

Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study

Hoosen M Coovadia, Nigel C Rollins, Ruth M Bland, Kirsty Little, Anna Coutsooudis, Michael L Bennish, Marie-Louise Newell

Summary

Background Exclusive breastfeeding, though better than other forms of infant feeding and associated with improved child survival, is uncommon. We assessed the HIV-1 transmission risks and survival associated with exclusive breastfeeding and other types of infant feeding.

Methods 2722 HIV-infected and uninfected pregnant women attending antenatal clinics in KwaZulu Natal, South Africa (seven rural, one semiurban, and one urban), were enrolled into a non-randomised intervention cohort study. Infant feeding data were obtained every week from mothers, and blood samples from infants were taken monthly at clinics to establish HIV infection status. Kaplan-Meier analyses conditional on exclusive breastfeeding were used to estimate transmission risks at 6 weeks and 22 weeks of age, and Cox's proportional hazard was used to quantify associations with maternal and infant factors.

Findings 1132 of 1372 (83%) infants born to HIV-infected mothers initiated exclusive breastfeeding from birth. Of 1276 infants with complete feeding data, median duration of cumulative exclusive breastfeeding was 159 days (first quartile [Q1] to third quartile [Q3], 122–174 days). 14·1% (95% CI 12·0–16·4) of exclusively breastfed infants were infected with HIV-1 by age 6 weeks and 19·5% (17·0–22·4) by 6 months; risk was significantly associated with maternal CD4-cell counts below 200 cells per μL (adjusted hazard ratio [HR] 3·79; 2·35–6·12) and birthweight less than 2500 g (1·81, 1·07–3·06). Kaplan-Meier estimated risk of acquisition of infection at 6 months of age was 4·04% (2·29–5·76). Breastfed infants who also received solids were significantly more likely to acquire infection than were exclusively breastfed children (HR 10·87, 1·51–78·00, $p=0\cdot018$), as were infants who at 12 weeks received both breastmilk and formula milk (1·82, 0·98–3·36, $p=0\cdot057$). Cumulative 3-month mortality in exclusively breastfed infants was 6·1% (4·74–7·92) versus 15·1% (7·63–28·73) in infants given replacement feeds (HR 2·06, 1·00–4·27, $p=0\cdot051$).

Interpretation The association between mixed breastfeeding and increased HIV transmission risk, together with evidence that exclusive breastfeeding can be successfully supported in HIV-infected women, warrant revision of the present UNICEF, WHO, and UNAIDS infant feeding guidelines.

Introduction

Breastfeeding remains an important route of acquisition of HIV-1 infection for infants; in 2005, an estimated 630 000–820 000 infants were newly infected, of whom around 280 000–360 000 would have been infected through breastfeeding.^{1–3} Comprehensive prevention of mother-to-child-transmission (PMTCT) programmes report substantially lower rates of perinatal transmission of between 2% and 5% in non-breastfed and breastfed populations.^{4–6} Yet, even where programmes are available that offer HIV testing to pregnant women and antiretroviral prophylaxis for those infected, many infants continue to become infected through breastfeeding. National and local recommendations for infant feeding by HIV-infected mothers in resource-poor countries have been confounded by the scarcity of accurate estimates of the risk of HIV acquisition through different infant feeding practices and the associated survival risks and benefits.⁷ In particular, the risks of exclusive breastfeeding by HIV-infected mothers in developing countries have not been adequately reported.

Observational data from a randomised trial of the effect of antenatal vitamin A supplementation on perinatal

transmission,⁸ suggested that the risk of postnatal HIV transmission was lower with exclusive breastfeeding than with mixed breastfeeding. Another trial of a vitamin A intervention showed that HIV postnatal transmission risk and mortality were higher in mixed breastfed infants than in those who were exclusively breastfed.⁹ Furthermore, both clinical trials and operational research settings have shown conflicting serious morbidity and mortality risks associated with the free provision of replacement feeds.^{10,11} In Botswana, cumulative all-cause infant mortality at 7 months was significantly higher in infants randomly assigned to formula-feeding than in those assigned to breastfeeding and zidovudine.¹⁰ In the first quarter of 2006, over 22 500 infants in 12 districts in Botswana had diarrhoea (compared with 9166 in the same period in 2005), and the number of deaths in children less than 5 years increased by about 20 times; almost all these infants were not breastfed,¹² and there were no reported changes in infant feeding practice over this time. In Côte d'Ivoire, however, infants of HIV-infected mothers who chose to formula feed had no greater mortality than did those who were breastfed.¹¹

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Centre for HIV/AIDS Networking (Prof H M Coovadia MD), Department of Paediatrics and Child Health (Prof N C Rollins MD, Prof A Coutsooudis PhD), and Africa Centre for Health and Population Studies (N C Rollins, R M Bland MB, Prof M L Newell PhD), University of KwaZulu-Natal, South Africa; Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, University College London, London, UK (K Little MSc, M-L Newell); and Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK (M L Bennish MD)

Correspondence to: Prof Nigel Rollins, Department of Paediatrics and Child Health, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella, 4013, South Africa rollins@ukzn.ac.za

These, and other similar reports, have confused rather than guided infant feeding policies and how health workers should counsel mothers in high HIV-prevalence settings on infant feeding options.¹³ We therefore implemented a study to assess the HIV transmission risks and survival associated with exclusive breastfeeding and other types of infant feeding.

Methods

Study design

HIV-infected and uninfected pregnant women attending antenatal clinics in KwaZulu Natal (seven rural, one semiurban, and one urban) were enrolled into a non-randomised intervention cohort study if they were 16 years of age or older, planned to stay in the study area for at least 3 months after delivery, and provided written informed consent. Uninfected women were included to establish the effect of HIV status on adherence to exclusive breastfeeding and other infant-feeding practices. The populations served by the rural and semiurban antenatal clinics were part of the surveillance area of the Africa Centre Demographic Surveillance System. Maternal sociodemographic and health information was recorded; antenatal CD4-cell counts were measured in HIV-infected women (September, 2001, to March, 2003, FACScan, Becton, Dickinson and Company, NJ, USA, thereafter Epics XL, Beckman Coulter, CA, USA).

Single-dose nevirapine was provided for all HIV-infected women and their infants any time after first booking or 28 weeks' gestation.¹⁴ All women were counselled antenatally about infant-feeding options.¹⁵ 6 months' supply of commercial infant formula (number of tins increased with age) was offered free through the KwaZulu Natal PMTCT programme from the end of 2002, and HIV-infected mothers could choose to access this supply any time in the first 12 months of the infant's life. For those initiating formula milk from birth, an initial supply was provided antenatally.

After delivery, all mothers, irrespective of HIV status, and their infants were visited at home by infant-feeding counsellors three or four times in the first 2 weeks of life and every 2 weeks thereafter until the infant was 6 months old. Counsellors and clinic-based study nurses supported mothers to breastfeed exclusively or to replacement feed exclusively according to antenatal or present choice. The counsellors also recorded information on breast health and breastfeeding technique (positioning and attachment) but no data on infant-feeding type, to avoid potential recording bias. Instead, mothers reported feeding practices to an independent group of field monitors who visited every week and documented all feeds (milks and solids), fluids, drugs, morbidity episodes, and attendances at health facilities for every day of the preceding week. Mothers kept food-intake and morbidity diaries for use during the field-monitor interview to corroborate the verbal report. Cumulative patterns of exclusive breast-

feeding were determined only at the time of analysis and not during the course of home visits. If a mother was not present for a counselling or monitoring visit, the study team returned on up to 2 consecutive days.

Neither feeding counsellors nor field monitors were aware of the mothers' HIV status. Mothers and infants also attended clinics 6 weeks after delivery and every month thereafter until 6 months. At every visit the study nurse measured the infant and recorded any morbidity events, a dried blood spot sample was obtained by heel or finger prick from the infant, and a breastmilk sample from the mother. A dried blood spot sample was also obtained within 72 h of delivery when possible. Clinic nurses and HIV counsellors advised HIV-infected women to stop all breastfeeding when infants were 6 months old. At the time of the study, highly active antiretroviral treatment was not available through the provincial health services. The study was approved by the biomedical research ethics committee of the University of KwaZulu-Natal.

Exclusive breastfeeding was defined as the infant receiving only breastmilk from birth (including expressed breastmilk) from his or her mother and no other liquids or solids, with the exception of drops or syrups consisting of vitamins, mineral supplements, or drugs.^{16,17} Our protocol, however, allowed water or formula milk to be given for up to a total of 3 days, either on separate or continuous days, without exclusion from the group (this allowance was included since we were unsure how well participants would be able to adhere to the strictest definition of exclusive breastfeeding). Periods of exclusive breastfeeding ended on the fourth day of a child receiving either water or formula milk. Infants who received porridge or other solid foods, even if only once, were excluded from the feeding group. Although the WHO definition allows all drugs, including those self-prescribed, we additionally stipulated that paracetamol could be given only for up to 7 days and oral rehydration solution as treatment for diarrhoea for up to 72 h. Replacement feeding was defined as provision of any non-human milk and the exclusion of all breastmilk, with or without other liquids or solids. Mixed breastfeeding was defined as giving breastmilk with non-human milk, other liquids, or solids.^{17,18}

HIV status was established by quantitative HIV RNA assay (Nuclisens HIV-1 QT, Organon Teknika, Boxtel, Netherlands, and Nuclisens EasyQ HIV-1, Biomerieux, Boxtel, Netherlands) with a sensitivity of 80 copies of HIV RNA per mL of blood (equivalent to 1600 copies HIV RNA per 50 μ L dried blood spot).¹⁹ If HIV RNA was not detectable (<80 copies per mL blood) the infant was regarded as HIV uninfected. Infants were regarded as HIV infected if two samples both had more than 4000 copies per mL.²⁰ If the second sample had less than 4000 copies per mL, a third sample was obtained for confirmation. For infants with detectable HIV RNA viral load at 6 months, stored samples were tested, if available, to assess when the infection was acquired.

Statistical methods

We captured data with optical imaging recognition software (Teleform, Cardiff, San Diego, CA, USA) into a Microsoft SQL Server database with custom written applications (Pericom, Durban, South Africa). Analysis was based on the database created on Oct 10, 2005. Infants with no feeding or test data were excluded from the analyses, and

infants were excluded if there were more than 5 days of missing feeding data in any 30 day period. Feeding categories were established from the analytical database by application of algorithms that first classified infant-feeding practices on every day of life and then measured the cumulative pattern from birth. Analyses were done with Stata (version 9.1). Continuous data with a normal

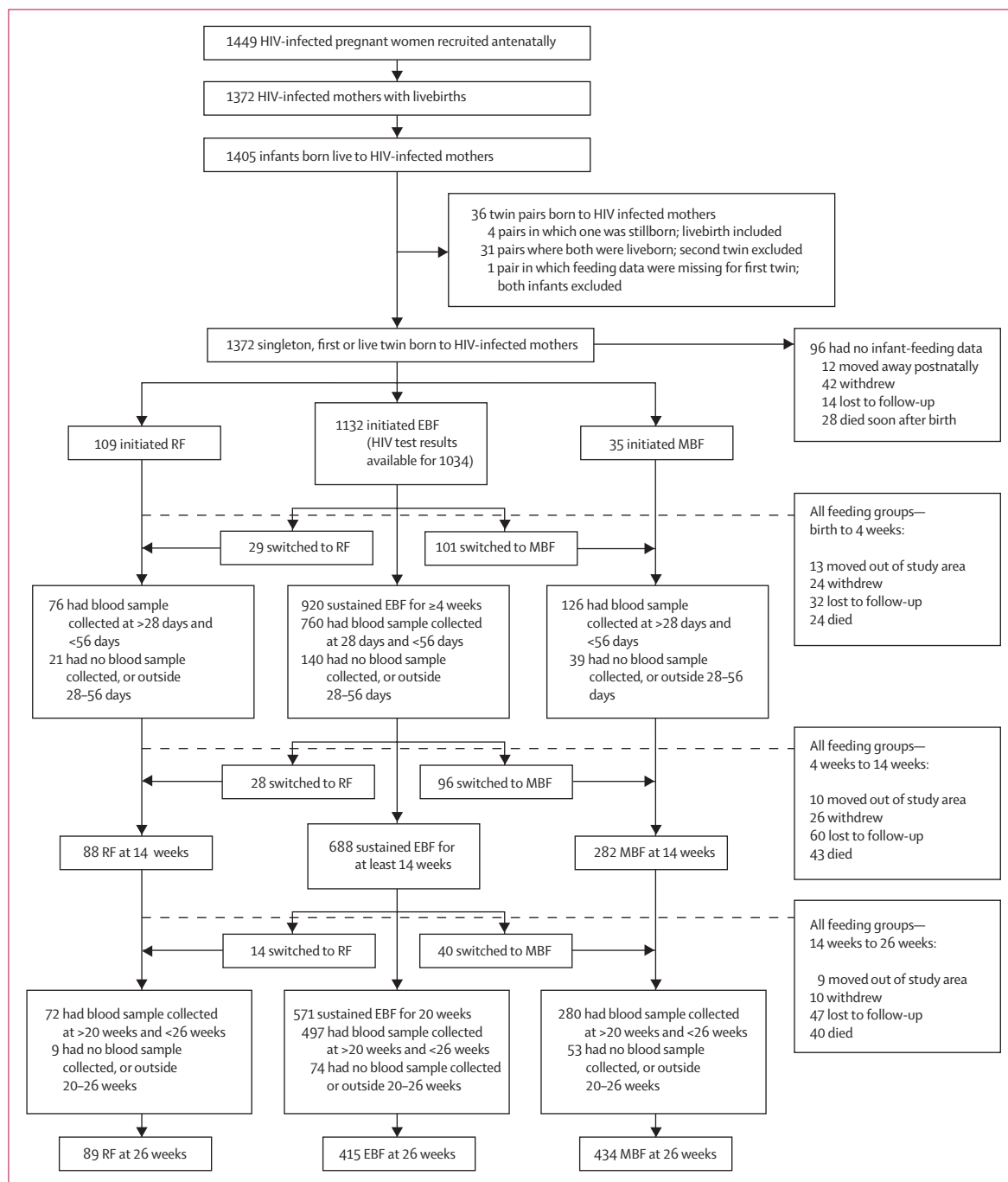


Figure 1: Flow diagram of infant-feeding groups over time and associated HIV testing

EBF=exclusive breastfeeding. RF=replacement feeding. MBF=mixed breastfeeding. Figure does not show infants who switched from RF (any time) to MBF. MBF numbers are included in text and table.

	Overall	Exclusive breastfeeding	Replacement feeding	Mixed breastfeeding	Feeding information unavailable	p*
Total	1372	1132 (83%)	109 (8%)	35 (3%)	96 (7%)	
Median maternal age (years)†	25.1 (16.1–45.8)	24.9 (16.1–45.8)	27.1 (16.7–41.4)	25.1 (16.3–37.2)	26.0 (18.0–40.0)	
Enrolment clinic						
Urban	257 (19%)	203 (18%)	35 (32%)	4 (11%)	15 (16%)	0.0003
Semiurban	514 (37%)	414 (37%)	41 (38%)	14 (40%)	45 (47%)	0.8312
Rural	601 (44%)	515 (45%)	33 (30%)	17 (49%)	36 (37%)	0.0023
Highest education attained						
None	18 (1%)	10 (1%)	5 (4.6%)	0	3 (3%)	0.0007
Some primary	292 (21%)	230 (20%)	29 (27%)	11 (31%)	22 (23%)	0.1233
7 years (primary)	467 (34%)	392 (35)	35 (32%)	6 (17%)	34 (35%)	0.5954
10 years	328 (24%)	286 (25%)	21 (19%)	7 (20%)	14 (15%)	0.1678
12 years (matriculation)	174 (13%)	138 (12%)	13 (12%)	8 (23%)	15 (16%)	0.9295
Post-matriculation	95 (7%)	76 (7%)	6 (6%)	3 (9%)	8 (8%)	0.6271
House type						
Brick	51 (4%)	41 (4%)	6 (6%)	1 (3%)	3 (3%)	0.3259
Concrete/cement/mixed	761 (55%)	637 (56%)	66 (61%)	20 (57%)	38 (40%)	0.3836
Board/mud/sticks/stones	423 (31%)	359 (32%)	27 (25%)	13 (37%)	24 (25%)	0.1366
Unknown/other/missing	137 (10%)	95 (8%)	10 (9%)	1 (3%)	31 (32%)	0.7799
Toilet type						
Flush/chemical	249 (18%)	202 (18%)	28 (26%)	4 (11%)	15 (16%)	0.0446
Pit latrine/bucket	792 (58%)	664 (59%)	65 (60%)	23 (66%)	40 (42%)	0.8332
None	289 (21%)	256 (23%)	12 (11%)	7 (20%)	14 (15%)	0.0049
Missing	42 (3%)	10 (1%)	4 (4%)	1 (3%)	27 (28%)	0.0077
Water type						
Piped indoors/in yard	536 (39%)	445 (39%)	54 (50%)	10 (29%)	26 (28%)	0.0366
Piped public tap	384 (28%)	319 (28%)	29 (27%)	13 (37%)	23 (24%)	0.7258
Borehole/rain water tank	105 (8%)	91 (8%)	9 (8%)	2 (6%)	3 (3%)	0.9241
River	297 (22%)	260 (23%)	13 (12%)	8 (23%)	16 (17%)	0.0077
Other/missing	50 (4%)	17 (2%)	4 (4%)	2 (6%)	27 (28%)	0.0891
Main income provider						
Mother	152 (11%)	117 (10%)	20 (18%)	5 (14%)	10 (10%)	0.0104
Household member at home	842 (61%)	716 (63%)	61 (56%)	23 (66%)	42 (44%)	0.1298
Household member, away, returns <monthly	151 (11%)	123 (11%)	14 (13%)	4 (11%)	10 (10%)	0.5392
Household member, away, returns >monthly	42 (3%)	39 (3%)	2 (2%)	0	1 (1%)	0.3574
Other/missing	185 (13%)	137 (12%)	12 (11%)	3 (9%)	33 (34%)	0.7357
Maternal CD4 cell count (per µL)‡	453 (2–1835)	462 (21–1835)	395.5 (15–1229)	526 (20–1433)	434 (2–1131)	
>500	547 (40%)	465 (41%)	33 (30%)	17 (49%)	32 (33%)	0.0283
200–500	606 (44%)	496 (44%)	48 (44%)	11 (31%)	51 (53%)	0.9711
<200	146 (11%)	111 (10%)	23 (21%)	5 (14%)	7 (7%)	0.0003
Missing	73 (5%)	60 (5%)	5 (5%)	2 (6%)	6 (6%)	0.7541
Maternal mid-upper arm circumference (cm)§	27 (16–56.4)	27 (16–56.4)	28.1 (21–43)	28.0 (22–38.5)	27.9 (16–37.5)	
Infant birthweight (g)	3100 (1150–4600)	3100 (1150–4500)	3200 (1500–4600)	3000 (1600–3800)	3100 (1700–4500)	
<2500	138 (10%)	116 (10%)	6 (6%)	7 (20%)	9 (9%)	0.1135
2500–3499	823 (60%)	704 (62%)	67 (62%)	18 (51%)	34 (35%)	0.8872
>3500	301 (22%)	249 (22%)	34 (31%)	5 (14%)	13 (14%)	0.0288
Missing	110 (8%)	63 (6%)	2 (2%)	5 (14%)	40 (42%)	0.0916
Child sex						
Male	676 (49%)	571 (50%)	55 (51%)	14 (40%)	36 (38%)	0.9905
Female	671 (49%)	554 (49%)	54 (50%)	21 (60%)	42 (44%)	0.9110
Missing	25 (2%)	7 (1%)	0	0	18 (19%)	0.4097

Data are number (%) or median (minimum, maximum). EBF=exclusive breastfeeding. RF=replacement feeding. *p for comparison between EBF and RF. †n=1368 and 1128 for maternal age groups overall and EBF. ‡n=1299, 1072, 104, 33, and 90 for overall, EBF, RF, mixed breastfeeding, and feeding information unavailable, respectively; §n=1068, 931, 88, 29, and 20 for overall, EBF, RF, mixed breastfeeding, and feeding information unavailable, respectively.

Table 1: Characteristics for liveborn children and HIV-infected mothers by infant-feeding practice at birth

distribution were assessed with *t* test, Mann-Whitney test for non-normal distributions, two-sample tests of proportions or χ^2 for categorical variables, and Fisher's exact test if numbers were small.

Cumulative transmission and mortality in the first 6 months of life were assessed by Kaplan-Meier analysis, and association with maternal and infant variables was quantified in a Cox regression analysis.²¹⁻²⁴ We assessed the proportional-hazards assumption of the Cox regression model with log-log plots and regression of the Schoenfeld residuals; the goodness-of-fit was assessed by the log-likelihood test.²² Transmission rate at 6 weeks and 6 months was based on infants' samples obtained at 4-8 weeks and 20-26 weeks, respectively.²⁵ For the Kaplan-Meier analyses, the estimated time of acquisition of infection was taken to be midway between the dates of the last negative test and the first positive test.²⁵ For some variables, a missing response was considered to mean that the interviewee was unsure of the answer, and in such cases missing was included as a category of the variable. The sample size was defined with a CI approach on the basis of a priori estimates. We assumed that 6-week transmission would be around 11%, and regarded an additional 5% postnatal transmission as of public-health significance. The sample size to detect whether the HIV-infection rate at 22 weeks of infants who were exclusively breastfed until 18 weeks was 16% \pm 2% variance with 80% power and 95% CI (Statcalc in EpiInfo) was 1344 exclusively breastfeeding mothers, assuming 65% exclusive breastfeeding adherence to 18 weeks.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 29, 2001, and April 16, 2005, 2722 women delivered 2779 liveborn infants—1372 HIV-infected mothers had 1405 infants, 1345 HIV-uninfected had 1369, and five had indeterminate HIV-infection status. Our analysis is based on HIV-infected mothers and their infants. There were 36 twin pairs born to HIV-infected mothers, of whom 32 were both liveborn, and four in which one of the twins was stillborn and one liveborn. Twins were concordant on feeding method with the exception of a pair in which feeding data were missing for the first born and the second born was reportedly exclusively replacement fed. The second born of liveborn twins were excluded, leaving 1372 liveborn infants from 1372 HIV-infected mothers. Complete feeding data from birth to 6 months were available for 1276 infants. Figure 1 shows recruitment of patients, feeding practices, and follow-up, including reasons for loss to follow-up and non-availability of infant-feeding data and HIV-transmission results.

	Maternal antenatal CD4 count by feeding method (cells per μ L)				HIV point prevalence rates
	<200	200-500	>500	Missing	
EBF (n=362)	30 (8%)	162 (45%)	155 (43%)	15 (4%)	55 (15%; 11.7-19.3)
RF (n=28)	10 (36%)	9 (32%)	8 (29%)	1 (4%)	2* (7%; 0.9-23.5)
MBF (starting <14 weeks; n=332)	40 (12%)	159 (48%)	113 (34%)	20 (6%)	89 (27%; 22.1-31.9)
MBF (starting >14 weeks; n=239)	30 (13%)	96 (40%)	101 (42%)	12 (5%)	61 (26%; 20.1-31.5)

Data are number (%) or number (%; 95% CI). EBF=exclusively breastfeeding. RF=replacement feeding. MBF=mixed breastfeeding. *Two children who switched from EBF to RF.

Table 2: Maternal antenatal CD4-cell counts and HIV point prevalence rates at 26 weeks by method of feeding at 26 weeks

Most of the mother-infant pairs initiated exclusive breastfeeding (table 1). Median duration of exclusive breastfeeding for women who initiated breastfeeding and whose infants had HIV test results available (n=1034) was 159 days (first quartile [Q1] to third quartile [Q3], 122-174 days); 847 (82%) exclusively breastfed for at least 6 weeks, 688 (67%) for at least 3 months, and 415 (40%) for 6 months. The proportions of infants with HIV results available who were fed a mixture of breastmilk and other fluids at 6 weeks, 3 months, and 6 months were 3.8%, 5.7%, and 15.4%, respectively. Some children were replacement fed from birth and appropriately switched to

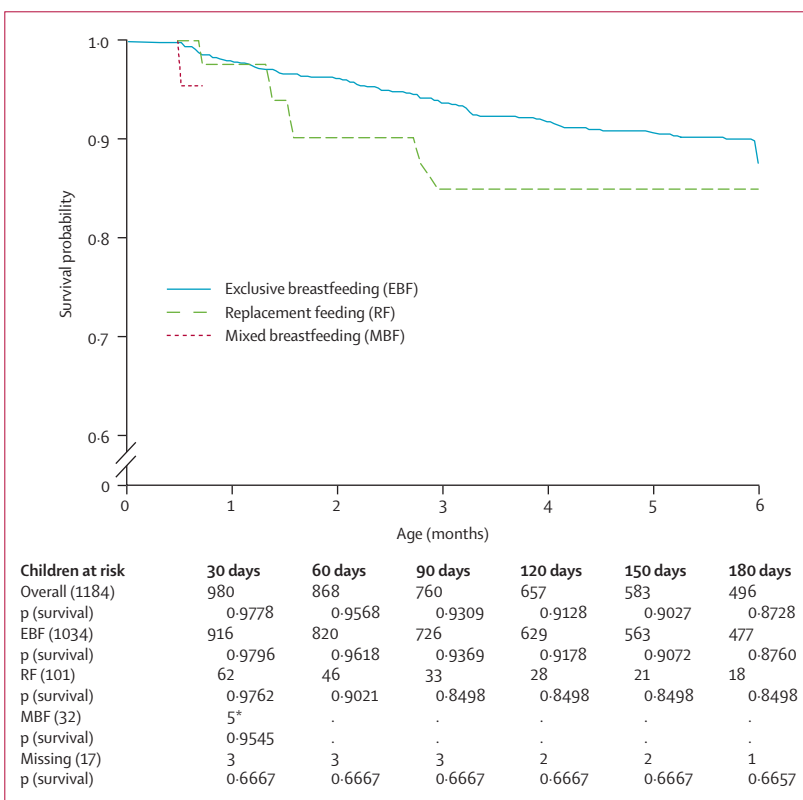


Figure 2: Survival probability by feeding type
*No further events beyond last time point of around 22 days. Note break in legend.

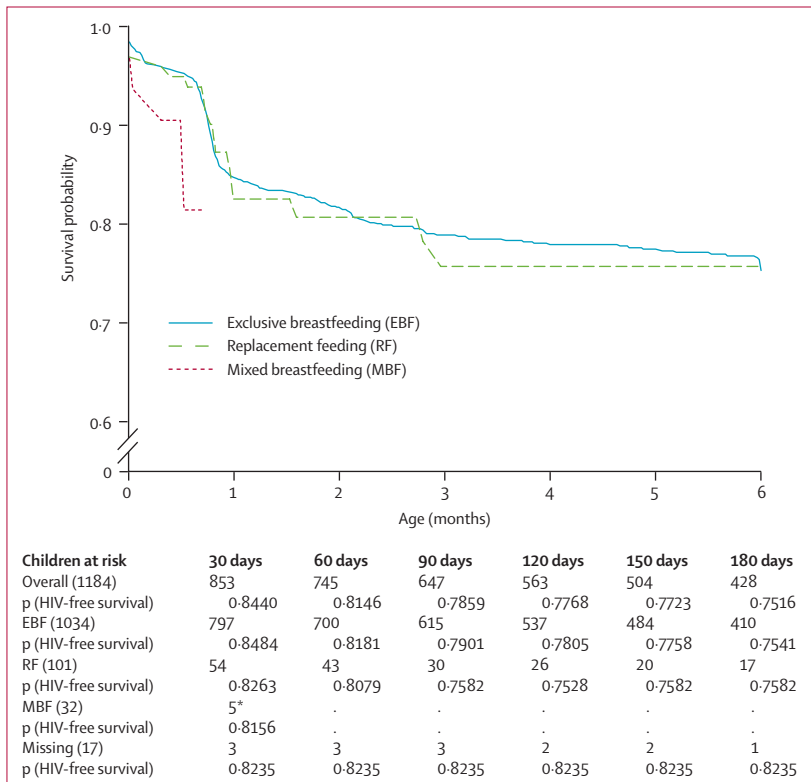


Figure 3: 6 month HIV-free survival by feeding type
 *No further events beyond last time point of around 22 days. Note break in legend.

replacement feeding without any mixed feeding; some were not included at some time points because HIV results were not available within the interval specified per protocol, even though feeding data might have been available. In 193 076 days of follow-up, exclusive breastfeeding was reported in 141 599 (74%) days and complete avoidance of breastmilk was reported in 37 143 (19%) days. Of the 114 women who initiated exclusive breastfeeding and who subsequently moved,⁴⁵ withdrew,³⁵ or were lost-to-follow-up,³⁴ about 68% were exclusively breastfeeding at the time of last contact. More women who chose to replacement feed had CD4-cell counts less than 200 cells per μ L than did those choosing to exclusively breastfeed ($p=0.003$; table 1).

At 4–8 weeks of age (median 44 days; Q1–Q3, 40–48 days), 998 infants (all feeding types) were tested for HIV infection. 150 were positive, giving a point prevalence rate of 15.0% (95% CI 12.9–17.4). At 20–26 weeks of age, 962 children were tested, of whom 208 (21.6%, 19.1–24.4) were infected. Of the 760 exclusively breastfed infants who were tested at 4–8 weeks of age, 114 (15.0%, 12.5–17.8) were infected; of the 497 infants exclusively breastfed for more than 20 weeks and tested at 20–26 weeks of age, 80 (16.0%, 13.0–19.6) were infected.

Of 1034 exclusively breastfed infants, 175 had been diagnosed with infection before 6 months of age (3686.63 person-months at risk postpartum). In Kaplan-

Meier survival analysis conditional on exclusive breastfeeding, cumulative infection rates were 14.1% (12.0–16.4) at 6 weeks of age, 18.1% (15.8–20.8) by 4 months, 18.6% (16.2–21.4) by 5 months, and 19.5% (17.0–22.4) by 6 months. In 723 exclusively breastfed infants who were HIV uninfected at or after 6 weeks, the estimated Kaplan-Meier cumulative risk of infection from 6 weeks of age was 1.1% (0.28–1.84) after 1 month, 2.2% (1.05–3.34) after 2 months, 2.7% (1.44–4.02) after 3 months, 3.3% (1.88–4.77) after 4 months, and 4.0% (2.29–5.76) after 5 months (ie, at about 6 months of age).

For infants who were HIV uninfected at or after 6 weeks of age, and accounting for a 2-week time lag between the end of a feeding episode and the estimated time of infection, the overall transmission rate per 100 child-days, including infants who were replacement fed and those with missing data excluded, was 0.032 (0.0222–0.0455). This rate varied from 0.0290 (0.0195–0.0442) for 100 days of exposure to exclusive breastfeeding and 0.0436 (0.0208–0.0915) for breastmilk plus other foods or fluids. This result equates to an estimated risk of 10.72 per 100 child-years of exposure to exclusive breastfeeding (or 0.89% per child-month). In Cox regression analysis with exclusive breastfeeding as reference, the hazard ratio (HR) for breastmilk plus other food or fluids was 1.56 (0.66–3.69, $p=0.308$).

Infants who were breastfed but also received solids (generally home-prepared cereal or commercial infant porridges) any time after birth, were nearly 11 times more likely to acquire infection than were exclusively breastfed children (HR 10.87, 1.51–78.00, $p=0.018$). 203 mothers started exclusively breastfeeding and later introduced solids; the median age at which solids were introduced was 147 days (Q1–Q3, 99–171 days). Similarly, infants who at 14 weeks of age were fed both breastmilk and formula milk were nearly twice as likely to be infected as exclusively breastfed infants at that time (1.82, 0.98–3.36, $p=0.057$). Table 2 shows maternal antenatal CD4-cell counts and HIV point prevalence rates at 26 weeks of age by method of feeding at 26 weeks.

In a further regression analysis with feeding classified as exclusive breastfeeding for 20 weeks or more, exclusive replacement feeding for 6 months, or mixed breastfeeding starting before 3 months and after 3 months, both early-mixed feeders ($n=356$) and late-mixed feeders ($n=257$) were at greater risk of being infected than were infants exclusively breastfed (1.54, 1.10–2.15, $p=0.011$ and 1.53, 1.07–2.20, $p=0.021$, respectively).

94 of the 1034 liveborn children who were initially exclusively breastfed died, with estimated cumulative mortality rates increasing from 1.92% (1.23–3.00) after 1 month, 3.60% (2.66–5.10) at 2 months, 6.13% (4.74–7.92) at 3 months, 8.01% (6.37–10.05) at 4 months, 9.20% (7.27–11.22) at 5 months, and 12.24% (10.06–14.85) at 6 months. Of these 94 infants who died, 73 (78%) were HIV infected. Eight of 101 children

who initiated exclusive replacement feeding from birth died. Kaplan-Meier mortality estimates by the end of 1 month were 4.22% (1.34–12.87), 9.90% (4.49–21.05) after 2 months, and 15.12% (7.63–28.73) after 3 months with no further events thereafter (figure 2). In a separate Cox regression model including both exclusive breastfeeding and replacement feeding, the HR for formula feeding was 2.06 (1.00–4.27, $p=0.051$). Overall, there were 223 (22%) deaths or infections in infants exclusively breastfed, resulting in an overall Kaplan-Meier estimated HIV-free survival of 75.4% at 6 months (figure 3).

In univariable and multivariable Cox's regression analyses conditional on exclusive breastfeeding, risk of transmission overall was strongly associated with maternal CD4-cell counts and less strongly with maternal age, birthweight below 2500 g, vaginal delivery, and long duration of ruptured membranes (table 3). The estimated transmission at 6 months in exclusively breastfed infants born to HIV-infected women with CD4-cell counts less than 200 per μL or 200 and greater cells per μL were 34% and 17%, respectively. Infant's sex, maternal socioeconomic status and education, location of residence, antenatal feeding intention, and duration of labour were not significantly associated with the overall risk of transmission, and there was no evidence of a significant trend over time. Data for nevirapine uptake and ingestion were inconsistent and are not included in any analysis.

In univariable regression analyses, maternal CD4-cell count (less than 500 per μL), low birthweight, infant's infection status, and place of birth (other or unknown) were significantly associated with increased mortality. In multivariable analyses, however, only infant's infection status was significantly associated with death; infected infants were 15 times more likely to die than uninfected children (15.28, 9.20–25.40, $p<0.0001$). In multivariable analyses, in a model with a significantly reduced goodness-of-fit (log likelihood -599.07 compared with -530.11) that excluded infant's infection status, the only significant factor was maternal CD4-cell count. Infants born to mothers with a CD4-cell count of 200–500 cells per μL were nearly twice as likely to have died (unadjusted HR 1.89, 1.16–3.08, $p=0.011$) than were those born to mothers with a CD4-cell count greater than 500 per μL . Those born to mothers with CD4-cell counts less than 200 per μL were more than three times as likely to have died (3.19, 1.73–5.88, $p=0.0001$).

Infants born to mothers with CD4-cell counts less than 200 per μL were almost four times more likely to acquire HIV or die than were those born to mothers with CD4-cell counts greater than 500 per μL , and those born to mothers with CD4-cell counts between 200 and 500 cells per μL were 2.2 times more likely to acquire HIV or die. Other variables significantly associated with decreased HIV-free survival were birthweight less than 2500 g, rupture of membranes for longer than 12 h, and maternal age between 20 and 30 years (table 4).

	n	Hazard ratio (95% CI)	p	Adjusted hazard ratio* (95% CI)	p
Maternal age (years)					
<20	162	1.0			
20–30	634	1.94 (1.17–3.23)	0.010	1.86 (1.11–3.11)	0.018
>30	234	1.42 (0.79–2.54)	0.239	1.25 (0.69–2.25)	0.466
Missing	4	3.21 (0.43–24.10)	0.258	2.23 (0.29–17.1)	0.437
Maternal CD4-cell count per μL					
>500	412	1.0		1.0	
200–500	463	2.43 (1.66–3.55)	<0.001	2.44 (1.67–3.58)	<0.001
<200	106	3.90 (2.43–6.26)	<0.001	3.79 (2.35–6.12)	<0.001
Missing	53	2.72 (1.42–5.22)	0.003	3.04 (1.58–5.86)	0.001
Birthweight (g)					
Over 3500	229	1.0		1.0	
2500–3500	649	1.12 (0.75–1.65)	0.584	1.07 (0.72–1.59)	0.748
Below 2500	108	2.10 (1.27–3.48)	0.004	1.81 (1.07–3.06)	0.026
Missing	48	1.63 (0.82–3.21)	0.164	1.10 (0.51–2.36)	0.803
Duration of rupture of membranes (h)					
<4	558	1.0		1.0	
4–12	125	0.63 (0.36–1.11)	0.107	0.68 (0.39–1.20)	0.181
>12	55	2.04 (1.22–3.43)	0.007	2.19 (1.29–3.70)	0.004
Missing	296	1.13 (0.81–1.59)	0.474	1.19 (0.84–1.68)	0.324
Method of delivery					
Vaginal	901	1.0		1.0	
Caesarean section	133	0.56 (0.32–0.97)	0.037	0.51 (0.29–0.90)	0.019
Place of delivery					
Hospital or clinic	865	1.0		1.0	
Home and other	149	1.49 (1.03–2.18)	0.037	1.29 (0.86–1.97)	0.220
Missing	20	2.35 (1.04–5.33)	0.041	2.04 (0.85–4.90)	0.110

*Adjusted for all variables in the table.

Table 3: Mother-to-child transmission risk during exclusive breastfeeding (n=1034)

Discussion

This study, with a rigorous design and implementation, accords with earlier reports that exclusive breastfeeding carries a significantly lower risk of HIV transmission than do all types of mixed breastfeeding.^{8,9} Infants who received formula milk in addition to breastmilk, before or after 14 weeks of age, were nearly twice as likely to be infected as were infants who received breastmilk only. Although the numbers in non-exclusively breastfeeding categories were small, infants who were breastfed but also received solids were nearly 11 times more likely to acquire HIV infection than were those who received breastmilk only.

The main objective of our study was to provide an accurate rate of mother-to-child transmission of HIV-1 and survival of exclusively breastfed infants born to women who were HIV infected at enrolment. We initiated an intervention cohort study that offered intensive infant-feeding support and ensured high-quality data through carefully designed data-collection forms and processes. We did not randomly assign infants to the different feeding groups for several reasons. We already knew that there is no transmission after 6 weeks (beyond perinatal acquisition) if mothers do not breastfeed,

	n	Hazard ratio (95% CI)	p	Adjusted hazard ratio* (95% CI)	p
Maternal age (years)					
<20	162	1.0			
20–<30	634	1.58 (1.04–2.40)	0.031	1.54 (1.01–2.35)	0.043
30+	234	1.19 (0.73–1.93)	0.479	1.10 (0.67–1.79)	0.714
Missing	4	2.15 (0.29–15.88)	0.451	1.35 (0.18–10.03)	0.772
Maternal CD4 cell count per µL					
>500	412	1.0		1.0	
200–500	463	2.25 (1.61–3.13)	<0.001	2.28 (1.63–3.18)	<0.001
<200	106	4.02 (2.68–6.02)	<0.001	3.97 (2.63–5.98)	<0.001
Missing	53	2.05 (1.09–3.85)	0.026	2.25 (1.19–4.24)	0.012
Birthweight (g)					
Over 3500	229	1.0		1.0	
2500–3500	649	1.22 (0.86–1.73)	0.273	1.16 (0.81–1.65)	0.421
Below 2500	108	2.17 (1.38–3.42)	0.001	1.93 (1.21–3.05)	0.005
Missing	48	1.34 (0.69–2.62)	0.387	1.14 (0.58–2.25)	0.697
Duration of rupture of membranes (h)					
Less than 4	558	1.0		1.0	
4–12	125	0.81 (0.51–1.28)	0.362	0.88 (0.56–1.40)	0.539
>12	55	2.11 (1.33–3.38)	0.002	2.32 (1.44–3.72)	0.001
Missing	296	1.22 (0.90–1.65)	0.190	1.29 (0.95–1.75)	0.104
Method of delivery					
Vaginal	901	1.0		1.0	
Caesarean section	133	0.60 (0.37–0.95)	0.031	0.50 (0.31–0.81)	0.005

*Adjusted for all variables in the table

Table 4: Factors associated with HIV-free survival in exclusively breastfed infants in the first 6 months of life (n=1034)

although many mothers who choose replacement feeding also breastfeed for various reasons.³ Most importantly, we believed a randomised study would be unethical because of the well-documented morbidity and mortality risks of mixed breastfeeding. We expected, however, that some women would choose replacement feeding whereas others would default to mixed breastfeeding, allowing some comparative analyses. Most women sustained exclusive breastfeeding for long periods, resulting in very few infants being mixed fed, and thus our ability to make comparative analyses was restricted. Our study design contrasts with the two studies in South Africa and Zimbabwe that examined infant feeding and HIV transmission, in which only 26% and 8% of infants, respectively, were still exclusively breastfed at 3 months.^{9,26}

Our results show that the Kaplan-Meier estimated risk of postnatal transmission of HIV by 20–26 weeks of age in exclusively breastfed infants who were negative at 6 weeks of age was 4.04%. This result is close to that of the Durban (South Africa) study, in which the cumulative probability of infection between 6 weeks and 6 months in the 118 infants who were exclusively breastfed for at least 3 months was 4.4%.²⁶ In the Harare (Zimbabwe) study the estimated risk of transmission in 156 infants who were exclusively breastfed for at least 3 months was only 1.3% compared with 3.9% in the entire cohort (n=2060) and 4.4% in infants who received mixed feeding.⁹

Methodological differences and the heterogeneity in risk factors for transmission and survival,²⁵ and differences in overall rates^{3,27,28} make direct comparisons difficult. The Durban and Harare studies were both randomised controlled trials of the effect of vitamin A supplementation on mother-to-child transmission. Estimation of the risk of HIV transmission associated with exclusive breastfeeding was a secondary objective, and infant-feeding data were obtained only at 6 weeks, 3 months, and 6 months. The long recall periods could have caused misclassification of feeding type.²⁹ Conversely, the frequent visits made by feeding counsellors in our study might have prompted mothers to report feeding practices falsely and over-represent exclusive breastfeeding. However, feeding information in this study was obtained by a separate team of fieldworkers who were unaware of the feeding counsellors' interactions with mothers. The Durban and Harare studies were urban-based studies and had substantially fewer infants exclusively breastfeeding beyond 3 months than in our study. Clade C, the dominant HIV type found in South Africa, is more likely to result in mother-to-child transmission than in clades A and B found elsewhere in Africa.^{30–32}

We implemented an intervention to improve exclusive breastfeeding practices in HIV-infected women who chose to breastfeed and in HIV-uninfected women. We achieved a much higher rate of exclusive breastfeeding than has previously been reported in any HIV-infected or HIV-uninfected cohorts using the most stringent of definitions and data-collection methods. In our experience, HIV-infected women, if given good support, are able to adopt appropriate and optimum feeding practices, including rapid cessation at 6 months (data not reported) to restrict both transmission and mortality risks present in the environment. Although many infants were withdrawn from the study and HIV test results were unavailable for some, their omission is unlikely to significantly affect the transmission risks and estimates. Feeding practices in these infants were very similar to those in their peers at the time of withdrawal or loss to follow-up, and demographic determinants such as maternal socioeconomic status and education and location of residence were not otherwise associated with transmission risks. Infant's sex did not affect either feeding practices or, in contrast with other studies,²⁷ the rate of postnatal transmission.

Exclusive breastfeeding ordinarily protects the integrity of the intestinal mucosa, which thereby presents a more effective barrier to HIV. Exclusive breastfeeding is also associated with fewer breast health problems than is mixed feeding, such as subclinical mastitis and breast abscesses, which in turn are associated with increased breastmilk viral load. The effect that small departures from exclusive breastfeeding have on the risk of HIV transmission is uncertain, although predominant breastfeeding (the introduction of non-milk fluids) was associated with reduced transmission in one study.⁹ Why is the addition of solids especially hazardous? Perhaps large and complex

proteins found in solid foods precipitate greater damage than do modified cows' milk proteins to gastrointestinal mucosa, which ease viral entry between cells, or regulate gut receptors differently, thereby increasing the likelihood of virus adherence and infection. Smith and Kuhn³³ have summarised some of these findings and mechanisms.

We noted that mortality in the first 3 months of life was roughly doubled in the group receiving replacement feeding compared with the exclusive breastfeeding group (15% vs 6%). This result remains a concern, even though mothers who chose to replacement feed were more likely to have CD4-cell counts less than 200 per μL than were mothers who exclusively breastfed. If these women also had clinical symptoms, their ability to care for their children appropriately could have been affected. A significant increase in early mortality in formula fed versus breastfed infants was also identified in studies in Kenya³⁴ (11% vs 9%) and Botswana³⁵ (7.6% vs 3.7%). However, in better resourced areas these differences have not been reported,³⁶ which reinforces the UNAIDS guidelines on replacement feeding—namely, “where replacement feeding is acceptable, feasible, affordable, sustainable, and safe, avoidance of all breastfeeding is recommended, otherwise exclusive breastfeeding is recommended for the first few months of life”.³⁷

The substantial mortality associated with not breastfeeding in this study emphasises the importance of developing feeding policies appropriate to background infant-mortality rates, which can be successfully supported by health services and to guide individual mothers. *The Lancet* series³⁸ on global dimensions of neonatal and child survival emphasised the need to consider overall survival and not only avoidance of HIV infection; breastfeeding remains a key intervention to reduce mortality. Even in countries with high HIV prevalence, breastfeeding could prevent 13% of deaths in children younger than 5 years; in countries with low HIV prevalence, 15% of under-5 deaths could be prevented. Furthermore, 210 000–270 000 of new infections attributable to breastfeeding every year could be averted if HIV-infected mothers who breastfeed stopped after 6 months of exclusive breastfeeding (around 4% transmission) rather than the more usual 18–24 months of mixed feeding (16% transmission). This finding also has relevance for the promotion of exclusive breastfeeding in HIV-uninfected mothers in high-prevalence settings; in addition to the proven survival benefits for the infant, if such mothers unknowingly become HIV infected while breastfeeding, exclusive breastfeeding will carry a lower risk of transmission than will mixed breastfeeding.

Infants exclusively breastfed by women with CD4-cell counts less than 200 per μL were twice as likely to become infected and almost four times more likely to die before 6 months of age than were infants exclusively breastfed by women with CD4-cell counts above 500 per μL . Similar findings in west,³⁹ east,^{27,40} and southern Africa⁹ provide a strong argument for intensification of efforts to identify

this susceptible group and immediately offer them highly active antiretroviral treatment for the health of both mothers and infants. Where such programmes exist, the referral and counselling of pregnant women and initiation of treatment should be an over-riding priority that is closely monitored as an indicator of overall programme effectiveness.

The key policy finding of our study is the definite demonstration that early introduction of solid foods and animal milks increases HIV transmission risks compared with exclusive breastfeeding from birth. These data, together with evidence that exclusive breastfeeding can be supported in HIV-infected women, warrant revision of the present UNICEF, WHO, and UNAIDS infant feeding-guidelines that were last revised in 2000.³⁷ The need for this review is reinforced by the reported drawbacks of free formula milk^{41,42} and WHO recommendations for the provision of highly active antiretroviral therapy to pregnant women with CD4-cell counts lower than 200 per μL .⁴³

Study Steering Committee

Janet Darbyshire (chair), Nono Simelela (SA National Department of Health), Victoria Sithole (Community Advisory Board) and the study investigators.

Data Monitoring and Safety Committee

Cathy Wilfert (chair), Elizabeth Glaser (Pediatric AIDS foundation), Carl Lombard (statistician, Medical Research Council, South Africa), Ames Dhai (Department of Obstetrics and Gynaecology and the Biomedical Ethics Unit, University of KwaZulu-Natal, South Africa), and Francis Crawley (Good Clinical Practice Alliance).

Contributors

H M Coovadia and A Coutsooudis contributed to the conception of the study. H M Coovadia, N C Rollins, R M Bland, A Coutsooudis, M L Bennish, and M-L Newell designed and implemented the study. N C Rollins and R M Bland project managed the study. H M Coovadia, N C Rollins, A Coutsooudis, and M-L Newell drafted the report. N C Rollins, K Little, and M-L Newell analysed the data. R M Bland, K Little, and M L Bennish reviewed the report. All authors read and approved the final version of the report.

Conflict of interest statement

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