variables influencing MTCT – maternal viral load and CD4 cell counts – were similar between the three groups. Some indices of social advantage (high school education, employment, electricity in the home, piped water in the home) were more frequent in those who were not breastfed com-

pared with infants on exclusive breastfeeding. It is difficult to envisage, except obliquely, a role for these characteristics in affecting MTCT. Serum retinol levels were also higher and a positive syphilis test lower in those not breastfeeding; these would favour lower transmission rates [20,21] in those never breastfed and higher transmission rates in those exclusively breastfed, and therefore the direction of bias is counter to the hypothesis we are attempting to establish.

The most serious limitation of this study was the measurement of actual feeding practice. The strongest evidence we have that women correctly reported their feeding practices was that among the formula-fed group there were only two new infections detected after 6 weeks and one of these was in a child only tested for the first time at 4 months (the other child was negative at 6 weeks and positive at 3 months). Infants in the exclusively breastfed group began acquiring infection after 6 months, once all exclusive breastfeeding had ceased. Measuring adherence will always be difficult. Frequent monitoring (at least weekly) may help to improve the validity of maternal recall.

The total duration of breastfeeding for mother-child pairs who exclusively breastfed for 3 months or more was longer (median 13 months) than for those who mixed breastfed to 3 months or more (median 9 months) and was considerably longer than for the whole cohort (median 6 months). This may be a cause for concern because we observed that once exclusive breastfeeding ended, if mixed breastfeeding continued, new mixed breastfeeding-associated infections began to occur among the previously exclusively breastfed children. Children were protected against breastfeeding transmission when breastfeeding was exclusive but not when it ceased to be exclusive even within the subgroup of 'exclusive' breastfeeders. This finding raises important questions about appropriate interventions for children older than 6 months once exclusive breastfeeding can no longer be offered.

We analysed morbidity among infants before they became HIV infected and found no evidence to support the idea that babies were switched from exclusive to mixed breastfeeding because they had been weakened by previous ill health, as suggested by a previous study [6].

We provide further data on the contribution of all breastfeeding to MTCT of HIV-1 in African women. From the difference in the cumulative probabilities of HIV infection between ever and never breastfed babies, we estimate the excess risk of MTCT as a result of breastfeeding to be 12% by 15 months or approximately 39% of all transmissions. This is in accordance with recent figures for the risks of MTCT caused by breastfeeding obtained in other studies [22]. Future

studies describing the risk of HIV transmission through breastfeeding must take care not only to describe the pattern of breastfeeding, but also the duration of breastfeeding, as the risk is not static but varies with the duration of breastfeeding. This information will allow mothers to make better informed choices throughout the duration of breastfeeding.

## Conclusion

Exclusive breastfeeding is not a static category nor is it a characteristic that can be determined at birth. We conducted a time-dependent analysis comparing exclusive and mixed breastfeeders at each age up to 6 months, allowing mother-child pairs to shift from exclusive to mixed categories whenever this transition occurred. The results of the time-dependent analysis were consistent with the analysis that used a fixed category. Because testing was on a regularly scheduled protocol, we elected to use Kaplan-Meier life tables using the child's age at the first positive HIV test as the event to take into account right-censoring in the data. If adherence to the study testing protocol is complete, this method can directly estimate the cumulative probability of HIV infection by the ages specified by the protocol. If full compliance with the study protocol is not achieved, this method will tend to underestimate the true underlying infection time because there will be a delay in detecting infection. As the focus of the analysis was on a comparison of transmission across feeding groups, missed tests are only relevant if differentially distributed across the groups.

We do not know the mechanisms through which exclusive breastfeeding may be safer than mixed breastfeeding. The latter may be associated with a decrease below a critical threshold of protective factors in breast milk as a result of the consumption of less breast milk, and replacement by formula and other liquids and solids. We favour the hypothesis that contaminated fluids and foods introduced in mixed breastfed babies damage the bowel and facilitate entry into the tissues of HIV in breast milk. A study in Guatemala of non-HIV-infected children [23] showed that gut damage was greatest with mixed breastfeeding and least with exclusive breastfeeding.

It is imperative that further studies be undertaken by other groups working at different sites to test the reproducibility of these findings that infants given exclusive breastfeeding over a period of 6 months have no excess risk of acquiring HIV infection than infants not given any breast milk. If these results are confirmed, then the public health benefits for HIV-infected women in developing countries is considerable.

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