Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival

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Objectives: The promotion of exclusive breastfeeding (EBF) to reduce the postnatal transmission (PNT) of HIV is based on limited data. In the context of a trial of postpartum vitamin A supplementation, we provided education and counseling about infant feeding and HIV, prospectively collected information on infant feeding practices, and measured associated infant infections and deaths.

Design and methods: A total of 14 110 mother–newborn pairs were enrolled, randomly assigned to vitamin A treatment group after delivery, and followed for 2 years. At baseline, 6 weeks and 3 months, mothers were asked whether they were still breastfeeding, and whether any of 22 liquids or foods had been given to the infant. Breastfed infants were classified as exclusive, predominant, or mixed breastfed.

Results: A total of 4495 mothers tested HIV positive at baseline; 2060 of their babies were alive, polymerase chain reaction negative at 6 weeks, and provided complete feeding information. All infants initiated breastfeeding. Overall PNT (defined by a positive HIV test after the 6-week negative test) was 12.1%, 68.2% of which occurred after 6 months. Compared with EBF, early mixed breastfeeding was associated with a 4.03 (95% CI 0.98, 16.61), 3.79 (95% CI 1.40–10.29), and 2.60 (95% CI 1.21–5.55) greater risk of PNT at 6, 12, and 18 months, respectively. Predominant breastfeeding was associated with a 2.63 (95% CI 0.59–11.67), 2.69 (95% CI 0.95–7.63) and 1.61 (95% CI 0.72–3.64) trend towards greater PNT risk at 6, 12, and 18 months, compared with EBF.

Conclusion: EBF may substantially reduce breastfeeding-associated HIV transmission.

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Introduction

Each year, 700 000 infants acquire HIV infection from their mothers [1]. A total of 280 000 of the infants become infected through breastfeeding, about 40% of total mother-to-child transmission.

Programmes to prevent mother-to-child transmission are expanding rapidly, particularly in southern Africa, where up to 40% of antenatal women are infected [2]. These programmes typically provide HIV counseling and testing, antiretroviral prophylaxis, and infant feeding counseling. However, infant feeding counseling is

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difficult to implement for reasons that include limited data on the risks associated with different feeding practices, limited follow-up after delivery, and stigma associated with replacement feeding and exclusive breastfeeding (EBF).

International guidance currently states that when replacement feeding is acceptable, feasible, affordable, sustainable and safe, the avoidance of all breastfeeding by HIV-infected mothers is recommended [3]. Otherwise, EBF is recommended during the first months of life. In Africa, replacement feeding that is acceptable, feasible, affordable, sustainable and safe is uncommon, and many HIV-positive women are choosing to breastfeed [4,5]. Finding ways to make breastfeeding safer for HIV-positive women living in resource-limited settings is an urgent priority [6,7].

Exclusive breastfeeding is recommended because it protects infants from morbidity and mortality whether or not HIV related [8–10]. In addition, Coutsoudis and colleagues [11] reported that HIV-exposed infants who were breastfed exclusively for at least 3 months had a lower risk of HIV infection than mixed-fed infants.

This report confirms the observations of Coutsoudis and colleagues [11], and provides additional data distinguishing the magnitude of risk of HIV transmission or death associated with different breastfeeding patterns.

Study participants and methods

The data were collected as part of the ZVITAMBO trial, designed to measure the impact of single-dose postpartum vitamin A supplementation on several maternal and neonatal health outcomes [12]. A secondary objective was to investigate the role of infant feeding practices in breastfeeding-associated HIV transmission. From 25 November 1997 to 29 January 2000, 14 110 postpartum mothers and their neonates were randomly assigned to one of four vitamin A treatment groups within 96 h of delivery [mean (SD) = 19 (16) h post-delivery] at one of 14 maternity clinics and hospitals in the greater Harare area in Zimbabwe.

Mother–baby pairs were eligible if neither had an acutely life-threatening condition, if the baby was a singleton with a birth weight of 1500 g or greater, and if the mother planned to stay in Harare after delivery. Written informed consent, including permission for HIV testing, was obtained from the mother. Mothers could receive their HIV test results at any time during the trial. Receiving HIV test results was encouraged but not required for joining the study because women may have understandable reasons not to know their status, and we believed that this should not exclude them from participating in a trial that might benefit them and their babies.

Socioeconomic and demographic data were collected by interview. Obstetric details were transcribed from hospital records. Gestational age was estimated using the Capurro method [13]. Infant birth weight and maternal mid-upper arm circumference (MUAC) were measured using methods described by Gibson [14]. Whole blood was collected from mothers and babies and processed within 2 h. Maternal plasma and infant plasma and cell pellets [Amplicor whole blood polymerase chain reaction (PCR) sample preparation method; Roche Diagnostics Systems, Alameda, CA, USA] were prepared and stored at −70 °C until analysis.

UNICEF conducted a pilot mother-to-child transmission prevention programme in two Harare antenatal clinics during a period that overlapped by 8 months with ZVITAMBO recruitment. During those months, 69 HIV-positive antenatal women received zidovudine, but none of them joined ZVITAMBO after delivery. Antenatal HIV testing and antiretroviral prophylaxis was not available in any other Harare public sector facility during our recruitment.

Education and counseling about infant feeding in the context of HIV

When the trial began, information about HIV transmission through breastfeeding was scant. In June 1998, new infant feeding guidelines were published by UNAIDS/UNICEF/WHO stating that HIV-positive mothers should be fully informed about the risks and benefits of infant feeding alternatives and empowered to make their best personal choice [15]. In response, we modified our procedures to provide a 24-h turnaround time for HIV test results. Study nurses were trained to counsel HIV-positive women about feeding options, and kitchens were established for teaching safe replacement feeding. Additional funding was obtained to conduct formative research to inform a more effective programme to educate mothers about infant feeding in the context of HIV. This programme [16] was fully implemented within the trial by 1 November 1999. The programme emphasized EBF for HIV-positive mothers who chose to breastfeed, optimal breastfeeding techniques to avoid cracked nipples, milk stasis, and mastitis, the prompt treatment of breast problems, and safe sex practices especially during the breastfeeding period. These four 'safer breastfeeding' practices were also promoted among all status-unknown and HIV-negative women. Known HIV-positive women were counseled to stop breastfeeding early.

Follow-up of subjects

Follow-up visits at 6 weeks, 3 months, and 3-monthly intervals for up to 24 months included maternal and infant blood collection following the same protocol used

at recruitment. Free clinical care included the treatment of acute infections with appropriate antimicrobial drugs, referral to government treatment facilities for suspect tuberculosis, counseling and antibiotic treatment for mastitis, and oral rehydration solution education for diarrhea. HIV-related and psychosocial counseling was available throughout the study.

Maternal HIV, CD4 cell count, and hemoglobin testing

Mothers were tested for HIV at baseline by two enzyme-linked immunosorbent assays (ELISA) run in parallel (HIV 1.0.2 ICE; Murex Diagnostics, Edenvale, South Africa, and GeneScreen HIV 1/2; Sanofi Diagnostics Pasteur, Johannesburg, South Africa). Discordant ELISA results were resolved by Western Blot assay (HIV Blot 2.2; Genelabs Diagnostics SA, Geneva, Switzerland), interpreted according to the manufacturer's guidelines. Quality control was monitored by the use of kit controls, the inclusion of an internal quality control sample on every plate, and participation in the quality control programme for HIV testing of the Zimbabwe Ministry of Health.

Maternal CD4 cells were enumerated using a Facscount (Becton Dickinson International, Erembodegem, Belgium) within 48 h of phlebotomy. Quality control was monitored by the inclusion of kit controls and two additional reference samples (high and low) provided by the Research Institute of the McGill University Health Center with every batch.

Hemoglobin was measured at baseline for women enrolled after 1 October 1998 (approximately 60% of the total sample) using a hemoglobinometer (HemoCue, Mission Viejo, CA, USA) on the day of collection.

Infant HIV testing

After all patient contact was completed, the last available specimen was tested [plasma by GeneScreen ELISA for samples collected at ≥ 18 months, or cell pellets by prototype Roche Amplicor version 1.5 qualitative PCR assay (Roche Diagnostic Systems) for samples collected at < 18 months]. If the last available sample was HIV negative, the baby was classified as uninfected and no further testing was carried out. If the last sample was positive, the 3-month pellet was tested. If this sample was positive, the baseline and 6-week samples were tested. If these samples were negative, sequential samples moving forward through time were tested until two consecutive samples tested positive.

Infant feeding practices

Breastfeeding initiation and pre-lacteal feeding information was collected at baseline. At 6 weeks, 3 months, and 6 months, detailed feeding information was collected, including whether or not any of 22 liquids (water, juice, tea, cooking oil), milks (formula, fresh, tinned), medicines (traditional, oral rehydration solution, prescribed), or solid foods (porridge, sadza, fruits, vegetables, meat, eggs) had ever been given to the infant.

The analysis reported here is based on data from mothers who were HIV positive at delivery, whose babies were alive and PCR-negative for HIV at 6 weeks, and who provided infant feeding data at birth, 6 weeks, and 3 months. Infants' breastfeeding patterns up to 3 months were classified using or adapting WHO definitions [17] as follows:

Exclusive breastfeeding (EBF) – the infant consumed only breast milk and no other liquids, milks or solid foods except vitamins or prescribed medicines, according to mothers' reports at all three timepoints, or at two of three timepoints. One lapse in the exclusivity of EBF at one of the three timepoints was allowed only if the non-breast milk item consumed was a non-milk liquid. Allowing one lapse in the definition of EBF is consistent with other studies (A. Coutsoudis, N. Rollins, personal communications).

Predominant breastfeeding (PBF) – the infant's predominant source of nourishment was breast milk, but non-milk liquids (e.g. water, tea, juice, cooking oil) were also consumed according to mothers' reports at all three timepoints, or at two of three timepoints. One lapse in PBF was allowed only if the mother reported EBF for the lapsed time period.

Mixed breastfeeding (MBF) – the infant consumed breast milk and either non-human milks, such as infant formula or cows' milk, or solid or semisolid foods or both, according to mothers' reports at one or more timepoints.

Statistical analysis

Statistical analysis was conducted using SAS Version 8.2 (Cary, NC, USA). Characteristics associated with early feeding practices were examined using chi-square and one-way analysis of variance for categorical and continuous variables, respectively. Turnbull methods [18] (using 2000 'bootstraps' to calculate confidence intervals [19]) were used to estimate postnatal transmission (PNT) in infants who were PCR negative at their 6-week visit (42 days). This reflects HIV infection that is unequivocally attributable to breastfeeding [20], and is hereafter referred to as postnatal HIV transmission (PNT). Infants who never had a positive HIV test were censored at the age of their last negative test result. Infants of mothers who died or stopped breastfeeding were censored 60 days after the mother's date of death or breastfeeding cessation, respectively [21]. PNT risks at 6, 12 and 18 months were calculated for each of the three feeding groups, and pairwise comparisons were made between the feeding groups at the same timepoints. Survival rates to 18 months were estimated using Kaplan–Meier methods.

Cox proportional hazards models were used to investigate the effect of the early breastfeeding pattern on PNT or PNT plus death, with and without adjusting for other explanatory variables, including maternal and neonatal vitamin A supplementation, household income, maternal baseline CD4 cell count, MUAC, hemoglobin, marital status, age, education, and death during the follow-up period, reported or diagnosed breast pathology (mastitis, cracked nipples), infant gestational age and birth weight, and a breastfeeding 'propensity score' that was developed using methods described by Joffe and Rosenbaum [22].

Briefly, the propensity scores were calculated using multinomial logistic regression with feeding pattern as the outcome and EBF as the reference category. Socio-economic, obstetric, and maternal and neonatal health variables were fit in the model. The propensity score was included in models to correct for baseline imbalances between feeding groups, because infants were not randomly assigned to specific breastfeeding practices.

The CD4 cell count and hemoglobin were analysed as categorical variables (< 200, 200–349, 350–499, ≥ 500, and 'missing') and (< 70, ≥ 70, and 'missing'), respectively. Cox models were also used to investigate the potential effect modification of early feeding practices by maternal baseline CD4 cell count. Independent factors were retained in the multivariate models at the $\alpha = 0.10$ level; interaction terms were retained at the 0.15 level.

Ethical approval

The Medical Research Council of Zimbabwe, the Medicines Control Authority of Zimbabwe, the Johns Hopkins Bloomberg School of Public Health Committee on Human Research, and the Montreal General Hospital Research Ethics Committee approved the study protocol.

Results

A total of 4495 mothers (31.9%) were HIV positive at enrolment (Fig. 1). One hundred and twenty-eight infants (2.8%) born to HIV-positive mothers provided no additional follow-up data. Among the remaining infants born to HIV-positive mothers, 918 tested PCR positive at baseline or 6 weeks, including 16 infants who died before their 6-week visit. An additional 64 infants were HIV negative at birth and died before their 6-week visit, and 515 infants were alive but missing PCR status at 6 weeks. Among the remaining 2870 infants, 810 were missing feeding data at one ($n = 578$), two ($n = 231$), or all three ($n = 1$) timepoints. The remaining 2060 infants were included in this analysis. Compared with the 810 excluded infants, those included in the analysis had significantly higher [mean (SD)] birth weights [2960 g (45) versus 2890 g (44), $P = 0.001$] and MUAC [25.8 cm (2.9) versus 25.4 cm (3.0)], but were similar in all other baseline characteristics.

Breastfeeding duration was prolonged: 99.1, 94.0, and 59.1% of mothers were still breastfeeding at 6, 12, and 18 months, respectively. However, only 156 babies (7.6%) were EBF for at least 3 months, compared with 490 (23.8%) and 1414 (68.6%) infants who were PBF and MBF, respectively, to at least 3 months (Table 1). A total of 93.2% of infants were MBF by 6 months. EBF mothers were slightly older and more likely to be unemployed. EBF infants also tended to have higher absolute birth weights ($P = 0.08$) but were no less likely to be low birth weight (< 2500 g) than other infants. As reported previously [16], the single strongest prognostic factor for EBF was enrolment in the trial after the full implementation of the education and counseling programme.

Between 6 weeks and 18 months, 199 infants became HIV infected (8, 35, and 156 in the EBF, PBF, and MBF groups, respectively) and an additional 48 infants died with their last PCR test being negative (2, 13, and 33 in the EBF, PBF, and MBF groups, respectively), totaling 247 postnatal HIV infection or death events. The total number of deaths (with or without infection) was 3, 16, and 52 in the EBF, PBF, and MBF groups, respectively. Kaplan–Meier estimates of mortality at 18 months (death with or without HIV infection) were 1.96% [95% confidence interval (CI) 0.64–5.95], 3.57% (95% CI 2.19–5.81) and 4.17% (95% CI 3.18–5.47) for the EBF, PBF, and MBF groups, respectively.

Postnatal transmission at 6, 12, and 18 months was 3.9% (95% CI 3.0–4.7), 7.7% (95% CI 6.6–9.3), and 12.1% (95% CI 10.5–14.0), respectively. A total of 68.2% of all PNT occurred after 6 months. Compared with EBF babies, MBF babies had a significantly greater PNT risk, and PBF babies tended to have a higher risk (not statistically significant) (Fig. 2 and Table 2). PNT rates were 5.1, 6.7, and 10.5 per 100 child-years of breastfeeding for EBF, PBF, and MBF, respectively. The overall PNT rate was 9.2 per 100 child-years of breastfeeding, comparable to a meta-analysis rate of 8.9 per 100 child-years of breastfeeding in nine other studies in Africa [23].

The final Cox proportional hazard ratios for the risk of PNT at 6, 12, and 18 months are shown in Table 3, and the results for HIV infection plus death are found in Table 4. The maternal CD4 cell count was an important predictor of PNT. In mothers with CD4 cell counts less than 200 cells/μl ($n = 216$), PNT was 33.7% (95% CI 22.9–44.1).

Maternal nutritional status was positively associated with infant outcomes. Each additional centimetre of maternal MUAC was associated with a 6–12% reduction in PNT

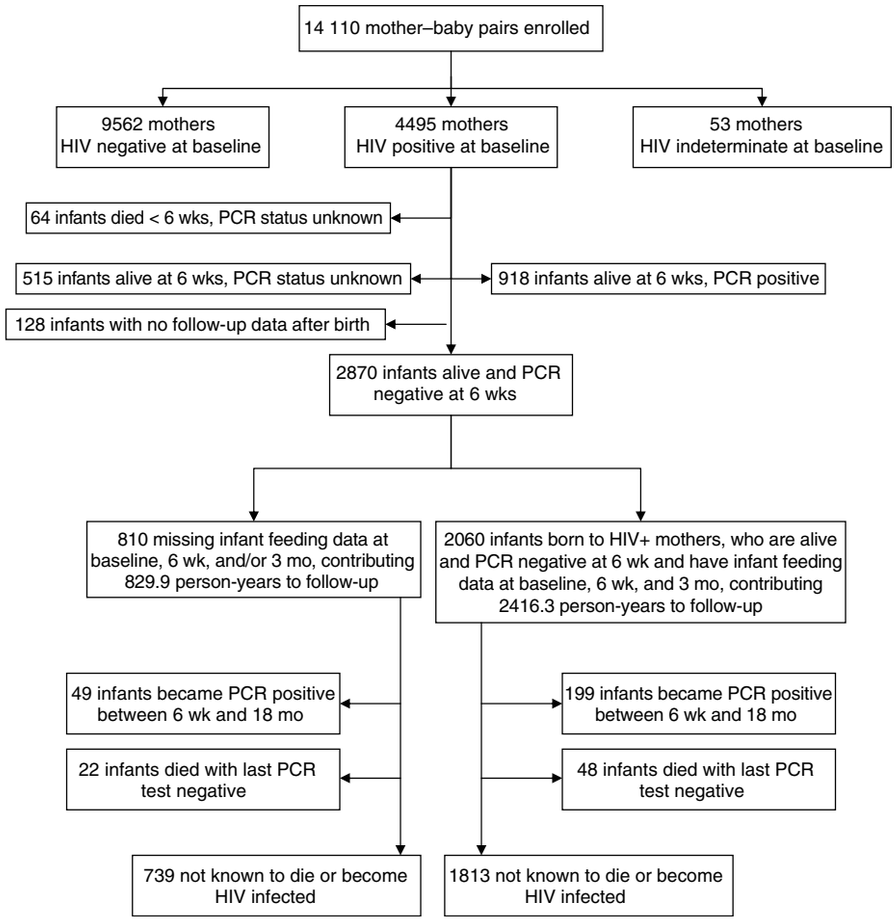

Fig. 1. Flow of participants through trial. PCR, Polymerase chain reaction.

(Table 3). Severe maternal anemia at baseline (hemoglobin < 70 g/l) was a significant positive predictor of PNT, with the greatest risk in the first 6 months (adjusted hazards ratio 6.93; 95% CI 2.39–20.10). Findings were similar in models when HIV infection plus death was the outcome (Table 4).

In the final models, MBF was associated with a fourfold increase in PNT and a threefold increase in the risk of PNT plus death at 6 months, compared with EBF. The protective effects of early EBF declined over time, but the risks posed by early MBF were still observed at 18 months ($P < 0.008$). Compared with EBF, early PBF was associated with a 2.6-fold and 1.6-fold increased risk of PNT, and a 2.4-fold and 1.7-fold increased risk of PNT plus death at 6 and 18 months, respectively, but these differences were not statistically significant. When the propensity score was included in the models, it was not significant ($P > 0.69$) and did not substantially change the hazard ratios associated with different feeding methods (data not shown). Interactions between early breastfeeding patterns and maternal CD4 cell count were not significant. However when the analysis was restricted to mothers with baseline CD4 cell counts less than 500 cells/μl, the adjusted hazard ratio for postnatal HIV transmission at 18 months for MBF compared with EBF was 3.19 (95% CI 1.30–7.82), suggesting that the protective effects of EBF may have been even greater among the women in whom PNT risks were highest. Vitamin A supplementation was not associated with the risk of PNT or PNT plus death at any time point (Table 3 and Table 4), as reported elsewhere [24].

Discussion

The findings of this study have three specific programmatic implications. First, the introduction before the age of 3 months of solid foods or animal milks to breastfeeding infants born to HIV-positive mothers was associated with a fourfold greater risk of PNT at 6 months compared with EBF. The protective effects of early EBF were still significant at 18 months post-delivery, with a 61% reduction in PNT compared with MBF. The risk of PBF over EBF varied from 1.6 to 2.7 over the 18-month period, reaching statistical significance at 12 months only. These findings indicate that the early introduction of

Table 1. Maternal and infant characteristics according to feeding mode during the first 3 months of life.

Variable	EBF ($n = 156$)	PBF ($n = 490$)	MBF ($n = 1414$)	P value overall	P value for EBF vs MBF
Birth weight (*n*)	155	489	1401		
Mean (g) (SD)	3040 (437)	3000 (422)	2966 (455)	0.08	0.05
< 2500 g	11.0 (17)	11.5 (56)	13.1 (183)	0.54	0.72
Gestational age (*n*)	154	483	1401		
< 37 weeks	5.8 (9)	7.7 (37)	8.5 (119)	0.48	0.48
Maternal CD4 cell count (*n*)	132	439	1238		
< 200 cells/μl	13.6 (18)	10.7 (47)	12.2 (151)	0.65	0.76
200–349	24.2 (32)	27.6 (121)	23.4 (290)		
350–499	28.8 (38)	27.1 (119)	27.3 (338)		
≥ 500	33.3 (44)	34.6 (152)	37.1 (459)		
Maternal arm circumference (*n*)	155	486	1404		
Mean (cm) (SD)	25.9 (2.7)	26.0 (3.0)	25.9 (2.8)	0.87	0.89
< 23.0 cm	9.7 (15)	12.6 (61)	11.5 (161)	0.60	0.80
Maternal hemoglobin (*n*)	92	258	761		
Mean (g/l) (SD)	114.0 (16.0)	113.7 (17.4)	112.3 (19.2)	0.25	0.41
< 110 g/l	34.8 (32)	36.4 (94)	39.4 (300)	0.53	0.26
< 70 g/l	1.1 (1)	1.6 (4)	2.8 (21)	0.38	0.40
Mode of delivery (*n*)	154	487	1410		
Vaginal	93.5 (144)	92.2 (449)	90.0 (1269)	0.17	0.10
Duration of membrane rupture (*n*)	149	472	1366		
> 4 h	29.5 (44)	34.3 (162)	38.0 (519)	0.07	0.04
Breast problems (*n*)	156	490	1414		
Reported or diagnosed at any time 6 weeks–18 months	9.0 (14)	14.3 (70)	13.8 (195)	0.21	0.09
Maternal formal education (*n*)	156	490	1411		
< 8 years	15.4 (24)	17.4 (85)	20.1 (284)	0.19	0.38
Maternal age (*n*)	156	489	1412		
Mean (years) (SD)	26.6 (5.1)	26.4 (5.1)	25.7 (4.9)	0.02	0.05
Household income (*n*)	106	343	1025		
< 1.20 US$/day	15.1 (16)	13.7 (47)	17.0 (174)	0.35	0.43
Maternal employment (*n*)	156	488	1410		
Unemployed	86.5 (135)	84.6 (413)	78.9 (1113)	0.004	0.006
Enrolled in trial after counseling and education intervention began (*n*)	156	490	1414		
Yes	34.6 (54)	10.2 (50)	5.9 (84)	< 0.0001	< 0.0001
Maternal vital status at 12 months (*n*)	156	480	1391		
Dead	0.6 (1)	2.0 (10)	1.6 (22)	0.47	0.64
Infant sex (*n*)	156	489	1413		
Male	57.7 (90)	50.3 (246)	52.8 (746)	0.26	0.24
Vitamin A treatment group	156	490	1414		
Mother VA/baby VA (Aa)	25.6 (40)	24.7 (121)	24.6 (348)	0.81	0.44
Mother VA/baby placebo (Ap)	26.9 (42)	25.5 (125)	24.3 (344)		
Mother placebo/baby VA (Pa)	26.9 (42)	24.9 (122)	24.5 (347)		
Mother placebo/baby placebo (Pp)	20.5 (32)	24.9 (122)	26.5 (375)		

EBF, Exclusive breastfeeding; MBF, mixed breastfeeding; PBF, predominant breastfeeding; VA, vitamin A.Values are [% (*n*)] except where noted otherwise.

non-human milks and solid foods conveys an especially high risk, but that even non-milk liquids are likely to increase the risk. Therefore, the more strictly HIV-positive mothers are able to breastfeed exclusively, the lower the risks of HIV or death will be for their infants.

Second, consistent with studies in west Africa [25], South Africa [26], and Tanzania [27], more than two-thirds of all PNT occurred after 6 months. Together, such studies provided strong justification for supporting early breastfeeding cessation among HIV-positive women. This can only be done, however, when women are socially supported to do so, and when safe nutritionally adequate alternatives are available.

Third, women with CD4 cell counts less than 200 cells/μl were five times more likely to transmit HIV during breastfeeding compared with women with CD4 cell counts over 500 cells/μl, confirming the findings of other studies that PNT is highly correlated with immune suppression [23, 28, 29]. Screening mothers for CD4 cell counts could help to identify infants at highest risk of PNT so that alternative feeding methods (or antiretroviral therapy for the mother) can be considered.

Three limitations of this study deserve mention. First, we did not collect data on maternal blood viral load during breastfeeding, which is an important determinant of the risk of PNT. Instead, we have used the maternal CD4 cell count, hemoglobin, MUAC, and death during the

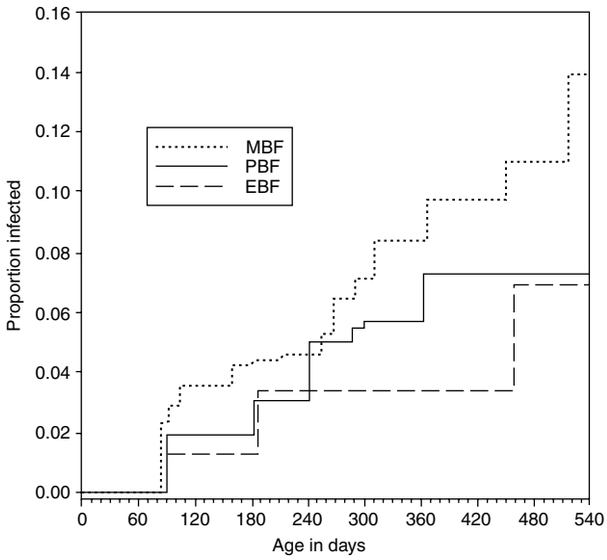

Fig. 2. HIV infection among infants born to HIV-infected mothers according to early breastfeeding practice using Turnbull methods [18]. Pairwise comparisons between feeding groups at 6, 12, and 18 months were calculated using the bootstrap method [19] using 2000 bootstraps:

	Estimated difference (95% CI)		
Pairwise comparison	6 months	12 months	18 months
EBF versus PBF	−1.7% (−4.1% to +0.7%)	−3.9% (−7.4% to +0.2%)	−1.6% (−7.6% to +5.2%)
EBF versus MBF	−3.1% (−5.0% to −0.7%)	−5.0% (−8.2% to −1.3%)	−7.0% (−12.5% to −0.4%)
PBF versus MBF	−1.4% (−3.3% to +0.7%)	−1.1% (−4.1% to +1.9%)	−5.4% (−9.4% to −1.4%)

CI, Confidence interval.

follow-up period as indicators of the severity of maternal disease. Second, we did not collect data on the frequency or quantity of non-breast milk foods and liquids consumed by infants, and therefore are unable to estimate whether there is a threshold in terms of non-exclusivity of breastfeeding that is associated with the increased risk of PNT. Third, feeding patterns were self-selected by mothers, raising the possibility of reverse causality (i.e. mothers who chose EBF might have been those already at a lower risk of transmission). The decision to breastfeed exclusively was indeed associated with some indicators of maternal and infant health (Table 1). However, adjusting for these variables, and including the propensity score in the final model, did not change the protective effect of EBF. Importantly, the strongest single predictor of EBF in this cohort and among the entire study population, including HIV-negative mothers [16], was enrolment in the study after implementation of the education and counseling programme, suggesting that choosing EBF was primarily the result of being better informed, not being healthier.

The practice of EBF was relatively uncommon for two probable reasons. It is the cultural norm to introduce liquids and solid foods very early in Zimbabwe [16]. Also, the methods used to define EBF in our study were very strict. Whereas most surveys use 24-h recall methods to define EBF, we used prospectively collected data that covered the entire first 3 months of life. Following a sensitivity analysis, we decided to allow one lapse in our strict definition of EBF, which could have biased our estimates towards the null. However, the PNT risk in EBF

Table 2. Cumulative percentage (95% confidence interval) with HIV infection between 6 and 18 months of age according to early breastfeeding pattern among infants born to HIV-infected mothers.

Breastfeeding pattern	*n*	6 months	12 months	18 months
Exclusive	156	1.31 (0.00–3.29)	3.42 (0.70–6.82)	6.94 (2.03–12.89)
Predominant	490	3.03 (1.56–4.75)	7.29 (4.95–9.76)	8.56 (5.47–11.63)
Mixed	1414	4.40 (3.30–5.52)	8.41 (6.83–10.23)	13.92 (11.63–16.26)

Turnbull point estimates [18] with 2000 'bootstraps' [19] for confidence intervals.

Table 3. Final Cox proportional hazards model for risk of HIV infection between 6 weeks and 6, 12, and 18 months.

Variable	HR HIV infection (95% CI) at 6 months	*P* value	HR HIV infection (95% CI) at 12 months	*P* value	HR HIV infection (95% CI) at 18 months	*P* value
No. events	76		157		197	
Early breastfeeding pattern[a]						
Exclusive	1.0		1.0		1.0	
Predominant	2.63 (0.59, 11.67)	0.20	2.69 (0.95, 7.63)	0.06	1.61 (0.72, 3.64)	0.25
Mixed	4.03 (0.98, 16.61)	0.05	3.79 (1.40, 10.29)	0.009	2.60 (1.21, 5.55)	0.02
Infant birth weight (kg)	*		*		0.75 (0.54–1.04)	0.09
Maternal age (years)	1.06 (1.01, 1.11)	0.02	1.04 (1.01, 1.08)	0.01	1.05 (1.02–1.08)	0.002
Maternal MUAC (cm)	0.88 (0.81–0.96)	0.005	0.93 (0.87–0.93)	0.009	0.94 (0.89–0.99)	0.03
Maternal CD4 cell count at delivery ($\times 10^6$/l)						
$\geq$500	1.0		1.0		1.0	
350 to < 500	*		1.73 (0.98–3.98)	0.06	1.51 (0.94–2.43)	0.09
200 to < 350	3.16 (1.63–6.11)	0.0006	3.06 (1.81–5.07)	< 0.0001	2.40 (1.53–3.75)	0.0001
< 200	9.12 (4.85–17.13)	< 0.0001	6.24 (3.67–10.63)	< 0.0001	5.28 (3.37–8.28)	< 0.0001
Maternal hemoglobin (g/l)						
< versus $\geq$ 70	6.93 (2.39–20.10)	0.0004	3.25 (1.30–8.13)	0.01	2.93 (1.18–7.28)	0.02
Maternal death	1.89 (0.91–3.94)	0.09	2.18 (1.25–3.80)	0.006	1.87 (1.10–3.18)	0.02
Maternal marital status: married vs single/widowed	0.51 (0.25–1.03)	0.06	*		*	
Vitamin A group[b]						
Aa versus Pp	1.30 (0.67–2.50)	0.44	0.97 (0.61–1.53)	0.88	1.04 (0.59–1.56)	0.86
Ap versus Pp	0.96 (0.48–1.90)	0.90	1.14 (0.74–1.77)	0.53	1.21 (0.82–1.79)	0.34
Pa versus Pp	1.27 (0.67–2.42)	0.47	1.01 (0.64–1.57)	0.98	1.02 (0.68–1.53)	0.92

CI, Confidence interval; HR, hazard ratio; MUAC, mid-upper arm circumference.
[a]Early is defined as the first 3 months of life. See text for definitions of feeding patterns.
[b]See Table 1.
*Not retained in the model.

Table 4. Final Cox proportional hazards model for the risk of HIV infection or death between 6 weeks and 6, 12, and 18 months.

Variable	HR HIV infection (95% CI) at 6 months	*P* value	HR HIV infection (95% CI) at 12 months	*P* value	HR HIV infection (95% CI) at 18 months	*P* value
No. events	96		196		244	
Early breastfeeding pattern[a]						
Exclusive	1.0		1.0		1.0	
Predominant	2.42 (0.71, 8.18)	0.16	2.36 (1.00–5.57)	0.05	1.73 (0.84, 3.52)	0.14
Mixed	3.03 (0.95, 9.69)	0.06	3.03 (1.34, 6.86)	0.008	2.48 (1.26, 4.84)	0.008
Infant birth weight (kg)	0.61 (0.38–0.98)	0.04	0.75 (0.54–1.04)	0.09	0.71 (0.53–0.96)	0.02
Maternal age (years)	*		*		1.03 (1.01–1.06)	0.01
Maternal MUAC (cm)	0.93 (0.86–1.00)	0.05	0.93 (0.88–0.99)	0.01	0.93 (0.89–0.98)	0.004
Maternal CD4 cell count at delivery ($\times 10^6$/l)						
$\geq$500	1.0		1.0		1.0	
350 to < 500	*		1.62 (1.01–2.60)	0.05	*	
200 to < 350	2.52 (1.47–4.32)	0.0008	2.64 (1.69–4.11)	< 0.0001	1.80 (1.31–2.48)	0.0003
< 200	6.23 (3.65–10.63)	< 0.0001	4.70 (2.95–7.48)	< 0.0001	3.26 (2.31–4.60)	< 0.0001
Maternal hemoglobin (g/l)						
< versus $\geq$ 70	6.97 (2.72–17.85)	< 0.0001	3.30 (1.44–7.57)	0.005	2.70 (1.18–6.17)	0.02
Maternal death	1.82 (0.92–3.63)	0.09	1.97 (1.17–3.32)	0.01	1.63 (0.99–2.69)	0.05
Maternal marital status: married vs single/widowed	0.44 (0.25–0.78)	0.005	0.63 (0.41–0.99)	0.05	0.63 (0.42–0.95)	0.03
Vitamin A group[b]						
Aa versus Pp	1.60 (0.89–2.87)	0.12	1.14 (0.75–1.73)	0.55	1.21 (0.83–1.76)	0.32
Ap versus Pp	0.96 (0.51–1.82)	0.91	1.22 (0.82–1.83)	0.33	1.33 (0.93–1.91)	0.12
Pa versus Pp	1.40 (0.78–2.53)	0.26	1.23 (0.82–1.84)	0.33	1.25 (0.86–1.80)	0.24

CI, Confidence interval; HR, hazard ratio; MUAC, mid-upper arm circumference.
[a]Early is defined as the first 3 months of life. See text for definitions of feeding patterns.
[b]See Table 1.
*Not retained in the model.

infants with and without one lapse of non-milk liquids was similar at 6 months (1.2 versus 1.5%), suggesting that allowing this one lapse had little effect.

Our findings underscore the importance of supporting EBF, particularly in areas of high HIV prevalence, where many women do not know their HIV status, and among HIV-positive mothers who choose to breastfeed. The early introduction of non-human milks and solid foods should be strongly discouraged because it increases the risk of HIV infection for babies of HIV-positive women and the risk of diarrhea and respiratory infections for all babies [8–10]. Among breastfeeding women known to be HIV positive, early breastfeeding cessation should be considered, along with support for nutritionally adequate, safe replacement feeding. HIV-positive mothers with CD4 cell counts less than 200 cells/μl should be strongly encouraged to consider antiretroviral treatment while breastfeeding, or replacement feeding from birth because of their very high risk of PNT.

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Contributors

P. Iliff participated in the conception, design, and implementation of the study, and drafting the manuscript. E. Piwoz participated in study conception and design, undertook most of the analysis and drafted the manuscript. N. Tavengwa participated in study design, implementation and interpretation. C. Zunguza contributed to study interpretation. E. Marinda and L. Moulton contributed to the statistical analysis. K. Nathoo participated in study design, implementation and interpretation. B. Ward contributed to conception, design and interpretation. J. Humphrey is the principal investigator of the ZVITAMBO trial, and participated in all aspects of the study, including conception, design, implementation, analysis and drafting of the manuscript. All primary authors reviewed the final manuscript. All members of the ZVITAMBO Team contributed to design and implementation.

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