Morbidity in relation to feeding mode in African HIV-exposed, uninfected infants during the first 6 mo of life: the Kesho Bora study[1–6]

Kirsten A Bork, Amandine Cournil, Jennifer S Read, Marie-Louise Newell, Cécile Cames, Nicolas Meda, Stanley Luchters, Grace Mbatia, Kevindra Naidu, Philippe Gaillard, and Isabelle de Vincenzi

ABSTRACT

Background: Refraining from breastfeeding to prevent HIV transmission has been associated with increased morbidity and mortality in HIV-exposed African infants.

Objective: The objective was to assess risks of common and serious infectious morbidity by feeding mode in HIV-exposed, uninfected infants ≤6 mo of age with special attention to the issue of reverse causality.

Design: HIV-infected pregnant women from 5 sites in Burkina Faso, Kenya, and South Africa were enrolled in the prevention of mother-to-child transmission Kesho Bora trial and counseled to either breastfeed exclusively and cease by 6 mo postpartum or formula feed exclusively. Maternal-reported morbidity (fever, diarrhea, and vomiting) and serious infectious events (SIEs) (gastroenteritis and lower respiratory tract infections) were investigated for 751 infants for 2 age periods (0–2.9 and 3–6 mo) by using generalized linear mixed models with breastfeeding as a time-dependent variable and adjustment for study site, maternal education, economic level, and cotrimoxazole prophylaxis.

Results: Reported morbidity was not significantly higher in nonbreastfed compared with breastfed infants [OR: 1.31 (95% CI: 0.97, 1.75) and 1.21 (0.90, 1.62) at 0–2.9 and 3–6 mo of age, respectively]. Between 0 and 2.9 mo of age, never-breastfed infants had increased risks of morbidity compared with those of infants who were exclusively breastfed (OR: 1.49; 95% CI: 1.01, 2.2; $P = 0.042$). The adjusted excess risk of SIEs in nonbreastfed infants was large between 0 and 2.9 mo (OR: 6.0; 95% CI: 2.2, 16.4; $P = 0.001$). Between 3 and 6 mo, the OR for SIEs was sensitive to the timing of breastfeeding status, i.e., 4.3 (95% CI: 1.2, 15.3; $P = 0.02$) when defined at end of monthly intervals and 2.0 (95% CI: 0.8, 5.0; $P = 0.13$) when defined at the beginning of intervals. Of 52 SIEs, 3 mothers reported changes in feeding mode during the SIE although none of the mothers ceased breastfeeding completely.

Conclusions: Not breastfeeding was associated with increased risk of serious infections especially between 0 and 2.9 mo of age. The randomized controlled trial component of the Kesho Bora study was registered at Current Controlled Trials (www.controlled-trials.com) as ISRCTN71468401. *Am J Clin Nutr* 2014;100:1559–68.

Keywords Africa, HIV/AIDS, diarrhea, infant feeding, infections

INTRODUCTION

Since 1981, the WHO has emphasized the protection and promotion of breastfeeding in resource-limited settings (1) because of its well-documented positive effects on infant infectious morbidity and mortality (2–4). However, accumulating data regarding risk of mother-to-child transmission of HIV type 1 through breast milk (5, 6) and the expanding HIV epidemic prompted United Nations agencies to recommend the

[1] From the Institut de Recherche pour le Développement (IRD), UMI233 IRD/Université de Montpellier 1, Montpellier, France (KAB, AC, and CC); the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, Bethesda, MD (JSR); the Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Somkhele, South Africa (M-LN); the Centre Muraz, Bobo-Dioulasso, Burkina Faso (NM); the International Centre for Reproductive Health, Mombasa, Kenya (SL); the Kenyatta National Hospital and University of Nairobi, Nairobi, Kenya (GM); the University of KwaZulu-Natal, Durban, South African Republic (KN); and the WHO, Reproductive Health and Research, Geneva, Switzerland (PG and IdV).

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[5] Present address for JSR: Division of Infectious Diseases, Department of Pediatrics, George Washington University School of Medicine, Washington, DC; for M-LN: Faculty of Medicine, University of Southampton, Southampton, United Kingdom; and for SL: Burnet Institute, Melbourne, Australia.

[6] Address correspondence to K Bork, UMI233, Institut de Recherche pour le Développement/Université de Montpellier 1, BP 64501, 34394 Montpellier Cedex 5, France. E-mail: kirsten.bork@ird.fr.

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complete avoidance of breastfeeding by HIV-infected mothers when safe and acceptable or, in women who started breastfeeding, to practice exclusive breastfeeding (EBF)[7] and wean rapidly by 4–6 mo postpartum (7, 8). Subsequently, evidence of high mortality of nonbreastfed or early weaned infants born to HIV-infected mothers has been published (9–11) as have data on the protective effect of antiretroviral use by mothers or infants with respect to the breast-milk transmission of HIV (12–14). Therefore, it is now recommended that breastfeeding in HIV-infected women be continued throughout infancy while using antiretrovirals (15).

Although several studies have quantified differences in morbidity by feeding mode in HIV-exposed infants (16–19), the potential drawback of reverse causality (i.e., mothers changing feeding mode in response to infant illness) has not been considered to our knowledge, and some studies did not distinguish between HIV-infected and -uninfected infants. Also, most published data were collected before the era of the antiretroviral-based prevention of mother-to-child-transmission during pregnancy and postpartum.

The objective of this analysis was to test for an association between the infant feeding mode and infectious morbidity of HIV-exposed, uninfected infants during the first 6 mo of life in the Kesho Bora trial conducted in Burkina Faso, Kenya, and South Africa. We hypothesized that *1*) not breastfeeding would be associated with increased morbidity risks compared with ongoing breastfeeding also in analyses that minimized reverse causality, *2*) EBF would be associated with lower risks than predominant and partial breastfeeding, and *3*) excess risks would be greatest during the first months of life.

SUBJECTS AND METHODS

Kesho Bora study

The Kesho Bora trial was a randomized controlled trial (RCT) (20). Pregnant, HIV-infected women at <32 wk of gestation with WHO clinical stage 1, 2 or 3 disease and a CD4 count of 200–500 cells/mm^3 were eligible for enrollment. The triple-antiretroviral group received a combination of 3 antiretrovirals from 28 to 36 wk pregnancy up to 6.5 mo postpartum (or breastfeeding cessation if earlier). The standard prophylaxis group received zidovudine and single-dose nevirapine. All infants received a dose of nevirapine preferably within 72 h of birth.

The study was conducted in Bobo-Dioulasso, Burkina Faso, Nairobi and Mombasa, Kenya, and Durban and a rural area of KwaZulu-Natal, South Africa. Enrollment took place from January 2005 to August 2008. Ethical clearance was given by the ethical and regulatory committees in Burkina Faso, Kenya, and South Africa and at the WHO and the U.S. CDC. The study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983, and all women provided written informed consent.

Infant feeding counseling was conducted antenatally and after delivery until the complete cessation of breastfeeding following WHO guidelines. Women were counseled to either breastfeed with complete cessation by 6 mo of age or formula feed from birth. The importance of EBF was emphasized together with the modalities of the weaning process to be conducted either over 2 wk or within 1–2 d (in South African sites) in accordance with national guidelines. Demonstrations of formula preparations were performed, and free formula was provided for infants from birth until 6 mo of age as appropriate.

In Bobo Dioulasso, Mombasa, and Nairobi, cotrimoxazole prophylaxis was provided to all infants from the age of 6 wk and throughout infancy except in case of adverse events. In South Africa, prophylaxis was stopped before the age of 6 mo in never-breastfed and early weaned infants (if HIV-uninfected at the age of 6 wk). All medical care was provided free of charge.

Data collection

Maternal background data were collected at enrollment (i.e., during pregnancy). Infant sex and birth weight were collected at a neonatal visit (at <7 d after delivery) from hospital files (birth weight was missing for home deliveries). Postnatal visits were scheduled at 2, 4, 6, and 8 wk of age and, thereafter, monthly until 1 y of age. At each visit, data were collected on items fed to the infant since the preceding visit (since birth for the 2-wk visit). Infant feeding data were collected by an interviewer different from the infant feeding counselor (except in the Bobo-Dioulasso study site). In case of breastfeeding cessation since the preceding visit, the precise date of cessation was noted.

Interviewers also inquired about infant morbidity since the preceding visit by using the following symptoms (according to mothers' definitions): acute diarrhea, chronic diarrhea (duration ≥ 2 wk), vomiting, fever, convulsions, and, for sites in Burkina Faso and Kenya only, runny or blocked nose, cough or dyspnea, constipation, and ear problems. Reported symptoms, clinical signs, and laboratory abnormalities were graded according to the Division of AIDS/National Institute of Allergy and Infectious Diseases adverse event tables (21). Clinical events of grade ≥ 3 (i.e., events associated with death, admission to hospital, prolongation of hospital stay, significant disability or incapacity, or life threatening) were defined as serious adverse events (SAEs). The date of onset of symptoms and any changes in infant feeding as a consequence of these symptoms were noted. All SAEs were followed up until complete recovery or death, and often more than one report per episode was available. For infant deaths, the breastfeeding status (yes compared with not) at the time of death was recorded. In addition to scheduled visits, mothers were encouraged to bring their infants to the clinic in case of illness.

The HIV-infection status of all infants was assessed by using a quantitative RNA– polymerase chain reaction test at age 6 wk and 12 mo (22). For infants confirmed positive at age 12 mo (or at the last stored sample in case of death or loss to follow-up before then), samples collected at the following ages were tested to estimate the timing of infection (14): at birth, 2 and 6 wk, and 3, 6, and 9 mo.

Study population and definition of variables

Live-born singleton infants (or the first born of multiple-gestation pregnancies) who had a neonatal visit form completed and were not lost to follow-up during the first month of life were considered in the analysis. Infants diagnosed with HIV infection by 6 wk of age were not eligible for the analysis because of their

[7] Abbreviations used: EBF, exclusive breastfeeding; LRTI, lower respiratory tract infection; RCT, randomized controlled trial; SAE, serious adverse event; SIE, serious infectious event.

high morbidity/mortality risk and the well-known association of infectious morbidity and breastfeeding (11, 19, 23).

Common infectious morbidity was defined as maternal-defined fever, acute or chronic diarrhea, or vomiting. Because cough and dyspnea were not collected in all sites, these symptoms could not be included in the main analysis but were included in a subanalysis for Bobo Dioulasso, Mombasa, and Nairobi. Morbidity declared at the first postnatal visit (i.e., at 2 wk of age) was not included in the analysis. Serious infectious events (SIEs) were defined as SAEs that resulted from gastroenteritis or a lower respiratory tract infection (LRTI). Infant age at an SIE was defined as the date of onset of the SIE minus the date of birth divided by 30.4. When the date of onset of the SIE was missing, it was replaced by date of notification of the SIE. SIEs that started during the early neonatal period (i.e., before age 7 d) were excluded from the analysis because the direction of causality between the illness and feeding mode was uncertain. Only the first report of a given SIE was included. The risk set for SIEs was composed of infants not lost to follow-up at the end of the monthly interval under study, even if they had not attended the scheduled visit, whereas the risk set for recalled morbidity was restricted to infants present at scheduled visits.

Breastfeeding mode was coded as exclusive, predominant, or partial. EBF was defined as no other foods or fluids other than breast milk except for modern medicine and vitamin drops. Predominant breastfeeding included breast milk and other fluids (e.g., water, sugar water, tea, and juice), whereas partial breastfeeding included breast milk plus other foods including formula (24). A cross-sectional infant feeding category was created for each visit on the basis of data available for that visit. Thereafter, a longitudinal (i.e., since birth) variable was created for each visit on the basis of data available from all visits up to that age as described previously (25). In the remainder of the current article, breastfeeding categories refer to the longitudinal definition.

Infant feeding mode was defined for each monthly interval. We first used the binary variable breastfeeding yes compared with no, and, thereafter, a variable with 5 modalities as follows: never breastfeeding, weaned, and partially, predominantly, and exclusively breastfeeding. In the analyses, nonbreastfeeding infants refer to infants who were not breastfeeding during the interval under study irrespective of whether they were weaned or never breastfed. Because few women reported prolonged breastfeeding into the second half of infancy, the analysis was restricted to the first 6 mo of life.

An indicator of economic status was constructed by using a multiple-correspondence analysis of 8 household assets (electricity, refrigerator, radio, television, telephone, source of water, type of toilet, and type of fuel for cooking) and divided into 3 groups of increasing economic levels by using country-specific tertiles.

Statistical analysis

Risk factors for morbidity were assessed by using a generalized linear mixed model [xtmelogit command in Stata software (version 12.1, Stata Corp.)] that took into account the time-dependent nature of infant feeding and cotrimoxazole prophylaxis and the possibility of repeated morbidity episodes per age period. The analysis considered 2 age periods (0–2.9 and 3.0–6.0 mo) separately because the impact of infant feeding on morbidity is likely to be age dependent. For both periods, the analysis included 3 intervals (i.e., 0–0.9, 1.0–1.9, and 2.0–2.9 mo and 3.0–3.9, 4.0–4.9, and 5.0–6.0 mo, respectively). Hence, at most, 3 morbidity episodes could be considered for a given age period (i.e., one episode per interval). Subjects were censored at the earliest event in the following: loss to follow-up, age 6 mo, and, for those who were HIV infected by 12 mo of age, at their last negative polymerase chain reaction test.

The dependent variable was infant morbidity of either reported morbidity or an SIE. For the former, a sensitivity analysis was conducted including cough/respiratory difficulties in the definition of morbidity for the 3 study sites in which these symptoms were collected (Bobo Dioulasso, Mombasa, and Nairobi) and the entire 0–6-mo age interval. For the latter, a sensitivity analysis was conducted after the exclusion of SIEs that led to infant death within the same month to make the analysis of morbidity independent of previously reported mortality (26). Because of the limited number of events, the 0–6-mo age interval was used for the latter analysis, and it was not possible to analyze lethal events independently.

The main independent variable was the infant feeding mode either as a binary variable (breastfeeding yes compared with no) or a 5-category variable, both of which were defined at the end of each monthly interval. However, for the analysis of SIEs, predominant and partial breastfeeding were pooled into one category of non-EBF because of a lack of events in some subgroups, and infants who died of gastroenteritis or LRTI during the interval were assigned the feeding mode defined at their last visit. Sensitivity analyses used the feeding mode before the onset of an SIE to reduce risk of reverse causality between a SIE and breastfeeding (binary variable only). First, breastfeeding status was defined at the beginning of the monthly interval in which SIEs were reported to begin and, thereafter, at 7 d before the reported onset of SIEs. Other independent variables were child sex, intervention arm, low birth weight ($<$2500 g), delivery mode (vaginal, emergency cesarean, and elective cesarean), maternal education (primary, secondary, and none), professional activity (any compared with none), CD4 cell count at delivery ($\leq$350 and $>$350/mm^3), economic level of the household, and cotrimoxazole prophylaxis (time dependent: any compared with none since the last visit). Missing values (for birth weight and CD4 cell count at delivery) were considered as specific modalities in the analysis. Factors were included in multivariate models if they were associated with morbidity with a P value <0.20 in univariate regression analyses for at least one of the 2 age intervals under study. The age group and study site were adjusted for in all analyses. The issue of reverse causality was also dealt with by using a direct approach whereby maternal reports of changes in feeding mode during SIEs were investigated including all reports of a given SIE.

Chi-square tests were used for the comparison of categorical variables across feeding categories and study sites. SAS (version 9.3; SAS Institute) and Stata (version 12.1; Stata Corp.) statistical software programs were used for the analyses, and tests were considered significant at $P < 0.05$.

RESULTS

Size and characteristics of study population

In 805 live-born infants of women enrolled in the Kesho Bora RCT, 6 infants died, 2 infants were lost to follow-up during the

first week of life, 8 infants moved or withdrew before 1 mo of age, and an additional 2 infants died between ages 1 wk and 1 mo (from an illness different from diarrhea and an LRTI). Thirty-six infants were diagnosed as HIV infected by 6 wk of age. Therefore, the analysis presented here comprised 751 infants (366 boys and 385 girls).

Characteristics of the study population are shown in **Table 1**. The socioeconomic level of mothers was generally low, albeit with important variations across study sites. Burkinabe mothers had the lowest levels of education and access to piped water and the highest rate of low-birth-weight infants. Conversely, South African mothers had the highest level of education and access to electricity. Breastfeeding initiation rates were greatest in Bobo Dioulasso and lowest in Durban (Table 1), but from 4 to 6 mo of age, breastfeeding was least frequent in Kenyan sites (78%, 32%, 33%, 41%, and 53% for Bobo-Dioulasso, Mombasa, Nairobi, Durban, and rural KwaZulu-Natal, respectively, at 4 mo of age; $P < 0.001$). The duration of cotrimoxazole prophylaxis also varied by study site; in Durban and rural South Africa, cotrimoxazole prophylaxis was discontinued by 4 mo of age for 95% of never-breastfeeding infants, whereas in the 3 other sites, virtually all infants still received prophylaxis at 6 mo of age irrespective of the infant feeding mode (results not shown).

Prevalence of morbidity

The average prevalence of reported infant morbidity (fever, acute or chronic diarrhea, and vomiting) was 179 and 241 events/1000 infant-months for infants 0–2.9 and 3–6 mo of age, respectively (**Table 2**). With consideration of the 2 most frequent symptoms independently, the prevalence was 135 compared with 169 events for fever and 49 compared with 98 events for acute diarrhea for the 2 age intervals, respectively.

When cough and dyspnea were added to the morbidity definition, the prevalence rose from 214 and 280 events/1000 infant-months for the 2 age intervals, respectively, to 316 and 412 events/1000 infant-months, respectively, for Burkinabe and Kenyan infants.

TABLE 1
Characteristics of infants and their mothers in the Kesho Bora RCT by study site[1]

Variables and modalities	Bobo Dioulasso, Burkina Faso (n = 225)	Mombasa, Kenya (n = 219)	Nairobi, Kenya (n = 41)	Durban, South Africa (n = 177)	Rural KwaZulu, South Africa (n = 89)	P-differences across sites	All sites (n = 751)
Maternal education						<0.001	
None	92 (40.9)	12 (5.6)	2 (4.9)	1 (0.6)	4 (4.5)		111 (14.8)
Primary	75 (33.3)	111 (50.7)	23 (56.1)	29 (16.4)	17 (19.1)		255 (34.0)
Secondary	58 (25.8)	96 (43.8)	16 (39.0)	147 (83.0)	68 (76.4)		385 (51.3)
Maternal occupation						<0.05	
Yes	71 (31.6)	74 (33.8)	6 (14.7)	61 (34.5)	19 (21.4)		231 (30.8)
No	154 (68.4)	145 (66.2)	35 (85.4)	116 (65.5)	70 (78.7)		520 (69.2)
Piped water						<0.001	
Yes	64 (28.5)	118 (53.9)	17 (41.5)	132 (74.6)	66 (74.2)		397 (52.9)
No	161 (71.6)	101 (46.1)	24 (58.5)	45 (25.4)	23 (25.8)		354 (47.1)
Flush toilet						<0.001	20 (22.5)
Yes	7 (3.1)	55 (25.1)	15 (36.6)	139 (78.5)			236 (31.4)
No	218 (96.9)	164 (74.9)	26 (63.4)	38 (21.5)	69 (77.5)		515 (68.6)
Electricity						<0.001	
Yes	133 (60.2)	122 (56.7)	20 (48.8)	151 (87.8)	69 (80.2)		505 (67.2)
No	88 (39.8)	93 (43.3)	21 (51.2)	21 (12.2)	17 (19.8)		246 (32.8)
Refrigerator						<0.001	
Yes	30 (13.3)	27 (12.3)	1 (2.4)	109 (61.6)	66 (74.2)		233 (31.0)
No	195 (86.7)	192 (87.7)	40 (97.6)	68 (38.4)	23 (25.8)		518 (69.0)
Mode of delivery						<0.001	
CS, elective	1 (0.4)	9 (4.1)	2 (4.9)	13 (7.3)	5 (5.6)		30 (4.0)
CS, emergency	6 (2.7)	7 (3.2)	10 (24.4)	27 (15.3)	9 (10.1)		59 (7.9)
Vaginal	218 (96.9)	203 (92.7)	29 (70.7)	137 (77.4)	75 (84.3)		662 (88.1)
Birth weight (g)						<0.001*	
<2500	35 (15.6)	8 (3.7)	5 (7.6)	12 (6.8)	4 (4.5)		64 (8.5)
≥2500	179 (79.5)	191 (87.2)	34 (82.9)	164 (92.7)	83 (93.3)		651 (86.7)
Missing	11 (4.9)	20 (9.1)	2 (4.9)	1 (0.6)	2 (2.3)		36 (4.8)
CD4 at delivery (/mm^3)						<0.001*	
<350	47 (20.9)	32 (14.6)	17 (41.5)	51 (28.8)	23 (25.8)		170 (22.6)
350–500	74 (32.9)	78 (35.6)	12 (29.3)	77 (43.5)	34 (38.2)		275 (36.6)
>500	84 (37.3)	93 (42.5)	8 (19.5)	46 (26.0)	26 (29.2)		257 (34.2)
Missing	20 (8.9)	16 (7.3)	4 (9.8)	3 (1.7)	6 (6.7)		49 (6.5)
Ever breastfeeding						<0.001	
Yes	210 (93.3)	171 (78.1)	31 (75.6)	101 (57.1)	72 (80.9)		585 (77.9)
No	15 (6.7)	48 (21.9)	10 (24.4)	76 (42.9)	17 (19.1)		166 (22.1)

[1]Values are n (%). *Chi-square test was performed without missing values. CS, cesarean delivery; RCT, randomized controlled trial.

TABLE 2
Rate of common infectious morbidity and selected clinical SIEs during the first 6 mo of life in African HIV-exposed, uninfected infants in the Kesho Bora RCT by infant feeding mode[1]

	Recalled infectious morbidity			Clinical SIEs		
Age interval and modality	Exposure, infant-months	Events	Rate/1000 infant-months	Exposure, infant-months	Events (deaths)	Rate/1000 infant-months
0–2.9 mo	2107	378	179	2206	27 (9)	12.2
Breastfeeding						
Yes	717	143	199	779	21 (7)	27.0
No	1390	235	169	1427	6 (2)	4.2
Mode						
Never breastfeeding	469	87	186	488	15 (5)	30.7
Weaned	247	56	227	291	6 (2)	20.6
Partially breastfeeding	185	46	249	151	1 (0)	6.6
Predominantly breastfeeding	363	63	174	387	2 (0)	5.2
EBF	843	131	15.5	889	3 (2)	3.4
3.0–6.0 mo	1972	476	241	2098	25 (9)	11.9
Breastfeeding						
Yes	1207	327	271	1304	22 (8)	16.9
No	765	149	195	794	3 (1)	3.8
Mode						
Never breastfeeding	432	109	252	467	4 (1)	8.6
Weaned	775	218	281	837	18 (7)	21.5
Predominant breastfeeding	319	61	191	211	1 (1)	4.7
Partial breastfeeding	170	31	182	230	0	0
EBF	276	57	207	353	2 (0)	5.7

[1]Infectious morbidity comprised all episodes of diarrhea, fever, and vomiting. SIEs comprised all diarrheal and lower respiratory disease episodes; early neonatal events (from 0 to 7 d of age) were not included. EBF, exclusive breastfeeding; RCT, randomized controlled trial; SIE, serious infectious event.

There were 27 events of severe morbidity (including 9 deaths) between 0 and 2.9 mo of age and 25 events of severe morbidity (including 9 deaths) between 3 and 6 mo of age or 12.2 and 11.9 events/1000 infant-months of exposure, respectively. Twenty-four infants suffered from gastroenteritis, and 28 infants suffered from an LRTI; 31 infants (59%) were hospitalized.

The intervention arm (i.e., triple antiretroviral compared with zidovudine and single-dose nevirapine) was not associated with either reported morbidity (180 compared with 178 and 247 compared with 236 events/1000 infant-months between 0 and 2.9 and 3 and 6 mo of age, respectively; $P > 0.20$ for both) or SIE (11.6 compared with 12.9 and 13.1 compared with 10.7 events/1000 infant-months between 0 and 2.9 and 3 and 6 mo of age, respectively; $P > 0.20$). Similarly, maternal CD4 cell count at delivery was not associated with infant morbidity ($P > 0.20$; data not shown). Thus, these variables were not included in subsequent analyses.

Infant feeding mode as a risk factor for reported morbidity

Nonbreastfeeding infants tended to have greater adjusted risk of maternal-reported morbidity (diarrhea, fever, or vomiting) between 0 and 2.9 mo of age as did infants in the lowest economic tertile, but excess risks were not significant (**Table 3**). However, never-breastfeeding infants had increased risks compared with those of infants who were exclusively breastfed ($P = 0.04$). Between 3 and 6 mo, no significant risk factors were identified. Cotrimoxazole prophylaxis and the level of maternal education were not independently associated with morbidity in either age interval.

For the entire 0–6-mo age interval, nonbreastfeeding infants tended to have greater morbidity risks than did breastfeeding infants (OR: 1.22; 95% CI: 0.99, 1.50; $P = 0.058$).

When acute diarrhea was analyzed independently, nonbreastfeeding infants had increased risks between 0 and 2.9 mo of age compared with those of breastfeeding infants (OR: 1.56; 95% CI: 1.01, 2.40; $P = 0.043$) as did infants who were never breastfed compared with EBF infants (OR: 2.14; 95% CI: 1.23, 3.73; $P < 0.01$). Between 3 and 6 mo of age, no significant differences were shown for either nonbreastfeeding infants (OR: 1.34; 95% CI: 0.89, 2.02; $P = 0.17$] or weaned or never-breastfeeding infants (data not shown). Between 0 and 6 mo of age, acute diarrhea was increased in nonbreastfeeding infants (OR: 1.40; 95% CI: 1.05, 1.88; $P = 0.024$) but not in those weaned or never breastfeeding (data not shown).

Fever was not independently associated with nonbreastfeeding in either age interval [OR: 1.29 (95% CI: 0.91, 1.81; $P = 0.15$) and 1.19 (0.68, 2.32; $P > 0.20$) between 0 and 2.9 and 3 and 6 mo of age, respectively] or from 0 to 6 mo of age (data not shown). However, between 0 and 2.9 mo of age, infants who were partially breastfed or weaned tended to have higher risks than EBF infants did [OR: 1.63 (95% CI: 0.97, 2.73; $P = 0.066$) and 1.57 (95% CI: 0.96, 2.55; $P = 0.072$), respectively].

In a subanalysis including cough and dyspnea for 3 of 5 study sites, excess risk for the 0–6-mo age interval was 1.28 (95% CI: 1.02, 1.61; $P = 0.035$) in nonbreastfeeding infants and 1.46 (95% CI: 1.00, 2.11; $P = 0.048$) in never-breastfeeding infants compared with exclusively breastfeeding infants.

Infant feeding mode as a risk factor for SIEs

Between 0 and 2.9 mo of age, not being breastfed was associated with 6-fold excess risk of experiencing at least one SIE ($P = 0.001$; **Table 4**). Infants who never breastfed had 9-fold

TABLE 3
Risk factors of recalled morbidity in HIV-exposed, uninfected African infants in the Kesho Bora RCT by using generalized linear mixed models[1]

	Model 1 (breastfeeding as binary variable)				Model 2 (5 modalities of infant feeding mode)			
	0–2.9 mo of age		3–6 mo of age		0–2.9 mo of age		3–6 mo of age	
	Values	*P*	Values	*P*	Values	*P*	Values	*P*
Breastfeeding status								
Not breastfeeding	1.31 (0.97, 1.75)	0.075	1.21 (0.90, 1.62)	0.20	—	—	—	—
Breastfeeding	1	—	1	—	—	—	—	—
Feeding mode								
PBF	—	—	—	—	0.99 (0.67, 1.47)	>0.20	0.74 (0.43, 1.27)	>0.20
Partial breastfeeding	—	—	—	—	1.41 (0.89, 2.24)	0.15	0.73 (0.47, 1.15)	0.17
Weaned	—	—	—	—	1.29 (0.84, 1.99)	>0.20	0.95 (0.62, 1.44)	>0.20
Never breastfeeding	—	—	—	—	1.49 (1.01, 2.19)	0.042	1.03 (0.64, 1.67)	>0.20
EBF	—	—	—	—	1	—	1	—
Cotrimoxazole								
Yes	0.85 (0.48, 1.50)	>0.20	0.75 (0.50, 1.12)	0.16	0.88 (0.50, 1.56)	>0.20	0.74 (0.48, 1.13)	0.17
No	1		1	—	1	—	1	—
Maternal education								
None	0.92 (0.57, 1.49)	>0.20	1.06 (0.69, 1.63)	>0.20	0.94 (0.58, 1.52)	>0.20	1.06 (0.69, 1.64)	>0.20
Primary	1.27 (0.92, 1.75)	0.15	1.27 (0.95, 1.69)	0.10	1.28 (0.93, 1.78)	0.13	1.27 (0.95, 1.69)	0.10
Secondary	1	—	1	—	1	—	1	—
Economic level								
First tertile	1.35 (0.96, 1.89)	0.086	1.07 (0.79, 1.44)	>0.20	1.35 (0.96, 1.90)	0.082	1.06 (0.79, 1.43)	>0.20
Second tertile	1.06 (0.75, 1.51)	>0.20	1.07 (0.79, 1.44)	>0.20	1.06 (0.75, 1.50)	>0.20	1.07 (0.79, 1.44)	>0.20
Third tertile	1	—	1	—	1	—	1	—

[1]All values are ORs; 95% CIs in parentheses. Infectious morbidity comprised all episodes of diarrhea, fever, and vomiting. Infant feeding mode and cotrimoxazole prophylaxis were time-dependent variables, defined at the end of each monthly interval. Events during the first 2 wk of life were not included. Model 1 included breastfeeding mode as a binary variable, whereas model 2 used infant feeding mode as a 5-modality variable. Both models were adjusted for study site and age interval. EBF, exclusive breastfeeding; PBF, predominant breastfeeding; RCT, randomized controlled trial.

greater risks, and infants weaned tended to have greater risks than exclusively breastfeeding infants did [OR of 8.8 (95% CI: 2.3, 33.8; $P = 0.001$) and OR of 4.3 (0.97, 19.2; $P = 0.056$), respectively]. Infants in the lowest economic tertile also had increased risks ($P = 0.03$), whereas maternal education was not independently associated with an SIE (Table 4). Between 3 and 6 mo of age, not being breastfed was associated with 4-fold higher risk of SIEs ($P = 0.02$; Table 4). Primary level maternal education was associated with a significantly increased OR compared with that for secondary level maternal education.

Between 0 and 6 mo, the OR for nonbreastfeeding infants was 5.3 (95% CI: 2.42, 11.8; $P < 0.001$), and no other variables were independently associated with an SIE. There was no interaction between the age period and breastfeeding status ($P = 0.20$).

When infant feeding was analyzed as a 4-modality variable, non-EBF (i.e., predominant and partial breastfeeding pooled) was not significantly associated with risk of SIEs compared with for EBF (Table 4). During the first 3 mo of life, never-breastfeeding infants had 9-fold increased risk of an SIE ($P < 0.01$), and weaned infants had 4-fold (nonsignificant) excess risk. Between 3 and 6 mo of age, neither weaned nor never-breastfeeding infants had significantly increased risks of an SIE (Table 4).

Sensitivity analyses that used breastfeeding status at the onset of each interval, instead of at the end provided similar ORs of SIEs associated with not breastfeeding between 0 and 2.9 mo of age (7.5; 95% CI: 2.7, 20.9; $P < 0.001$) but lower and nonsignificant excess risks between 3 and 6 mo of age (2.0; 95% CI: 0.8, 5.0; $P = 0.13$). The interaction between age and breastfeeding status was significant in analyses of the entire 0–6-mo age period ($P = 0.048$; data not shown).

Breastfeeding status at 7 d of age before SIEs did not differ from that defined at the onset of intervals between 0 and 2.9 mo of age, whereas the OR between 3 and 6 mo of age was 3.2 (95% CI: 1.2, 8.6; $P = 0.02$).

When lethal events ($n = 18$) were excluded from the analysis, nonbreastfeeding infants still had significantly greater risks of an SIE than did their breastfed counterparts between 0 and 6 mo of age (OR: 4.0; 95% CI: 1.6, 10.1; $P = 0.004$), and this excess risk was similar for never-breastfeeding and weaned infants compared with for infants who were exclusively breastfeeding [OR: 4.5 (95% CI: 1.2, 16.3; $P = 0.023$) and 4.3 (95% CI: 1.1, 16.2; $P = 0.033$) respectively]. No excess risk was shown for infants who were predominantly or partially breastfeeding [OR: 1.6 (95% CI: 0.3, 9.6) and 0.9 (95% CI: 0.1, 8.6), respectively].

Qualitative evidence of reverse causality between breastfeeding and SIE

More than 94% of mothers (49 of 52) reported not having changed the feeding mode as a consequence of SIEs. Two mothers reported changes in the feeding of their neonate during an SIE whereby one mother had to formula feed temporarily during hospitalization (thereby shifting to partial breastfeeding) and the other mother had stopped breastfeeding briefly without giving formula. A third mother stopped formula feeding temporarily in response to infant diarrhea at 3.5 mo postpartum. The exclusion of these infants did not change the conclusions of the analyses (results not shown).

TABLE 4
Risk factors of SIEs in HIV-exposed, uninfected African infants in the Kesho Bora RCT by using generalized linear mixed models[1]

	Model 1				Model 2			
	0–2.9 mo of age		3–6 mo of age		0–2.9 mo of age		3–6 mo of age	
	Values	*P*	Values	*P*	Values	*P*	Values	*P*
Breastfeeding status								
Not breastfeeding	6.0 (2.2, 16.4)	0.001	4.3 (1.2, 15.3)	0.024	—	—	—	—
Breastfeeding	1	—	1	—	—	—	—	—
Feeding mode								
Non-EBF	—	—	—	—	1.3 (0.3, 7.2)	>0.20	0.4 (0.1, 4.9)	>0.20
Weaned	—	—	—	—	4.3 (0.97, 19.2)	0.056	3.8 (0.8, 18.2)	0.092
Never breastfeeding	—	—	—	—	8.8 (2.3, 33.8)	0.001	1.6 (0.3, 9.0)	>0.20
EBF	—	—	—	—	1	—	1	—
Maternal education								
None	1.2 (0.2, 6.9)	>0.20	1.4 (0.2, 7.8)	>0.20	1.3 (0.2, 7.3)	>0.20	1.3 (0.2, 7.7)	>0.20
Primary	0.8 (0.3, 2.2)	>0.20	2.9 (1.1, 7.6)	0.028	0.9 (0.3, 2.3)	>0.20	2.8 (1.1, 7.3)	0.037
Secondary	1	—	1	—	1	—	1	—
Economic level								
First tertile	3.6 (1.1, 11.8)	0.03	0.8 (0.3, 2.5)	>0.20	3.6 (1.1, 11.5)	0.031	0.8 (0.3, 2.3)	>0.20
Second tertile	2.1 (0.6, 7.0)	>0.20	1.8 (0.7, 4.7)	>0.20	2.0 (0.6, 7.1)	>0.20	1.7 (0.6, 4.5)	>0.20
Third tertile	1	—	1	—	1	—	1	—

[1]All values are ORs; 95% CIs in parentheses. SIEs comprised all diarrheal and lower respiratory tract infection episodes; events during the early neonatal period (from 0 to 7 d of age) were not included. Model 1 included breastfeeding mode as a binary variable, and model 2 included infant feeding mode as a 4-modality variable. Non-EBF comprised predominant and partial breastfeeding because of an insufficient number of events in some subgroups. Breastfeeding mode was a time-dependent variable defined for monthly intervals and since birth on the basis of all visit reports up to the end of the time interval. Both models were adjusted for study site and age group. EBF, exclusive breastfeeding; RCT, randomized controlled trial; SIE, serious infectious event.

DISCUSSION

This analysis of infectious morbidity in 751 HIV-exposed, uninfected African infants showed that the absence of breastfeeding was associated with only slightly increased risks of maternal-reported morbidity and 4- to 6-fold greater risks of SIEs in the primary analysis. However, between 3 and 6 mo of age, results were less consistent in that excess risk of SIEs did not reach statistical significance in 1 of 2 sensitivity analyses.

In a recent analysis of mortality in the first 18 mo of age in the same population, nonbreastfeeding children had 7-fold greater mortality than that of their breastfeeding counterparts, and this difference arose mainly from the 0–6-mo age interval (26). In the current report, HIV-infected infants were not included in the analysis, and excess risk remained when SIEs leading to death were excluded. Taken together, these results strongly suggest that serious morbidity and mortality are more breastfeeding dependent than are less-severe forms. In this way, the results differ from those of other studies of HIV-exposed infants. In Tanzania, full breastfeeding (i.e., exclusive or predominant as compared with partial or none) was associated with a strong reduction in incidences of reported cough, acute diarrhea, fever, and outpatient visits between 0 and 6 mo of age but not of hospitalizations (27). In a clinic in South Africa, the incidence rate ratio of clinically diagnosed diarrhea was greatly reduced in 0–3-mo-old infants if they were breastfed (28). However, both of these analyses included both HIV-uninfected and -infected infants, and the latter infants are known to be very vulnerable to the absence of breastfeeding. In Kenyan uninfected HIV-exposed infants, the protection provided by breastfeeding was also greater for all pneumonia cases than pneumonia-related hospitalization (29).

Breast milk contains numerous specific and nonspecific immunologic agents (30, 31) that protect against infection, particularly gastroenteritis and pneumonia (32–34). The biological pathways through which breastfeeding might protect against the negative outcomes of acquired infectious disease include *1*) a better nutritional status, which enhances immunologic defenses and buffers weight loss, *2*) the frequent suckling pattern practiced in less-developed countries (35), which prevents dehydration and compensates for electrolytic loss caused by increased fecal losses and high fever, and *3*) the maintenance of infant desire for breastfeeding during illness when anorexia for solid and semisolid foods is frequently observed (36). This advantage of breastfeeding might also exist compared with formula.

We investigated the existence of so-called reverse causality (i.e., when the mother changes feeding mode in response to a morbidity episode). We showed very limited direct evidence of such behavior in that few mothers reported to have made changes in infant feeding because of an SIE. As mentioned previously, defining breastfeeding at the onset of monthly intervals, instead of at the end, led to lower, nonsignificant adjusted excess risks of an SIE for nonbreastfeeding infants in the 3–6-mo age interval. This result might be explained by the following 2 phenomena: either a significant part of the excess risk was actually due to reverse causality (i.e., mothers who stopped breastfeeding in response to infant illness) or a significant proportion of SIEs occurred shortly after breastfeeding cessation (i.e., within the same month).

With consideration that significant excess risk of SIEs was obtained when breastfeeding status was defined at age 7 d before the reported onset of the SIEs, it seems unlikely that the relation is explained by stopping breastfeeding in response to concomitant illness. However, we could not exclude that some mothers stopped breastfeeding because they perceived their infants as frail, perhaps in response to earlier illness episodes. Indeed, in a previous article, we showed that, in infants breastfed by 1 mo of age, those whose mothers reported an illness episode during neonatal life were more likely to be weaned between 1 and 3 mo of age (25). In that context, it is reassuring that, in the previously mentioned publication on the relation between feeding mode and mortality risk up to age 18 mo, sensitivity analyses accounting for previous (reported) morbidity showed that such morbidity had little effect on estimated excess risks (26).

The current study had several strengths. First, particular attention was paid to ruling out reverse causality. All mothers were asked whether they had made changes in the infant feeding mode since the onset of an SIE, and sensitivity analyses were conducted to assess whether associations with nonbreastfeeding were maintained beyond the weeks after weaning. Second, the analytic approach enabled us to take into account the occurrence of several events per child within each 3-mo period (i.e., $\leq$1 event/mo) while making optimal use of frequently collected data on infant feeding and its time dependency.

Some weaknesses are also worth mentioning. Reported morbidity data may have been affected by reporting bias in that nonbreastfeeding mothers might have been more anxious during infant-illness episodes and, therefore, more likely to report them than were breastfeeding women. SIEs were probably much-less sensitive to reporting bias because very precise standardized definitions were used. Infant feeding data were also potentially subject to reporting bias in that breastfeeding mothers were strongly advised to practice EBF, and some mothers might have avoided reporting non–breast-milk fluids or foods consumed by their infants. The advice to EBF also meant that we had limited statistical power to estimate ORs associated with partial and predominant breastfeeding.

In conclusion, reported morbidity overall differed only slightly by breastfeeding status at $\leq$6 mo of age, but nonbreastfeeding was associated with substantially increased risk of serious infectious morbidity in HIV-exposed uninfected infants living in 5 culturally and socioeconomically diverse settings in sub-Saharan Africa. We showed no clear evidence of reverse causality. Strong excess risk of SIEs associated with not breastfeeding is worrying because of the considerable human and technical resources invested in this RCT in mainly urban study clinics. Fatality rates associated with serious morbidity are likely to be higher in programmatic settings.

Members of the Kesho Bora Study Group are listed in **Appendix A**.

The authors' responsibilities were as follows—IdV, PG, JSR, NM, CC, and KAB: designed the research; SL, KN, GM, NM, M-LN, IdV, PG, and CC: conducted the research; KAB and AC: analyzed data; KAB: wrote and had primary responsibility for the final content of the manuscript; AC, M-LN, and JSR: provided input on the writing of the manuscript; and all authors: commented on earlier drafts and read and approved the final version of the manuscript. None of the authors reported a conflict of interest.

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APPENDIX A

THE KESHO BORA STUDY GROUP

Study sites

1) Bobo Dioulasso, Burkina Faso (Centre Muraz): Nicolas Meda (principal investigator), Paulin Fao, Odette Ky-Zerbo, Clarisse Gouem (study coordinators), Paulin Somda, Hervé Hien, Patrice Elysée Ouedraogo, Dramane Kania, Armande Sanou, Ida Ayassou Kossiwavi, Bintou Sanogo, Moussa Ouedraogo, Issa Siribie (investigators), Diane Valéa (Laboratory Coordinator), Sayouba Ouedraogo and Roseline Somé (data managers), and François Rouet (intersite laboratory coordination).

2) Durban, South Africa (University of KwaZulu Natal): Nigel Rollins (principal investigator), Lynne McFetridge, and Kevi Naidu (study coordinators).

3) Mombasa, Kenya (International Centre for Reproductive Health): Stanley Luchters, Marcel Reyners (principal investigators), Eunice Irungu (study coordinator), Christine Katingima, Mary Mwaura and Gina Ouattara (investigators), Kishor Mandaliya, Sammy Wambua (laboratory coordinators), and Mary Thiongo (data manager).

4) Nairobi, Kenya (Network for AIDS Researchers in East and Southern Africa): Ruth Nduati (principal investigator), Judith Kose (study coordinator), Ephantus Njagi (laboratory coordinator), and Peter Mwaura (data manager).

5) Somkhele, South Africa (Africa Centre for Health and Population Studies, University of KwaZulu Natal): Marie-Louise Newell (principal investigator), Stephen Mepham (study coordinator), Johannes Viljoen (laboratory coordinator), Ruth Bland (investigator), and Londiwe Mthethwa (data manager).

Supporting institutions

1) Agence Nationale de Recherches sur le SIDA et les hépatites virales, France: Brigitte Bazin and Claire Rekacewicz (sponsor representatives).

2) CDC, United States: Allan Taylor (sponsor representative and co-investigator) and Nicole Flowers, Michael Thigpen, Mary Glenn Fowler, and Denise Jamieson (co-investigators).

3) *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, United States: Lynne M Mofenson (sponsor representative) and Jennifer S Read (co-investigator).

4) Institut de Recherche pour le Développement, Montpellier, France: Kirsten Bork, Cécile Cames, and Amandine Cournil (nutrition coordination).

5) International Centre for Reproductive Health, Ghent University, Ghent, Belgium: Patricia Claeys, Marleen Temmerman, and Stanley Luchters (sponsor representatives);

6) Université Montpellier 1, EA 4205 "Transmission, Pathogenèse et Prévention de l'infection par le VIH"; and

Centre Hospitalier Universitaire de Montpellier, Laboratoire de Bactériologie-Virologie, Montpellier, France: Philippe Van de Perre, Pierre Becquart (until December 2006), Vincent Foulongne, and Michel Segondy (laboratory coordination).

Study coordination

WHO, Geneva, Switzerland: Isabelle de Vincenzi (study coordinator), Philippe Gaillard (site coordinator), Tim Farley (project manager), Ndema Habib (study statistician), and Sihem Landoulsi (study analyst).