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Linear Growth Faltering Among HIV-Exposed Uninfected Children.

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Running Title: Growth of HIV-exposed Botswana children

Abstract

Background: HIV-exposed uninfected (HEU) children experience increased mortality compared with their HIV-unexposed uninfected (HUU) peers. It is unclear whether HEU children are also at increased risk for undernutrition, a modifiable risk factor for mortality.

Methods: We conducted a cross-sectional, population-based survey of children under 5 years of age in five health districts in Botswana. Linear mixed-effects models were used to assess continuous outcomes while generalized estimating equations were used to estimate relative risks of stunting, wasting, and underweight between HEU (n=396) and HUU (n=1,109) children. Secondary analyses examined potential mediation by low birthweight.

Results: The association between maternal HIV-exposure and child stunting varied significantly by child age ($p<0.01$). HEU children <1 year and ≥2 years of age had 1.85 (95% CI: 1.03-3.31; p=0.04) and 1.41 (95% CI: 1.06-1.88; p=0.02) times the risk of stunting compared with HUU children after multivariate adjustment, respectively. During the period of 1-2 years of age, when breastfeeding cessation occurred among HUU children, HUU children had increased risk of stunting compared with HEU children who were predominantly formula fed (RR: 1.56; 95% CI: 1.05-2.32; p=0.03). A mediation analysis estimated 67% of the excess risk of stunting among HEU children ≥2 years was attributable to low birthweight (p=0.02). There was no difference in risk of wasting or underweight.

Conclusion: HEU children are at increased risk of stunting compared with their HUU peers; however, interventions to increase birthweight may significantly ameliorate this excess risk. Interventions to support optimal growth during weaning are needed for all breastfed children.

Keywords: HIV, child, malnutrition, stunting, birthweight, infant

Introduction

In 2013 the World Health Organization's guidelines for the prevention of mother-to-child HIV transmission (PMTCT) were changed to recommend all HIV-infected women initiate triple antiretrovirals in pregnancy and continue antiretrovirals for their lifetime, including throughout breastfeeding.[1] If this plan is implemented consistently worldwide, this public health approach holds the promise of virtually eliminating mother-to-child HIV transmission, but will also translate to over 1.5 million children being born HIV-exposed uninfected (HEU) on an annual basis.[2,3] In resource-limited settings, HEU children experience 2- to 3-fold higher mortality compared with children born to HIV-uninfected women.[4-7] Unless the etiologies underlying this health disparity and interventions to ensure the health and survival of HEU children in resource-limited settings are identified, PMTCT successes and the expansion of antiretroviral use in pregnancy may be partially overshadowed by excess mortality among HEU children.

An estimated 3.1 million children under the age of 5 years (U5) died in 2011 as a result of underlying undernutrition, or ~45% of total U5 deaths.[8] In sub-Saharan Africa, undernutrition has been associated with one-third of all U5 deaths.[9] In addition to the mortality consequences, restricted linear growth or stunting during early childhood has also been linked to reduced cognition, educational attainment, and lower lifetime earnings.[10,11] There is significant overlap in sub-Saharan Africa between geographic areas where U5 undernutrition predominates and generalized HIV epidemics. Higher mortality among HEU children in resource-limited settings like sub-Saharan Africa, where undernutrition is a leading cause of mortality, highlights the importance of understanding the association, if any, between HIV-exposure and undernutrition.

In this study we utilize data from a cross-sectional population-based survey of U5 children in Botswana to examine differences in anthropometric growth, comparing HEU children with HIV-unexposed uninfected (HUU) children. We also investigate whether the strength of the associations differ by child age and explore if differences in birthweight between HEU and HUU children potentially mediates differences in postnatal growth.

Methods

Study design

The Determinants of Malnutrition (DoM) study was conducted between September 2013 and February 2014 within five health districts in Botswana experiencing medium to high rates of undernutrition: Francistown, Ghanzi, Kgalagadi South, Kweneng East and Selebi Phikwe. The Republic of Botswana's Ministry of Health collaborated with Botswana Harvard AIDS Institute Partnership to carry-out the study funded by the United States Centers for Disease Control and Prevention. The DoM study was a cross-sectional study enrolling U5 children and their caregivers as they attended child welfare clinics (CWCs). Parents or guardians, referred to as caregivers, provided informed written consent to participate in the study with the child or children they accompanied to CWCs. This study of HIV-exposure is limited to children enrolled in the DoM study whose mothers' HIV status during the pregnancy was known and, if the child was born to an HIV-infected mother, the child had HIV testing that was negative for HIV. HIV-infected children and children with unknown HIV status were excluded from this analysis.

Botswana has an extensive network of government run hospitals, clinics, health posts and mobile stops. CWCs are held at almost all of these health locations and are free of charge for Botswana citizens. CWCs are structured to be attended monthly by U5 children as well-child clinics. During CWCs, a child's weight is evaluated monthly, length/height is evaluated twice a year, and children receive scheduled immunizations. In addition, caregivers are provided with age specific nutritional supplements for children, so long as the children are between the ages of 6 months and 5 years. As part of the national PMTCT strategy, exclusive formula feeding is promoted for all HIV-exposed infants, with provision of free infant formula throughl 12 months of age. Formula feeding has high uptake among HIV-infected women in Botswana.

Health facility and participant selection

A two-stage stratified sampling process using probability proportional to size (PPS) sampling technique was used. The sampling frame was obtained from Botswana's Ministry of Health and it comprised all the 176 health facilities and active mobile stops that conduct CWCs in the 5 districts. In the first stage facilities with average monthly under-5 CWC attendance of 75 or higher were divided by district and type of the health facility (hospital, clinic, health post, and mobile stop). A total of 36 health facilities (primary sampling unit) across five districts were then selected. The number of selected primary sampling units in each district was proportional to the average monthly volume in all facilities in the district. Within each district, the number of units sampled by each type of health facility was fixed at 55 children, except for the highest volume health clinic located in Mogoditshane, within the Kweneng East District Clinic, where a sample size of 110 was assigned. Facility classification as urban, peri-urban, or rural was based upon analysis provided by Botswana's Department of Town and Regional Planning housed within the Ministry of Local Government and Rural Development. In the second stage, caregivers attending CWCs were randomly selected each day until the target number of U5 children was reached. Children were only allowed to be sampled once during the study.

Survey instrument and data collection

Study activities included a questionnaire for the caregiver and chart abstraction from the child's under-5 health booklet to obtain birth weight, immunization records, sick visit and hospitalization data, HIV testing results of the child, if born to an HIV-infected mother, and maternal HIV status during the pregnancy. Data collected included sex and age of the child at enrollment, birth weight, birth order of the child, feeding choice in infancy as either breast, formula feeding or a combination of breast and formula feeding, duration of infant breastfeeding, history of up to the last five episodes of diarrheal illness or respiratory infection requiring outpatient care or hospitalization of the child if applicable, location of facility, maternal marital status, primary caregiver (i.e. mother, grandmother, aunt), household income, access to tap water, flush toilet, electricity and refrigerator in the home where the child resides, and access to gas or electricity as a cooking source compared with paraffin stove or wood in the home where the child resides. Additional data included number individuals in the household where the child resides eating from the same pot (communal eating), report of food insecurity in the household where the child resides either on the day of the study visit or within the past month, and maternal age at time of the child's birth. Food insecurity was assessed by caregiver-report of insufficient access to food either on the day of the study visit or in the past month. These two food insecurity questions have been used in past Botswana surveys, but are not part of a validated food security instrument.

Study staff trained in the acquisition of anthropometric measures used calibrated scales for weight assessment, length boards for recumbent assessment of length for children < 24 months of age, and stadiometers for height assessment of children ≥ 24 months of age. Study procedures required assessment of the child's weight and length/height three consecutive times at the same visit and the average of the three results was used as the final weight and length/height for the child. Length/height-for-age z score (LAZ/HAZ), weight-for-length z-score (WLZ/ WHZ), and weight-for-age z score (WAZ) were calculated using WHO child growth standards.[12] Stunting, wasting, and underweight were defined as a LAZ/HAZ, WLZ/WHZ, and WAZ of 2 or more standard deviations below the WHO population median, respectively.

Statistical methods

Maternal, caregiver, and child characteristics were compared between HEU and HUU children using the Wilcoxon rank-sum test for continuous variables and the $\chi 2$ test for categorical variables. We then assessed mean differences in LAZ/HAZ, WLZ/WHZ, and WAZ for HEU versus HUU children using linear mixed effects models (PROC MIXED) to account for clustering by facility due to sampling methods. Generalized estimating equations (GEEs) (PROC GENMOD) with log-links and exchangeable correlation matrices were used to account for clustering by facility and obtain relative risk estimates for the binomial outcomes of stunting, wasting, and underweight.[13,14] Multivariate models were defined *a priori* and included covariates for maternal age (<25, 25-30, and 30+ years), maternal marital status (married, single, divorces/widowed), location of enrollment (urban, peri-urban, rural), household income <1,000 Pula per month (yes/no), electricity (yes/no), refrigerator (yes/no), tap water (yes/no), electric or gas cooking (yes/no), flush toilet (yes/no), child sex, and birth order (first born, 2-4, or 5+). We also examined child age as an effect modifier using interaction terms in multivariate models with statistical significance of effect modification assessed using the likelihood ratio test. If statistically significant effect modification was determined, all models were presented stratified by child age.

We conducted an exploratory mediation analyses to determine the potential of low birthweight (LBW), defined as a birthweight of <2500 grams, to mediate differences in risk of child stunting between HEU and HUU children. In order to do so, we first created a multivariate base model to estimate the independent association of maternal HIV-exposure with the binary outcome of stunting. Next, we added a covariate for LBW to the base model to evaluate the potential mediating effect of LBW on the association between HEU children and stunting. We then calculated the mediation proportion and its p-value using the publicly available %Mediate macro (http://www.hsph.harvard.edu/donna-spiegelman/software/mediate/).[15] The mediation proportion is defined as the proportion of excess risk of stunting for HEU children relative to HUU children that can be attributed to elevated prevalence of low birth weight among HEU children. We also present the relationship of LBW with stunting stratified by maternal HIV status, in order to confirm the assumption of no effect modification by the mediation variable.

In all analyses, missing data for covariates was retained in the analysis using the missing indicator method for variables missing greater than 1% of the observations. All p-values were 2-sided and $p<0.05$ was considered statistically significant. Statistical analyses were performed using the SAS v 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethics

The study was approved by the Botswana Health Research Development Committee, Center for Global Health at the Centers for Disease Control in Atlanta, USA and the Massachusetts General Hospital's Human Subjects Committee.

Results

At total of 1,703 children <5 years of age were enrolled in the DoM study, of these 1,109 (65.1%) were born to HIV-uninfected mothers, 432 (25.4.8%) to HIV-infected mothers, and 162 (9.5%) to mothers with unknown HIV status in pregnancy. Among HIV-exposed children, 396 (91.7%) were HEU, 7 (1.6%) HIV-infected, and 29 (6.7%) were never tested or had an unknown HIV status at the time of the study visit. This study provides an evaluation of growth for the 396 HEU children and 1,109 HUU children.

Table 1 presents maternal, caregiver, and child characteristics for HEU and HUU children. Mothers of HEU children tended to be older than mothers of HUU children (30.1 versus 25.9 years) and slightly more HEU children attended CWCs in urban areas (47.1% versus 42.0%). HEU children also tended to have lower socioeconomic status compared with HUU children. A significantly higher proportion of HEU children resided in households where the monthly household income was less than 1000 pula per month (equivalent to ~$120 US Dollars) (37.5% versus 25.5%) and HEU households were less likely to have electricity, tap water, and refrigeration. As for child characteristics, HEU children tended to have higher birth order and were more likely to be born with low birthweight (<2500g) compared with HUU children (18.3% versus 10.8%). In addition, 94.7% of HEU children were exclusively formula fed from birth due to the national PMTCT strategy, while only 21.7% of HUU children received infant formula.

We examined the association of HIV-exposure with linear growth (LAZ/HAZ) and determined the strength of association significantly varied by child age (p-value for effect modification: <0.01). As shown in Figure 1, the prevalence of stunting was greater for HEU children compared with HUU children during the first year of life and from 2-5 years of age. During the period of 1-2 years of age, HUU children had increased prevalence of stunting. Table 2 presents univariate and multivariate mean differences in LAZ/HAZ and relative risk of stunting for HEU versus HUU children stratified by child age. Among children <1 year of age, HEU children had significantly increased risk of stunting compared with HUU children after multivariate adjustment (RR: 1.91; 95% CI: 1.17-3.09; p=0.01). For children 1-2 years of age, HEU children had reduced risk of stunting (RR: 0.64; 95% CI: 0.43-0.95; p=0.03) compared with HUU children in multivariate models. Among children 2-5 years of age, multivariate models indicated HEU children had significantly increased risk of stunting (RR: 1.42; 95% CI: 1.07-1.87; p=0.01) compared with HUU children.

In order to explore potential mechanisms leading to this qualitative change in the direction of the association by child age, we examined the relationship of time since breastfeeding cessation with stunting among HUU children. Among HUU children aged 1-3 years, those who were currently breastfed had a prevalence of stunting of 18.9%, while among similarly aged children who had stopped breastfeeding for <3 months the prevalence of stunting sharply increased to 36.0% (see Supplemental Table 1, which shows stunting prevalence by time since breastfeeding cessation). The prevalence of stunting gradually decreased with increased time since breastfeeding cessation to 22.5% for HUU children who had not breastfed for >12 months. In multivariate analyses, HUU children who had stopped breastfeeding within the last 3 months had 1.76 times the risk of being stunted at the time of the study visit (95% CI: 0.96-3.22; p=0.07) compared with similarly aged breastfed HUU children (see Supplemental Table 2, which shows the association of time since breastfeeding cessation with stunting).

We also conducted exploratory analyses to determine the potential for low birthweight to mediate the observed increased risk of child stunting among HEU children <1 years and ≥ 2 years. Table 3 presents mediation analysis results. HEU children <1 year and ≥ 2 years had roughly twice the prevalence of LBW (<2500g) compared with similarly aged HUU children, and within both age strata, LBW was strongly associated with increased risk of stunting. Among children <1 years, LBW was found to be a significant mediator of the relationship of HIV-exposure with stunting and the estimated mediation proportion was 35% (p=0.04). For children ≥ 2 years, 67% of the excess risk of stunting for HEU children relative to HUU children could be attributed to LBW (p=0.02). We found no significant difference in the association of LBW with stunting among HEU children 2-5 years (RR: 1.77; 95% CI: 0.57-5.53) versus HUU children 2-5 years (RR: 2.33; 95% CI: 1.33-4.14) (p-value for interaction: 0.22), which confirms the assumption of no effect modification by the mediation variable.

In Table 4 we present mean differences in WLZ/WHZ and WAZ, along with relative risk of wasting and underweight for HEU versus HUU children. We found no significant evidence of effect modification by child age for all analyses (all p-values for interaction > 0.05) and therefore results are presented without age stratification. There was no significant difference in risk of wasting or underweight or difference in WLZ/WHZ for HEU versus HUU children in multivariate models (all p-values >0.05). In univariate analyses HEU children had significantly decreased mean WAZ (-0.15; 95% CI:-0.29 - -0.01;

p=0.03), and a similar magnitude of the association was found in multivariate models but the results did not reach statistical significance (-0.13; 95% CI: -0.27-0.02; p=0.09).

Discussion

In this study we found maternal HIV-exposure increased the risk of stunting for Botswana children who were under 1 year or greater than 2 years of age. In secondary mediation analyses, increased prevalence of LBW among HEU children was found to be a significant mediator of the stunting association. During the period of 1-2 years of age, when weaning typically occurs in Botswana among children born to HIV-uninfected mothers, HUU children had an increased prevalence of stunting compared with HEU children. We did not find significant differences in risk of wasting and underweight between HEU and HUU children in multivariate analyses.

In this study we determined maternal HIV-exposure was associated with increased risk of stunting, but the strength of the relationship was dependent on child age. Previous studies comparing linear growth of HEU and HUU children have reported mixed results, but the majority of studies have found no association.[16-23] Nevertheless, there are a few studies which have noted growth deficits in HEU children, including a recent cross-sectional survey of HEU Ugandan infants (mean age 5 months) which found significantly increased risk of both stunting and wasting.[18] A Kenyan study also found significantly lower HAZ for HEU infants as compared to HUU infants at 1.5 months after birth.[19] A few potential mechanisms, independent of maternal sociodemographic differences, which may have led to increased risk of linear growth faltering among HEU children compared with HUU children include: exposure to antiretroviral drugs, deficits in immune responses to vaccination as well as pathogens, and increased exposure to other infections.[24-29]

To our knowledge we are the first study to utilize mediation analyses to estimate the proportion of stunting attributable to low birthweight, a potentially modifiable risk factor. In our cohort, we estimated 67% of the excess risk of stunting for HEU children over 2-5 years of age could be attributed to increased prevalence of LBW compared with HUU children. We have previously found that HEU children in Botswana exposed to combined antiretroviral treatment (cART) *in utero* had lower length at birth, 6 and

24 months of age compared with zidovudine monotherapy-exposed HEU infants.[29,30] Accordingly, there may be a greater impact on linear growth when triple antiretrovirals are provided to HIV-infected mothers in pregnancy compared with monotherapy. The majority of previous HEU child growth studies were conducted before the availability of cART in resource-limited settings, which may partially explain their null associations.[16,20-23] Nevertheless, a few of these studies noted lower birthweights among HEU children compared with HUU children[20,21]. Research is urgently needed to identify mechanisms by which cART during pregnancy influences birth weight and impairs postnatal linear growth, so that the safest combination of triple antiretrovirals for HIV-infected pregnant women and their children can be identified.

In this study, we noted a sharp increase in the prevalence of stunting among HUU children 1-2 years of age, particularly during the initial months after cessation of breastfeeding. There is a large body of literature indicating the importance of continuing breastfeeding and providing nutritious complementary foods during the first 2 years of life for child survival and growth.[32-36] A prospective cohort study of Kenyan children (mean age of 14 months at cohort entry) found children who continued breastfeeding throughout a 6 month follow-up period had significantly better length and weight outcomes compared with children who breastfed for <3 months of the follow-up period.[35] As almost all HEU children were formula fed in our study population, we were not able to examine breastfeeding cessation as a risk factor for stunting among HEUs; however, there is evidence that continued breastfeeding during the first two years of life also improves growth among HEU breastfed populations.[36] Overall, there is a strong programmatic need for monitoring growth and providing support during the period of complementary feeding introduction and breastfeeding cessation regardless of the HIV-exposure status of the child.

There are a few limitations to this study. First, due to the HIV-testing algorithm there may be a small amount of misclassification of child HIV status. The HIV testing algorithm in this population is a DNA PCR at 6 weeks with a follow-up ELISA at 18 months for non-breastfed HEU infants. As a result, there is a possibility that a very small number of children became HIV-infected after a 6 week negative HIV test but were not yet retested at 18 months; however in this population of almost all formula fed

HEU infants, the number of children who seroconverted after 6 weeks is likely very small. In addition, due to the cross-sectional nature of the study, we did not have information on the duration and type of antiretrovirals received by HIV-infected mothers during pregnancy. Further, we also did not have access to other maternal health indicators including height, body mass index, and anemia. As a result, poorer maternal health in pregnancy for HEU children may partially explain our observed differences in linear growth. Given the fact that an individual's overall growth and health is strongly influenced by the first 1,000 days of life, from conception to their second birthday, it is imperative to optimize maternal health, if we want optimize the growth of children. We also did not have data on the birth length of the child, which may be a significant mediator of postnatal growth, independent of LBW. In a previous study we found HEU children exposed to cART *in utero* had lower length at birth compared with zidovudine monotherapy-exposed children.[30,31] The cross-sectional nature of the study has some limitations but we also note that the children sampled in this survey are likely more representative of the general HEU child population than other studies using secondary analyses of clinical trials and follow-up studies which often provide improved medical care and growth monitoring.

Overall, we found HEU Botswana children under 1 year and 2-5 years of age had increased risk of stunting compared with their HUU peers. A mediation analysis indicated that a significant proportion of this excess risk appears to be linked to increased prevalence of low birthweight among HEU children. As a result, future research needs to determine the underlying mechanisms leading to low birthweight among children of HIV-infected mothers, which includes determining optimal cART regimens for the health of pregnant women as well as growth of their HEU children. This research is urgently needed as the number of HEU children is rapidly expanding due to continued success of PMTCT programs and the increasing number of countries transitioning to Option B+ in their national PMTCT guidelines.

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Figure Titles and Captions

Figure 1 Title. Prevalence of stunting (HAZ < -2) for HEU vs HUU children by child age

Figure 1 Caption.

Abbreviations: HAZ: height-for-age z-score; HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected

Table 1. Maternal, caregiver, and child characteristics for HEU (n=396) and HUU (n=1,109) children <5 years of age.

	HEU children (n=396) Mean ± SD or Frequency (%)	HUU children (n=1,109) Mean ± SD or Frequency (%)	p-value
Maternal and household characteristics			
Maternal age in years	30.1 ± 5.7	25.9 ± 6.0	<0.01
Marital status			
Single	331 (85.5)	932 (84.0)	0.18
Married	52 (13.5)	174 (15.7)	
Divorced or widowed	4 (1.0)	4 (0.3)	
Primary caregiver is parent or grandparent	336 (86.7)	975 (87.9)	0.53
Location of enrollment health facility			
Urban	182 (47.1)	466 (42.0)	0.04
Peri-urban	138 (35.5)	384 (34.6)	
Rural	67 (17.4)	259 (23.4)	
Household income <1,000 Pula per month	145 (37.5)	282 (25.5)	<0.01
No electricity in the home	165 (42.7)	383 (34.5)	<0.01
No refrigerator in the home	197 (51.0)	484 (43.6)	0.01
No electric or gas cooking source	131 (33.8)	326 (29.4)	0.11
No tap water in the home	258 (66.5)	677 (61.0)	0.05
No flush toilet	242 (62.5)	642 (57.8)	0.10
Report of food insecurity in past month	112 (29.0)	251 (22.8)	0.01
Child characteristics			
Male	213 (55.1)	509 (45.9)	<0.01
Child age in years	2.1 ± 1.3	2.1 ± 1.4	0.37
Birth order			
Firstborn	78 (20.3)	496 (44.7)	<0.01
2-4th child	268 (67.7)	552 (49.8)	
5+ child	41 (10.5)	62 (5.6)	
Low birthweight <2500grams	71 (18.3)	120 (10.8)	<0.01
Exclusively formula fed	375 (94.7)	241 (21.7)	<0.01

Table 1 Caption

Abbreviations: HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected; SD: standard deviation

Table 2. Mean difference in LAZ/HAZ and relative risk of stunting for HEU children (n=396) as compared to HUU children (n=1,109) stratified by child age (p-value for effect modification by age <0.01).

	Mean LAZ/HAZ ± SD or % stunting	Univariate[1] mean difference or relative risk (95% CI)	p-value	Multivariate[1,2] mean difference or relative risk (95% CI)	p-value
Mean difference LAZ/HAZ					
Under 1 year of age					
HEU children (n=100)	-0.54 ± 1.8	-0.20 (-0.58-0.18)	0.30	-0.22 (-0.64-0.19)	0.30
HUU children (n=283)	-0.25 ± 1.6	Ref.		Ref.	
1-2 years of age					
HEU children (n=109)	-1.13 ± 1.3	+0.03 (-0.29 – 0.35)	0.86	+0.07 (-0.28-0.41)	0.71
HUU children (n=282)	-1.18 ± 1.6	Ref.		Ref.	
2-5 years of age					
HEU children (n=187)	-1.44 ± 1.3	-0.25 (-0.45- -0.06)	0.01	-0.20 (-0.41- 0.01)	0.06
HUU children (n=544)	-1.19 ± 1.2	Ref.		Ref.	
Relative risk of stunting					
Under 1 year of age					
HEU children (n=100)	20.0%	1.76 (1.12-2.76)	0.01	1.91 (1.17-3.09)	0.01
HUU children (n=283)	11.0%	Ref.		Ref.	
1-2 years of age					
HEU children (n=109)	22.9%	0.76 (0.53-1.08)	0.13	0.64 (0.43-0.95)	0.03
HUU children (n=282)	29.8%	Ref.		Ref.	
2-5 years of age					
HEU children (n=187)	31.0%	1.47 (1.18-1.83)	<0.01	1.42 (1.07-1.87)	0.01
HUU children (n=544)	20.8%	Ref.		Ref.	

Table 2 Captions

[1]Univariate and multivariate models accounted for clustering by facility.

[2]Multivariate models accounted for clustering by facility and adjusted for maternal age (<25, 25-30, and 30+ years), maternal marital status (married, single, divorces/widowed), location of enrollment (urban, peri-urban, rural), household income <1,000 Pula per month (yes/no), electricity (yes/no), refrigerator (yes/no), tap water (yes/no), electric or gas cooking (yes/no), flush toilet (yes/no), child sex, and birth order (first born, 2-4, or 5+)

Abbreviations: CI: confidence interval; HAZ: height-for-age z-score; HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected; LAZ: length-for-age z-score; SD: standard deviation

Table 3. Mediation analysis of the association of child HIV-exposure with stunting by low birth weight stratified by child age

Child age	% Stunted	% LBW <2500g	Multivariate[1,2] adjusted relative risk (95% CI) of stunting for LBW of <2500g versus birth weight ≥2500g	Multivariate[1] adjusted relative risk (95% CI) of stunting for HEU vs. HUU (not adjusted for LBW)	Multivariate[2,3] adjusted relative risk of stunting for HEU vs. HUU (adjusted for LBW)	Estimated mediation proportion for LBW (p-value)
<1 year (HEU n=100) (HEU n=283)	HEU: 20.0% HUU: 11.0%	HEU: 15.0% HUU: 8.1%	2.81 (1.45-5.34)	1.63 (1.07-2.47)	1.49 (0.88-2.52)	35% (p=0.04)
2-5 years (HEU n=187) (HEU n=544)	HEU: 31.0% HUU: 20.8%	HEU: 21.1% HUU: 11.6%	2.02 (1.41-2.88)	1.43 (1.08-1.90)	1.12 (0.85-1.49)	67% (p=0.02)

Table 3 Captions

[1] Models restricted to children with a non-missing birth weights. Multivariate models were adjusted for maternal age (<25, 25-30, and 30+ years), maternal marital status (married, single, divorces/widowed), location of enrollment (urban, peri-urban, rural), household income <1,000 Pula per month (yes/no), electricity (yes/no), refrigerator (yes/no), tap water (yes/no), electric or gas cooking (yes/no), flush toilet (yes/no), child sex, birth order (first born, 2-4, or 5+).

[2]Includes adjustment for maternal HIV status

[3]Includes low birth weight (<2500g)

Abbreviations: CI: confidence interval; HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected; LBW: low birth weight

Table 4. Mean difference in WHZ and WAZ and relative risk of wasting and underweight for HEU children (n=396) as compared to HUU children (n=1,109)

	Mean z-score ± SD or %	Univariate[1] mean difference or relative risk (95% CI)	p-value	Multivariate[1,2] mean difference or relative risk (95% CI)	p-value
Mean difference WLZ/WHZ					
HEU children (n=396)	-0.20 ± 1.5	-0.06 (-0.22-0.10)	0.44	-0.03 (-0.21- 0.14)	0.69
HUU children (n=1,109)	-0.15 ± 1.4	Ref.		Ref.	
Relative risk of wasting[3]					
HEU children (n=396)	9.6%	1.29 (0.96-1.72)	0.09	1.15 (0.95-1.38)	0.15
HUU children (n=1,109)	7.5%	Ref.		Ref.	
Mean difference WAZ					
HEU children (n=396)	-0.79 ± 1.2	-0.15 (-0.29- -0.01)	0.03	-0.13 (-0.27-0.02)	0.09
HUU children (n=1,109)	-0.63 ± 1.3	Ref.		Ref.	
Relative risk of underweight[2]					
HEU children (n=396)	15.9%	1.26 (1.00-1.58)	0.05	1.02 (0.80-1.29)	0.87
HUU children (n=1,109)	12.5%	Ref.		Ref.	

Table 4 Captions

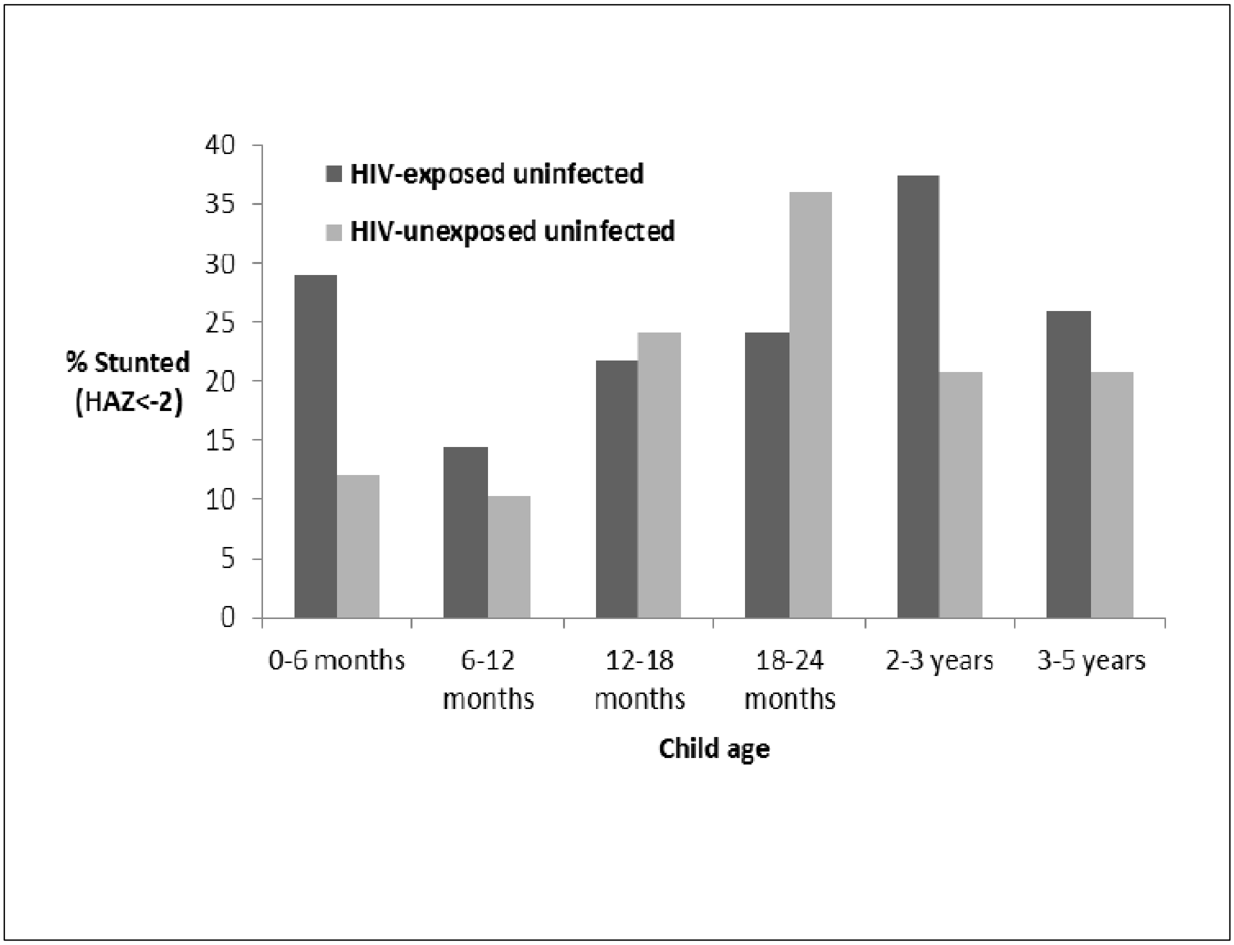
[1]Univariate and multivariate models accounted for clustering by facility.

[2]Multivariate models adjusted for maternal age (<25, 25-30, and 30+ years), maternal marital status (married, single, divorces/widowed), location of enrollment (urban, peri-urban, rural), household income <1,000 Pula per month (yes/no), electricity (yes/no), refrigerator (yes/no), tap water (yes/no), electric or F

[3]Wasting defined as WHZ < -2 and underweight defined as WAZ < -2.

Abbreviations: CI: confidence interval; HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected; SD: standard deviation; WAZ: weight-for-age z-score; WHZ: weight-for-height z-score; WLZ: weight-for-length z-score

Figure 1 Title. Prevalence of stunting (HAZ < -2) for HEU vs HUU children by child age



Abbreviations: HAZ: height-for-age z-score; HEU: HIV-exposed uninfected; HUU: HIV-unexposed uninfected

Online Supporting Material

SUPPLEMENTAL TABLE 1. Prevalence of stunting by time since cessation of breastfeeding for HIV-unexposed uninfected children 1-3 years of age (n=376)

Time since cessation of breastfeeding	% Stunted (Number stunted/total)
Current breastfeeding	18.9% (11/58)
No breastmilk <3 months	36.0% (18/50)
No breastmilk 3-6 months	26.5% (9/34)
No breastmilk 6-12 months	34.4% (33/96)
No breastmilk 12 months +	22.5% (31/138)