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A Prospective Cohort Study of Common Childhood Infections in South African HIV-exposed Uninfected and HIV-unexposed Infants

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Background: Much evidence of HIV-exposed uninfected (HEU) infant infectious morbidity predates availability of maternal combination antiretroviral therapy and does not control for universal risk factors (preterm birth, low birth weight, suboptimal breastfeeding and poverty).

Methods: This prospective cohort study identified HIV-infected and HIV-uninfected mothers and their newborns from South African community midwife unit. The primary outcome, infectious cause hospitalization or death before 6 months of age, was compared between HEU and HIV-unexposed (HU) infants and classified for type and severity using validated study-specific case definitions. Adjusted odds ratios (aORs) were calculated by logistic regression including stratified analyses conditioned on breastfeeding.

Results: One hundred and seventy-six (94 HEU and 82 HU) mother–infant pairs were analyzed. HIV-infected mothers were older (median, 27.8 vs. 24.7 years; $P < 0.01$) and HU infants more often breastfed (81/82 vs. 35/94; $P < 0.001$). Groups were similar for maternal education, antenatal course, household characteristics, birth weight, gestational age and immunizations. The primary outcome occurred in 17 (18%) HEU and 10 (12%) HU infants [aOR, 1.45; 95% confidence interval (CI): 0.44–4.55]. In stratified analysis restricted to breastfed infants, the aOR for hospitalization due to very severe infection or death was 4.2 (95% CI: 1.00–19.2; $P = 0.05$) for HEU infants. Hospitalization for diarrhea was more common in HEU than HU infants [8/94 (8.5%) vs. 1/82 (1.2%); $P = 0.04$].

Conclusion: The difference between HEU and HU infants in the probability of infectious cause hospitalization or death in the first 6 months of life was not significant. However, among breastfed infants, severe infectious morbidity occurred more often in HEU than HU infants.

Key Words: HIV-exposed uninfected, HIV unexposed, infants, infectious morbidity, South Africa

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Thirty percent of pregnant South African women are HIV-infected.[1] As a result of an effective public vertical transmission prevention programme, 97% of the 300,000 HIV-exposed South African infants born annually are HIV-uninfected at 6 weeks of age.[2,3] The existing evidence, largely from the pre-antiretroviral therapy (ART) era, suggests that HIV-exposed but uninfected (HEU) infants experience greater infectious morbidity and mortality than HIV-unexposed (HU) infants.[4–19] Southern African HEU infants experience the same universal risk factors for infectious morbidity as HU infants. There is evidence that some of these universal risk factors occur more often in HEU than HU infants, specifically poor birth outcomes (preterm birth, small for gestational age and low birth weight), suboptimal breastfeeding, maternal mortality and poverty.[20–25] Additionally, HEU infants experience unique exposures that may set them on an altered biologic trajectory to HU infants. These include an in utero environment perturbed by exposure to HIV, maternal immune compromise and antiretroviral drugs.[26–33]

Irrespective of HIV exposure, the majority of South African infants are vulnerable to high rates of infectious morbidity and mortality.[34] Considering the size of the South African HEU infant population, a moderate additional increase in morbidity adds to the burden on the public healthcare sector. This study sought primarily to determine whether HEU infants experience a higher probability of infectious cause hospitalizations or death compared with HU infants in the first 6 months of life. A central assumption was that universal infant risk factors, specifically socioeconomic position, poor birth outcomes and suboptimal breastfeeding, do not account for all of the differences between HEU and HU infants, and that HEU-unique exposures play a role. In addition, we sought to understand the severity of infections experienced.

METHODS

The Mother Infant Health Study was a prospective cohort study of HEU and HU infants in Kraaifontein, South Africa. From July 2012 to June 2013, HIV-infected and HIV-uninfected mothers and their newborns were enrolled from a single community midwife obstetric unit providing care for low-risk pregnancies. Eligibility was restricted to mothers from 4 well-defined low socioeconomic neighbourhoods and newborns with gestation ≥34 weeks and birth weight >2000 g. HIV-infected and HIV-uninfected mothers were frequency

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matched on race/ethnicity to limit between group variation in smoking and alcohol use. HU infants were matched to HIV-exposed infants with the closest date of birth, no more than 30 days apart, to control for seasonal infectious morbidity. HIV-infected infants were withdrawn and referred for HIV treatment. The South African National HIV Programme provides free care to all HIV-infected people.[35] At the time of the study, this included combination ART (cART) for adults including pregnant women with cluster of differentiation 4 (CD4) cell count <350 cells/μL, the standard first-line regimen being tenofovir, lamivudine and efavirenz. Zidovudine-based vertical transmission prevention prophylaxis was provided to pregnant women with CD4 ≥350 cells/μL not eligible for maternally indicated cART. All HIV-exposed infants received cotrimoxazole preventive therapy for at least 6 weeks or until cessation of breastfeeding.

At enrollment, within 72 hours of delivery, maternal data were abstracted from obstetric and HIV records, and a maternal interview was conducted. Follow-up occurred at 2 weeks, 2 months, 4 months and 6 months. At the 2-week visit, a maternal health history, socioeconomic and household questionnaire were conducted. Infant health and feeding history and physical examination were conducted at all visits. CD4 count was collected on all mothers at enrollment. At the 2-week visit, HIV status of HIV-uninfected mothers was confirmed with a 4th generation HIV enzyme-linked immunosorbent assay. Results of HIV-exposed infant HIV-polymerase chain reaction tests performed at 6 weeks of age according to the National programme were retrieved. At 6 months of age, HIV-polymerase chain reaction was repeated on HIV-exposed infants and HIV enzyme-linked immunosorbent assay on HU infants. Dried blood spot cards were stored at each visit for retrospective exclusion of HIV infection in infants lost to direct follow-up.

The Western Cape Province electronic hospital administrative system was searched for records of hospital admission and the Western Cape Province mortality registry for registered deaths. This allowed complete outcome determination on all infants including those lost to face-to-face contact. Two pediatricians, blinded to HIV-exposure status, independently classified the type and severity of hospitalization events according to study-specific case definitions. Mild–moderate events were those that did not fulfill criteria for hospitalization by World Health Organization Integrated Management of Childhood Illnesses or local child health management guidelines; severe events met Integrated Management of Childhood Illnesses or local guidelines for hospitalization; for very severe events, hospitalization criteria persisted for at least 48 hours after admission.[36] Validity and reliability of these definitions were evaluated in a separate study (manuscript in preparation). The Division of AIDS (DAIDS) Grading of Adult and Paediatric Adverse Events was also assigned.[37]

Infant breastfeeding status was categorized as exclusively breastfed, partially breastfed or not breastfed according to maternal 7-day recall at each study visit. The primary outcome was infant hospitalization for a presumed or confirmed infection or death before 194 days of age (upper limit of the 6-month study visit window). The secondary outcomes were severe or very severe infectious cause hospitalizations or death. Emergency department visits not leading to hospitalization were included as sick clinic visits and not hospitalizations. For multivariable analysis, severe infectious cause hospitalizations included very severe events, and all deaths were included as very severe events irrespective of cause.

Infants returning for the first study visit at 2 weeks of age and confirmed HIV-negative at the end of follow-up were included in the analytic cohort. A 2-sided α of 0.05 was set as the limit of statistical significance for hypothesis tests without correction for multiple comparisons. The incidence rate of sick clinic visits included all reported sick clinic visits over the number of days of directly observed follow-up. Logistic regression was used to determine adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Maternal, household and infant factors were evaluated for confounding. Priority was given to controlling for established determinants of infectious morbidity, decided a priori to include preterm birth, low birth weight (below 2500 g), breastfeeding exposure and socioeconomic circumstances. It was decided a priori not to adjust for maternal CD4 count or infant growth parameters as these may be on the causal pathway between HIV exposure and infant infectious morbidity. Infant breastfeeding status at 2 weeks and 6 months was controlled for in separate models, and a stratified analysis conditioned on breastfeeding status at 2 weeks and 6 months was conducted.

The sample size calculation was based on a test of proportions for independent groups. The targeted sample size, 325 mother–infant pairs, assumed an absolute risk difference of 15% for at least 1 infectious cause hospitalization before 195 days of age, 23% in HEU infants compared with 8% in HU infants.[38] Using a 2-tailed $\alpha = 0.05$ and power = 0.8, this sample size allowed for attrition of one-third of infants by 6 months and withdrawal due to HIV infection in 10% of HIV-exposed infants. Statistical analysis was performed using R version 3.1.0 (2014 R Foundation for Statistical Computing, Vienna, Austria). Gender-specific World Health Organization child growth standard Z scores were calculated.[39] Mothers provided written informed consent for themselves and their infant. Research ethics committees of Stellenbosch University (S12/01/009), Western Cape Province (2012RP22) and the University of British Columbia (H12-01181) approved the study.

RESULTS

During the enrollment period, 1384 deliveries occurred at Kraaifontein midwife obstetric unit including 363 (26.2%) HIV-infected women. Two hundred and sixty-four mothers (19.1%) were enrolled, 136 HIV-infected mothers and 128 HIV-uninfected mothers and their newborns. Eighty percent of the enrollment target was reached. Four HIV-exposed infants (2.9%) were HIV-infected and excluded. Ninety-four HEU infants (71.2%) and 82 HU infants (64.1%) returned at 2 weeks of age and were included in this analysis. Baseline characteristics did not differ significantly between mothers and infants who returned at 2 weeks and those who did not (data not shown).

HIV-infected mothers were significantly older than HIV-uninfected mothers (Table 1). Fifty-one percent of HIV-infected mothers (48/94) were diagnosed with HIV before pregnancy. Half of HIV-infected mothers (47/94) received maternally indicated cART, 43% (20/47) of whom initiated cART before pregnancy and 94% (44/47) received a nonnucleoside reverse transcriptase inhibitor-based first-line regimen. No mothers required hospitalization or died during follow-up. Household characteristics and asset possession were similar (data not shown).

Infant gender, gestational age and birth weight did not differ significantly between HEU and HU infants (Table 1). Almost 8% of infants had incomplete immunizations at 6 months of age. One HU infant never breastfed compared with 62.8% (59/94) of HEU infants. The median duration of breastfeeding in breastfed infants was no different between HEU and HU infants at 112 days [interquartile range (IQR), 56–194]. HEU infants had significantly lower length-for-age Z scores at birth and 6 months. There was no difference between the 2 groups in mean weight-for-age, weight-for-length, head circumference or mid–upper-arm-circumference Z scores.

The median number of all-cause or sick clinic visits per infant did not differ between HIV-exposure groups (Table 1). Neither did the incidence rates of all-cause, respiratory-specific or diarrhea-specific sick clinic visits with rate ratios of 0.82 (95% CI: 0.58–1.16), 0.75 (95% CI: 0.48–1.18) and 1.13 (95% CI: 0.42–3.24), respectively, in

TABLE 1. Maternal and Infant Characteristics Compared by Infant HIV-exposure Group

Characteristic	Total N = 176	HEU N = 94	HU N = 82	*P*
Maternal characteristics				
Demographic characteristics				
Age, median (IQR)	26.8 (23.3–30.4)	27.8 (23.8–31.1)	24.7 (21.8–29.7)	0.008
Black African race (%)	161 (91.5)	87 (92.6)	74 (90.2)	0.78
Married (%)	53 (30.1)	25 (26.6)	28 (34.1)	0.38
Monthly income in ZAR, median (IQR)	1060 (285–2265)	1060 (280–2290)	1040 (385–2180)	0.79
Education				0.10
None or some primary (%)	9 (5.1)	6 (6.4)	3 (3.7)	
Some secondary (%)	104 (59.1)	61 (64.9)	43 (52.4)	
Completed secondary (%)	63 (35.8)	27 (28.7)	36 (43.9)	
Health characteristics				
Primiparous (%)	41 (23.3)	16 (17.0)	25 (30.5)	0.05
Gestation at 1st antenatal visit (weeks), median (IQR)	20 (16–27)	20 (16–26)	20 (17–28)	0.19
Number of antenatal visits, median (IQR)	5(4–6)	5(4–6)	4(3–5)	0.03
Postnatal BMI (kg/m^2), median (IQR)	26.6 (23.2–29.2)	26.6 (23.1–28.9)	26.5 (23.6–29.5)	0.42
Maternal CD4 at delivery				
Absolute count (cells/μL), median (IQR)	409 (303–592)	343 (235–501)	467 (363–675)	<0.001
Percent, median (IQR)	33.4 (24.5–40.6)	26.1 (21.1–32.4)	39.3 (36.2–45.9)	<0.001
Categorized (cells/μL)				<0.001
<350 (%)	67 (38.1)	49 (52.1)	18 (22.0)	
350–499 (%)	51 (29.0)	21 (22.3)	30 (36.6)	
≥500 (%)	58 (33.0)	24 (25.5)	34 (41.5)	
Infant **c**haracteristics				
Birth characteristics				
Male (%)	84 (47.7)	46 (48.9)	38 (46.3)	0.85
Gestational age, mean (SD)	38.91 (1.5)	38.7 (1.5)	39.1 (1.5)	0.06
Birth weight in grams, mean (SD)	3171 (409)	3118 (375)	3231 (440)	0.07
Low birth weight <2500 g (%)	9 (5.1)	6 (6.4)	3 (3.7)	0.51
Feeding characteristics				
Mother's intention to exclusively breastfeed (%)	117 (66.5)	37 (39.4)	80 (97.6)	<0.001
Feeding at 2 wk: (N = 176)				<0.001
Exclusive breastfeeding (%)	99 (56.3)	32 (34.0)	67 (81.7)	
Partial breastfeeding (%)	17 (9.7)	3 (3.2)	14 (17.1)	
No breastfeeding (%)	60 (34.1)	59 (62.8)	1 (1.2)	
Feeding at 6 mo: (N = 132)				<0.001
Exclusive breastfeeding (%)	7 (5.3)	4 (5.5)	3 (5.1)	
Partial breastfeeding (%)	44 (33.3)	7 (9.6)	37 (62.7)	
No breastfeeding (%)	81 (61.4)	62 (84.9)	19 (32.2)	
Growth, WHO Z scores				
Birth				
WAZ, median (IQR)	–0.28 (0.88)	–0.40 (0.83)	–0.17 (0.92)	0.08
LAZ, median (IQR)	–0.22 (1.88)	–0.57 (1.87)	0.18 (1.88)	0.008
Age 6 mo				
WAZ, median (IQR)	0.18 (1.19)	0.09 (1.17)	0.29 (1.23)	0.35
LAZ, median (IQR)	–0.53 (1.03)	–0.71 (0.93)	–0.31 (1.10)	0.03
Clinic visits				
All-cause clinic visits per infant, median (IQR)	6 (5–7)	6 (5–7)	6 (5–7)	0.78
Sick clinic visits per infant, median (IQR)	1 (0–1)	1 (0–1)	1 (0–2)	0.12
Infants with at least 1 sick clinic visit				
All cause (%)	93 (52.8)	47 (50.0)	46 (56.1)	0.43
Diarrhea (%)	17 (9.7)	11 (11.7)	6 (7.3)	0.53
Respiratory (%)	55 (31.3)	26 (27.7)	29 (35.4)	0.88
Fever (%)	8 (4.5)	4 (4.3)	4 (4.9)	1.00
Immunizations incomplete at 6 mo of age (N = 132) (%)	10 (7.6)	5 (6.9)	5 (8.5)	0.91

BMI indicates body mass index; LAZ, length-for-age Z score; SD, standard deviation; WAZ, weight-for-age Z score; WHO, World Health Organization; ZAR, South African rand (during the study 1 US dollar = ~10 ZAR).

HEU relative to HU infants. Seventeen (18.1%) HEU and 10 HU (12.2%) infants had a primary outcome event; all 17 HEU infants and 8 HU infants had single infectious cause hospitalizations, 1 HU infant had 2 infectious cause hospitalizations and 1 HU infant had a sudden death not associated with hospitalization. The median age at first primary outcome event was similar in HEU and HU infants [48.5 days (IQR, 27.5–135) vs. 49.0 days (IQR, 21–77); $P = 0.29$]. The median length of hospital stay was 2 days longer in HEU than HU infants [6 days (IQR, 2–7) vs. 4 days (IQR, 2–4); $P = 0.09$]. The majority of primary outcome events occurred in the first 3 months of life in both groups, 58.8% (10/17) of HEU infants and 90% (9/10) of HU infants (Fig. 1). Between 91 and 194 days, an additional 7 (7.4%) HEU infant outcome events occurred, compared with a single (1.2%) HU infant event (Fisher exact test; $P = 0.07$).

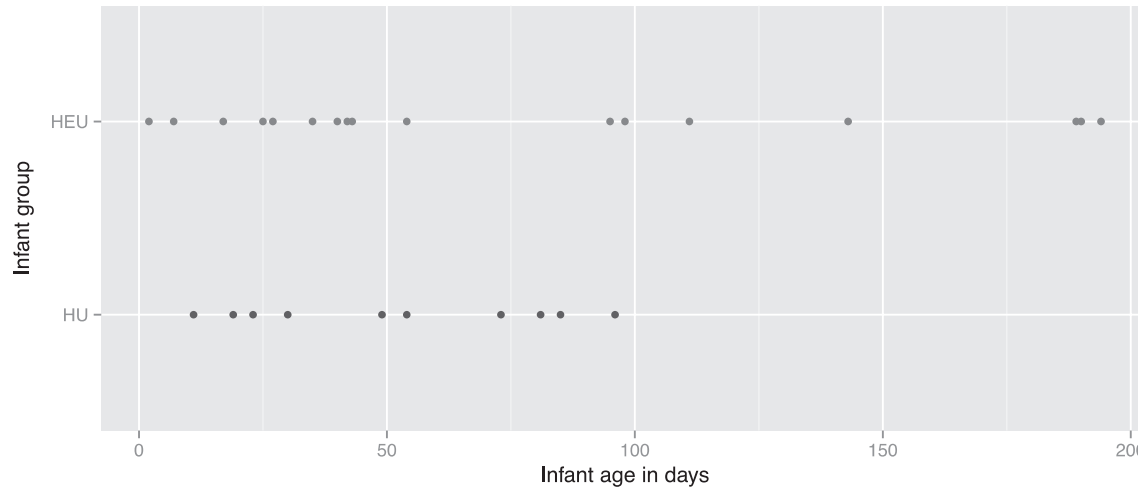

FIGURE 1. Infant age at time of primary outcome event.

Three hospitalizations were graded mild–moderate [2/94 (2.1%) HEU vs. 1/82 (1.2%) HU; $P = 1.00$], 9 as severe [5/94 (5.3%) HEU vs. 4/82 (4.9%) HU; $P = 1.00$] and 15 as very severe events [10/94 (10.6%) HEU vs. 5/82 (6.1%) HU; $P = 0.26$). All hospitalizations met criteria for a DAIDS grade 3 event. The proportion of infants hospitalized at least once with a lower respiratory tract infection did not differ [10/94 (10.6%) HEU vs. 7/82 (8.5%) HU; $P = 0.83$]. More HEU than HU infants were hospitalized with diarrhea [8/94 (8.5%) HEU vs. 1/82 (1.2%) HU; Fisher exact test; $P = 0.04$]. Although 22% (18/82) of HIV-uninfected mothers had CD4 counts <350 cells/μL, no infectious events occurred in HU infants born to these mothers.

In unadjusted analysis, HEU infants had a 59% greater odds (OR, 1.59; 95% CI: 0.69–3.82) of at least 1 infectious cause hospitalization or death compared with HU infants. Maternal age, maternal CD4 count and breastfeeding had the strongest effect on the primary outcome (Table 2). Adjustment for maternal age and any breastfeeding at 2 weeks of age (Table 3, model A) or at 6 months of age (Table 3, model B) trivially reduced the OR for HEU relative to HU infants. The unadjusted OR for very severe infectious cause hospitalization or death was 1.83 (95% CI: 0.62–6.11) that increased further after adjusting for maternal age and any breastfeeding at 2 weeks of age (aOR, 2.49; 95% CI: 0.60–10.19).

In stratified analysis, among infants not breastfeeding at 6 months of age, there was no difference between HEU and HU infants for the probability of the primary or secondary outcomes (Table 4). At 2 weeks of age, it was only possible to compare breastfeeding HEU and HU infants, as all but one of the HU infants were breastfed at this time point. Among only those infants receiving any breastfeeding at 2 weeks of age, HEU infants had an increased odds relative to HU infants of very severe infectious cause hospitalization or death (aOR, 4.21; 95% CI: 1.00–19.22; $P = 0.05$).

DISCUSSION

Firm conclusions to resolve whether a difference in infectious morbidity exists between HEU and HU infants cannot be drawn from this study. Of note, though is the consistency in the

TABLE 2. Association Between Maternal and Infant Characteristics and Infectious Cause Hospitalization or Death, Adjusted for HIV Exposure

Characteristic	aOR (95% CI)	*P*	HEU aOR (95% CI)	*P*
Maternal characteristics				
Maternal age in years	0.94 (0.86–1.02)	0.14	1.82 (0.78–4.48)	0.18
Gestation in weeks at 1st ANC visit	0.99 (0.92–1.05)	0.70	1.56 (0.68–3.77)	0.30
Number of ANC visits	1.00 (0.80–1.24)	0.97	1.49 (0.64–3.63)	0.36
Smoking during pregnancy	1.40 (0.30–5.00)	0.62	1.54 (0.67–3.74)	0.32
Absolute CD4 count (cells/μL)	1.00 (1.00–1.00)	0.009	2.22 (0.92–5.74)	0.09
CD4 >500 cells/μL	2.94 (1.24–7.05)	0.01	1.96 (0.83–4.90)	0.14
Body mass index (kg/m^2) postnatal	1.01 (0.92–1.11)	0.84	1.58 (0.68–3.81)	0.29
Infant characteristics				
Female	0.58 (0.25–1.33)	0.20	1.57 (0.68–3.79)	0.30
Gestational age in weeks	0.87 (0.66–1.14)	0.30	1.50 (0.65–3.63)	0.35
Birth weight in kg	1.26 (0.45–3.59)	0.66	1.63 (0.71–3.97)	0.26
Low birth weight (<2500 g)	0.63 (0.03–3.71)	0.68	1.61 (0.70–3.81)	0.27
Immunizations incomplete at 6 mo	0.95 (0.14–3.92)	0.95	1.61 (0.61–4.56)	0.35
No breastfeeding (reference any breastfeeding)				
2 wk	1.49 (0.49–4.67)	0.51	1.25 (0.39–3.77)	0.69
6 mo	1.52 (0.54–4.50)	0.44	1.28 (0.48–3.58)	0.56

Each row represents a logistic model including the variable in the row and the term for infant group, HEU relative to HU. The first aOR is for the effect of the variable in the row and the second HEU aOR is for the effect of being an HEU infant relative to HU infants.
ANC indicates antenatal clinic.

TABLE 3. Logistic Regression Models for the Odds of Infectious Cause Hospitalization or Death in HEU Infants Relative to HU Infants

Variable	Unadjusted OR (95% CI)	Model A aOR (95% CI)	Model B aOR (95% CI)
Primary outcome—any infectious cause hospitalization or death ≤ 194 d old			
HEU infant	1.59 (0.69–3.82)	1.45 (0.44–4.45)	1.47 (0.54–4.25)
Maternal age (yr)	0.95 (0.87–1.03)	0.94 (0.86–1.02)	0.94 (0.86–1.02)
Any breastfeeding at 2 wk	0.59 (0.26–1.39)	0.70 (0.22–2.06)	-
Any breastfeeding at 6 mo	0.57 (0.22–1.36)	-	0.65 (0.21–1.85)
Secondary outcome 1—severe infectious cause hospitalization or death ≤ 194 d old			
HEU infant	1.42 (0.59–3.59)	1.61 (0.48–5.09)	1.31 (0.45–4.02)
Maternal age (yr)	0.95 (0.86–1.03)	0.94 (0.85–1.02)	0.94 (0.86–1.02)
Any breastfeeding at 2 wk	0.78 (0.32–1.98)	0.98 (0.29–3.08)	-
Any breastfeeding at 6 mo	0.61 (0.22–1.51)	-	0.66 (0.21–2.01)
Secondary outcome 2—very severe infectious cause hospitalization or death ≤ 194 d old			
HEU infant	1.83 (0.62–6.11)	2.49 (0.60–10.19)	1.37 (0.39–5.57)
Maternal age (yr)	0.92 (0.82–1.02)	0.91 (0.80–1.01)	0.91 (0.80–1.01)
Any breastfeeding at 2 wk	0.78 (0.26–2.36)	1.19 (0.30–4.48)	-
Any breastfeeding at 6 mo	0.34 (0.08–1.13)	-	0.37 (0.07–1.52)

Model A: the odds of an outcome in HEU compared with HU infants adjusted for maternal age (continuous variable) and any breastfeeding at 2 weeks (binary variable). Model B: the odds of an outcome in HEU compared with HU infants adjusted for maternal age (continuous variable) and any breastfeeding at 6 months (binary variable).

TABLE 4. Stratified Analysis of the Effect of HIV Exposure on Infectious Cause Hospitalization or Death, Conditioned on Breastfeeding and Adjusted for Maternal Age

Breast-feeding status	Primary Outcome		Secondary Outcome 1		Secondary Outcome 2	
	n/N	aOR (95% CI)	n/N	aOR (95% CI)	n/N	aOR (95% CI)
No breastfeeding						
2 wk	HEU: 11/59 HU: 1/1	Model not possible*	HEU: 8/59 HU: 1/1	Model not possible*	HEU: 5/59 HU: 1/1	Model not possible*
6 mo	HEU: 15/80 HU: 4/25	1.34 (0.42–5.19)	HEU: 12/80 HU: 4/25	1.06 (0.32–4.18)	HEU: 9/80 HU: 3/25	1.04 (0.27–5.09)
Any breastfeeding						
2 wk	HEU: 6/35 HU: 9/81	1.96 (0.59–6.22)	HEU: 6/35 HU: 8/81	2.16 (0.64–7.01)	HEU: 5/35 HU: 4/81	4.21 (1.00–19.22)
6 mo	HEU: 2/14 HU: 6/57	1.82 (0.24–10.2)	HEU: 2/14 HU: 5/57	2.07 (0.27–12.0)	HEU: 1/14 HU: 2/57	3.75 (0.14–6.0)

Primary outcome: at least 1 infectious cause hospitalization or death ≤194 days old. Secondary outcome 1: at least 1 severe infectious cause hospitalization or death ≤194 days old. Secondary outcome 2: at least 1 very severe infectious cause hospitalization or death ≤194 days old.

*Single HU infant not breastfeeding at 2 weeks and thus not possible to model this relationship.

observed pattern of increased severity of infectious events in HEU infants with a greater proportion of these infants experiencing infectious cause hospitalizations, very severe infectious cause hospitalizations and a longer length of hospital stay, despite a similar rate of out-patient sick clinic visits to HU infants. There is evidence from this cohort of 3 significant findings: (1) in the presence of breastfeeding, HEU infants had a 4 times greater odds of very severe infectious cause hospitalization compared with HU infants; (2) infectious morbidity in HEU infants that are suboptimally breastfed was no different to that of HU infants also suboptimally breastfed and (3) a low maternal CD4 count was not associated with infectious morbidity in this cohort.

The 18% hospitalization rate observed in HEU infants corresponds with reports from Botswana, South America and the Caribbean.[12,40] In contrast, the rate observed in HU infants (12%) was higher than previously observed in South Africa (7%) and Botswana (6%).[12,38] These data are reassuring that the difference in hospitalization between HEU and HU infants in our cohort was unlikely overestimated. A similar hospitalization rate was observed in a recent Mozambican study (rate ratio (RR), 1.51; 95% CI: 0.71–3.18) also with no difference in out-patient sick clinic visits between HEU and HU infants.[16]

Significantly, more HEU infants were hospitalized with diarrhea than HU infants. The number of infants with diarrhea was too small to interrogate the association between diarrhea and breastfeeding, the most obvious explanation. However, the rate of diarrhea-associated sick clinic visits was no different in HEU and HU infants, indicating similar frequency of diarrhea, but HEU infants experienced a greater severity of diarrhea, more often requiring hospitalization. If the deprivation of the protective effects of breastfeeding and the risk of contamination of formula milk were the major drivers of this difference, it might be expected that the frequency of all diarrheal episodes, including sick clinic visits, would be increased in HEU infants. That was not the case in this cohort.

The majority of morbidity in both HEU and HU infants in this cohort was due to lower respiratory tract infections. Studies have noted the burden of respiratory tract infections in HEU infants, but without HU control groups, it has not been possible to understand whether HEU infants experience morbidity as expected or morbidity in excess of expected for their context.[41–43] In Southern Africa, HEU infants have the same rate of all pneumonia as HU infants but have a greater risk for severe pneumonia and empiric pneumonia treatment failure.[8,13,16,18] Early studies observed a larger relative difference in infant mortality than in morbidity or hospitalization when comparing HEU and HU infants, possibly indicating more severe disease in HEU infants.[10–12] Our observation that HEU infants have a higher probability of very severe infections even when breastfed supports previous observations. The current study is the first to specifically attempt to understand the severity of infections. A difference in severity would have been missed using the DAIDS grading that poorly discriminated the severity of hospitalization events. The observed pattern potentially indicates that it is not more frequent exposure to common infectious pathogens, but an altered immunologic or inflammatory response to the pathogens that drives severity of infection in HEU infants.

In our cohort, irrespective of HIV exposure, those infants not breastfed experienced a 50% greater probability of infectious morbidity than infants who were breastfed, as expected (Table 2). HEU infants in our cohort receiving any breastfeeding for a similar duration to HU infants though had a substantially greater odds of very severe infectious morbidity (Table 4). In the context of similar breast milk exposure in HEU and HU infants, a mechanism other than the lack of protection afforded by breast milk should be considered. In addition, the majority of infants, both HEU and HU, who experienced an infectious cause hospitalization were born to mothers with a CD4 count of 500 cells/μL or greater. In HU infants, this may not be a clinically meaningful observation, but may be important in HEU infants.

With no difference in the proportion of HEU and HU infants hospitalized by 3 months of age, between 3 and 6 months of age, HEU infants continued to experience infectious cause hospitalizations while events tapered in HU infants. Similarly, in a large Zimbabwean study, the highest absolute mortality in HEU infants occurred in the neonatal period but the greatest relative increase in HEU mortality was from 2–6 months of age.[10] In South Africa, there is no difference in rates of all-cause neonatal sepsis in HEU and HU infants.[44] Combined, this evidence points to the period of greatest relative vulnerability in HEU infants being in the postneonatal, mid-infant period. This observation could help to define a focus for study of immunologic mechanisms of vulnerability in HEU infants.

We possibly did not identify a substantial difference in infectious morbidity because of inclusion of the lowest risk HIV-infected mothers and infants at lowest risk for infectious morbidity. However, our intent was to match HEU and HU infant environments in an attempt to identify unique events related to HIV exposure in the absence of HIV infection. For logistic reasons, our sample size was smaller than anticipated with substantial early attrition. These deficiencies in the power of the study were countered, somewhat, by the well-matched infant groups that limited confounding by differences in birth outcomes, maternal, household and socioeconomic factors. It is recognized that the potential for unmeasured differences and residual confounding remains. The marked early attrition could have introduced selection bias; however, similar proportions of HEU and HU infants were lost following enrollment, and baseline characteristics of mothers and infants lost were no different to those retained.

This study has helped to identify a more specific pattern to HEU infectious morbidity not previously described in the absence of HU control groups and discriminating outcome measurement tools. In this cohort of term HEU and HU infants experiencing similar social and household circumstances, breastfeeding HEU infants had a substantially greater probability of very severe infectious morbidity than breastfeeding HU infants. HEU infants tended to experience a greater severity of infectious morbidity in the context of a similar frequency of infections, the greatest relative difference between HEU and HU infants occurring between 3 and 6 months of age. The observed pattern could be in keeping with mechanisms related to HEU-unique exposures including deficient transplacental acquisition of maternal antibodies, delayed functional immune development in HEU infants or altered quality of the breast milk of HIV-infected mothers.

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