Early childhood diarrhea and growth among HIV-exposed, uninfected infants in Kenya

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Abstract

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Background: Diarrhea and poor linear growth are leading causes of childhood morbidity and mortality in low- and middle- income countries. While global childhood mortality has been significantly reduced in the past decades, further reductions will likely require a better understanding of the causes of and relationship between diarrhea and linear growth in vulnerable populations. The massive success of programs aimed at preventing mother-to-child transmission of HIV has resulted in a growing number of HIV-infected women giving birth to uninfected children. These HIV-exposed, uninfected (HEU) children, are an understudied and uniquely vulnerable population who experience higher rates of death and disability than their unexposed counterparts. These HEU children may be an important group to target for reducing diarrhea morbidity and linear growth faltering in order to further decrease global burden.

Methods: This dissertation used data from a historical cohort of HIV-infected mothers and their uninfected infants followed from pregnancy to 12 months postpartum in Nairobi, Kenya. Infant and maternal illness, including diarrhea, were ascertained at monthly study visits and sick visits. Infant length was recorded at monthly study visits. Anderson-Gill Cox models assessed maternal, environmental, and infant correlates of diarrhea, moderate-to-severe diarrhea (MSD; diarrhea with dehydration, dysentery, or related hospital admission), and prolonged/persistent diarrhea (Chapter 1). Length-for-age z-scores (LAZ) were used to measure infant linear growth. Mixed-effects models estimated the difference in monthly LAZ from 0-12 months by environmental,

maternal, and infant characteristics (Chapter 2). The relationship between diarrhea severity, treatment, burden, and timing and infant linear growth in the following month was tested throughout the first year of life using mixed-effects models (Chapter 3).

Results: Chapter 1: Over the 12 month follow-up period, HEU infants (n=373) experienced a mean 2.09 (95% Confidence Interval [CI]: 1.93, 2.25) episodes of diarrhea, 0.47 (95% CI: 0.40, 0.54) episodes of MSD, and 0.34 (95% CI: 0.29, 0.42) episodes of prolonged/persistent diarrhea. Postpartum maternal diarrhea was associated with increased risk of infant diarrhea (Hazard Ratio [HR]: 2.09; 95%: 1.43, 3.06) and infant MSD (HR: 2.89; 95% CI: 1.10, 7.59). In addition, maternal antibiotic use was a risk factor for prolonged/persistent diarrhea in these infants (HR: 1.63; 95% CI: 1.04, 2.55). Chapter 2: Among 372 HEU infants, mean LAZ decreased from -0.33 (standard deviation [SD]: 1.47) to -0.96 (SD: 1.23) between 0-12 months. Greater declines in LAZ were associated with household crowding and neonatal pneumonia, while higher maternal education and height were associated with greater gains in LAZ. Infants with low birthweight and birth stunting experienced some improvements in linear growth during infancy, with residual deficits at 12 months of age. Chapter 3: Diarrhea was associated an average loss of 0.07 (95% CI: -0.14, -0.00) in LAZ following the episode. More severe diarrhea (MSD) episodes were associated with greater declines in LAZ (adjusted difference [AD]: -0.18; 95% CI: -0.31, -0.06) compared to those without any diarrhea. Infants with any diarrhea (AD: -0.07 95% -0.16, 0.01) and MSD (AD: -0.22 95% -0.39, -0.04) not treated with antibiotics also experienced greater linear growth faltering than children with no diarrheal episodes and also no with antibiotics for any reason.

Conclusions: HEU children are at risk for diarrhea and linear growth faltering and among these children, diarrhea contributes to linear growth faltering. In addition to improved community and nutritional support for these infants, interventions targeted at improving maternal health and education may decrease diarrhea and improve growth.

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INTRODUCTION

In 2017 alone an estimated 5.4 million children under the age of five died, 75% within the first year of life [1]. Despite increasing numbers of live-births, childhood mortality has substantially decreased in recent decades. However, sub-Saharan Africa lags behind in this progress, with one in 13 children continuing to die before their fifth birthday [2]. Diarrheal illness and other acute preventable or treatable infectious diseases are responsible for more than half of under-five child deaths [2–4]. In addition, among surviving children, linear growth faltering is associated with risk of mortality and multiple morbidities, including poor cognitive development and reduced adult productivity [5–11]. Diarrheal disease contributes to childhood mortality both as an independent cause of death and by contributing to malnutrition, including poor linear growth [12–15]. Understanding the contribution of childhood diarrhea, including severity [16], treatment [17], burden [12,18], and timing [19,20], on linear growth among vulnerable populations remains critical to developing effective interventions to optimize childhood linear growth. Reducing global childhood mortality and long term outcomes requires a better understanding of the causes of and relationship between diarrhea and linear growth in vulnerable populations with high risk for early death.

While poor sanitation and low socioeconomic status are established risk factors for poor infant health, maternal health and disease may also influence infant risk of morbidity and mortality. Recent studies suggest improving maternal health may decrease infectious disease risk among their children [21,22]. In addition, as much as 20% of stunting at 12 months of age has origins in the fetal period [23]. Understanding the relative contributions of both pre- and postnatal causes of diarrhea and linear growth faltering may identify novel points of intervention to reduce childhood morbidity and mortality.

As implementation of effective measures to prevent mother-to-child transmission (PMTCT) of HIV have increased, children born to HIV-infected mothers are much less likely to acquire HIV than in previous decades [24–26]. The result is a growing number of HIV-exposed, uninfected (HEU) children, many of whom experience higher rates of death than their unexposed counterparts, primarily from infectious causes [27–40]. Many regions with highly prevalent HIV, such as sub-Saharan Africa, also bear the burden of high under-five mortality, diarrheal diseases, and poor linear growth [41,42], highlighting overlapping vulnerabilities in this population.

Children born to HIV-infected mothers may have a unique set of risk factors for acute infection and malnutrition resulting from impaired immune development, increased exposure to other infectious pathogens, and fewer social and economic resources. Despite these increased risks among HEU infant, few studies comprehensively characterize the risk factors, particularly maternal health, of infant diarrhea and linear growth. Chapter 1 assessed the role of maternal diarrhea and maternal antibiotic use plays in the risk of diarrhea of differing severities within the same cohort. Chapter 2 assesses the risk factors for infant linear growth trajectory and the effect of infant birth size and early infant illness on LAZ across the first year of life. Chapter 2 contributes to the knowledge base about potential catch-up growth among HEU infants after being born small and the effect of neonatal illness on this potential growth recovery. Finally, Chapter 3 tests the effect of diarrhea severity, treatment, burden, and timing on linear growth among HEU infants. Few studies have tested characteristics of diarrhea that may contribute to the effect on childhood linear growth in any population nor in a high risk population such as HEU infants.

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Chapter 1: Factors associated with increased risk of diarrhea among HIV-exposed, uninfected infants in Kenya

INTRODUCTION:

Diarrhea remains a significant cause of morbidity and mortality among children living in sub-Saharan Africa, contributing to nearly 10% of under-five deaths in the region [43,44]. Increasing evidence suggests diarrhea, particularly moderate-to-severe (MSD) and prolonged/persistent diarrhea, is associated with considerable long term morbidity, including growth compromise, increased frequency of other infections, and poor cognitive development [12,16,45,46].

In 2016, young women accounted for 290,000 new HIV infections in sub-Saharan Africa [47]. Widespread implementation of programs to prevent mother-to-child transmission (PMTCT) of HIV have successfully reduced risk of HIV transmission to children resulting in a growing population of children exposed to HIV but uninfected (HEU) [24]. Being born or living with an HIV-infected mother may present unique risk factors for diarrhea risk, such as frequent bouts of maternal diarrhea and/or increased maternal household antibiotic use. With 1.2 million HEU infants born each year, a reduction in diarrhea among this uniquely vulnerable population would contribute to the global decline in diarrhea burden [48]. We determined incidence and risk factors for diarrhea, MSD, and prolonged/persistent diarrhea in a cohort of HEU infants.

METHODS

Study design

This analysis utilized a historic cohort of HIV infected mothers and their infants. The parent study enrolled HIV positive pregnant women with gestation ≥28 weeks who attended Kenyatta National Hospital in Nairobi from 1999-2002. Further details regarding this cohort have been previously described [49,50]. Consistent with national guidelines at the time, participants received short-

course zidovudine for PMTCT of HIV and women with severe immunosuppression (CD4 count <200 cells/μl) were provided with cotrimoxazole prophylaxis and referred to HIV treatment programs. Per contemporaneous Kenyan guidelines during the parent study, infants did not receive additional prophylaxis nor did mothers receive ART during breastfeeding.

Eligibility criteria for our analysis included singleton birth or firstborn twin and at least one negative infant HIV PCR test at a recorded study follow-up visit. Infants testing positive for HIV by one month of age were considered perinatally infected and excluded from the analysis.

Data collection

At enrollment (about 32-weeks gestational age), sociodemographic and pregnancy medical history were collected using a standardized questionnaire, and blood samples were collected and processed for CD4 and HIV RNA viral load (VL) determination. After delivery, mothers were seen in the clinic with their infants at two weeks, four weeks, and then monthly up to 12 months (study visits). Additionally, mothers were encouraged to return to the clinic if the child was sick, from which morbidity diagnoses were recorded (sick visits). Infant morbidity and breastfeeding status were assessed by questionnaires and clinical exams at study visits. Maternal anthropometric measurements, CD4 count, and VL were collected at months 1, 3, 6, 9, and 12. Mothers were assessed for intercurrent illness at all study visits.

Statistical analysis

Definitions

Infant diarrhea was defined as maternal report since the last study visit or clinician diagnosis at any study or sick visit. Clinician diagnosis of diarrhea at sick visits was considered even if diarrhea was not the primary diagnosis. Reports of diarrhea within 14 days of a previous report were counted as the same episode to avoid double counting episodes. MSD was defined as diarrhea

with dehydration or dysentery, or a diarrhea associated hospitalization [3]. Diarrhea duration was ascertained in categories of ≥2 days and >1 week. We defined prolonged/persistent diarrhea to be episodes of >1 week.

Burden

Incidence of infant diarrhea was calculated by censoring at the last study visit at 12 months or at the last negative HIV test before the child was lost to follow-up, died, or had a positive HIV test, whichever came first. Interval censoring methods were used to censor person-time for missed visits and seven days before and after a diarrhea episode. Analyses were carried out for all infant diarrhea relative to no diarrhea, as well as the subset of only MSD and only prolonged/persistent diarrhea relative to no diarrhea or mild diarrhea not meeting MSD or prolonged/persistent diarrhea definitions.

Correlates

Hazard ratios (HR) and 95% confidence intervals (95% CI) for potential correlates of incident infant diarrhea were estimated using Anderson Gill Cox proportional hazard models with the Efron method for ties and robust standard errors. This model allows for repeated infant diarrhea events and time varying factors.

Potential correlates of diarrhea were specified *a priori* from established and suspected risk factors [51]. Maternal health indicators during pregnancy included low maternal CD4 count (<200, 200-499, versus ≥500 cells/μl), high VL (≥4 $\log_{10}$), low mid-upper arm circumference (MUAC, <23.5 cm) at enrollment, as well as report of antibiotic use and diarrhea. Antibiotic use and maternal diarrhea reported during the first year were also included. Infant factors included low birthweight (<2500 g) and breastfeeding at study visits (ever versus never; currently versus not currently breastfeeding). Other key covariates included household crowding (≥2 persons/room), type of

toilet (pit latrine versus flush), shared toilet (versus household), and maternal education (>primary education) measured at baseline. Maternal indicators during the first year of life (diarrhea and antibiotic use) were not collected at sick child visits and thus sick child visits were not included in time-varying analyses for these variables. Separate models tested the effect of time-varying maternal diarrhea and antibiotic use reported one month before and concurrent with infant diarrhea.

Crude and adjusted models were constructed to determine independent correlates of all infant diarrhea, MSD, and prolonged/persistent diarrhea. The following variables, in order, were considered for inclusion in the adjusted models: crowding, enrollment maternal CD4 count, and maternal MUAC. These covariates were retained in the adjusted model if the HR differed from the crude model by more than 10%. Adjusted models are presented as final models unless addition of the potential confounders did not change the crude model, in which case the crude was presented as the final model. Baseline maternal characteristics between infants lost to follow-up before the first visit and those included in the analysis were compared using Welch's t-tests (continuous variables) and Fischer's exact tests (binary variables). All analyses used two-sided hypothesis tests with an alpha of 0.05. Analyses were conducted in STATA 14 (StataCorp, College Station, Texas).

RESULTS

Study population

The parent cohort enrolled 510 pregnant HIV-infected women and recorded 468 live births of singleton or firstborn twins (6 second born twins were excluded). Among the 468 infants, 70 were perinatally HIV infected, 17 were lost to follow-up before the first visit, and eight had no regular visit negative HIV test (Figure 1). Mothers of infants who were lost to follow-up before the first visit had a slightly higher mean log VL (difference in means =0.42 $\log_{10}$ copies/ml; 95% CI: 0.03, 0.81),

but were not statistically different in any other characteristics at baseline. The remaining 373 HEU infants were included in this analysis, including 355 who remained uninfected in the first year and 18 who subsequently acquired HIV at median age of 181 days (interquartile range [IQR]: 90-354; Figure 1), whose visits after the last negative HIV test were censored.

At enrollment, most mother-infant pairs (84%) reported living in crowded households and used a pit latrine (52%). Mothers' median age at enrollment was 25 years (IQR: 22-28) and 42% had more than a primary school education. Prior to delivery, mothers' median CD4 count was 444 cells/μl (IQR: 317-618) and median HIV VL was 4.7 $\log_{10}$ copies/ml (IQR: 4.1-5.1); additionally, 14% were defined as undernourished (MUAC <23.5 cm). During pregnancy, 6% of mothers reported at least one episode of diarrhea and 20% reported using antibiotics. Postpartum, 22% of mothers ever reported diarrhea, among whom 127 total episodes were recorded (incidence rate 0.40 episodes/year). The majority of women (78%) reported antibiotic use for treatment at least once for a total of 813 occasions and 13% of women reported any cotrimoxazole prophylaxis use indicating severe immunosuppression (Table 1).

Infant diarrhea burden

Enrolled infants contributed 319.3 infant-years and 666 episodes of diarrhea. Of the 666 diarrhea episodes, 22% were considered MSD and 17% prolonged/persistent diarrhea (Table 2). The majority of infants (70%) experienced at least one diarrhea episode and the cohort experienced a mean 2.09 (95% CI: 1.93, 2.25) episodes of diarrhea, 0.47 (95% CI: 0.40, 0.55) episodes of MSD, and 0.34 (95% CI: 0.29, 0.42) episodes of prolonged/persistent diarrhea in the first year (Table 3). The incidence of all diarrhea peaked at ten months of age when the rate of diarrhea was 3.04 per infant-year (95% CI: 2.40, 3.85). The incidence of MSD was low in the first month (0.31 episodes/infant-year; 95% CI: 0.17, 0.57) and after two months of age the rate was stable

at approximately 0.50 episodes/infant-year. Incidence of prolonged/persistent diarrhea fluctuated throughout the first year with an average of about 0.33 episodes/infant-year (Figure 2).

Correlates of diarrhea, moderate-to-severe diarrhea, and prolonged/persistent diarrhea

All diarrhea

Household environment played an important role in risk of infant diarrhea. Infants living in households with pit latrine or in crowded homes had almost a 1.5-times higher risk of diarrhea than infants with a flush toilet or non-crowded households (HR: 1.44; 95% CI: 1.19, 1,74; HR: 1.35; 95% CI: 1.04, 1.76, respectively Table 3). Shared versus household toilet was not significantly associated with infant diarrhea.

Maternal diarrhea during follow-up was associated with an approximately 2-fold increase of infant diarrhea (HR: 2.09; 95% CI: 1.43, 3.06). Other maternal indicators, such as more than a primary education, maternal CD4, VL, diarrhea, or antibiotic use during pregnancy were not associated with infant diarrhea.

MSD

MSD was associated with toilet type, however, not with crowding. Infants living in a home with a pit latrine had a 49% increased risk for MSD (HR: 1.49; 95% CI: 1.04, 2.14) relative to those with a flush toilet, and children with a shared toilet had a 91% increased risk for MSD compared to those with a household toilet, with a trend for an association (HR: 1.68; 95% CI: 0.92, 3.04).

Maternal diarrhea during follow-up was associated with a 3-fold increase in infant MSD (HR: 2.89; 95% CI: 1.10, 7.59). Higher maternal VL at enrollment was associated with an increased risk of infant MSD (HR: 1.87; 95% CI: 1.04, 3.34), but maternal CD4 count was not (<200 cells/μl HR: 0.79; 95% CI: 0.36, 1.72 and 200-499 cells/μl HR: 1.01; 95% CI: 0.70, 1.45).

Currently breastfeeding infants had a 42% decreased risk of MSD (HR: 0.58; 95% CI: 0.39, 0.86), and infants who had ever been breastfed had a 31% decreased risk of MSD compared to those who never received breastmilk, with a trend for association (HR: 0.69: 95% CI: 0.47, 1.00, p-value=0.051).

Prolonged/Persistent Diarrhea

In contrast to all diarrhea and MSD, prolonged/persistent diarrhea was not associated with household environment factors. However, infants born to mothers with more than a primary education had a 58% decreased risk of prolonged/persistent diarrhea (HR: 0.42; 95% CI: 0.22, 0.80). Infants with mothers reporting antibiotic use had a 63% increased risk for prolonged/persistent diarrhea (HR: 1.63; 95% CI: 1.04, 2.55).

DISCUSSION

In this cohort of HEU infants, diarrhea, including MSD and prolonged/persistent diarrhea, occurred frequently during the first year of life. Risk factors varied by type of diarrhea with any diarrhea primarily associated with likelihood of infectious exposure (toilet type, crowding, and maternal diarrhea), whereas risk factors for MSD included likelihood of an infectious exposure (toilet type and maternal diarrhea) and factors potentially associated with a child's ability to fight the infection (high maternal VL and breastfeeding). Prolonged/persistent diarrhea was linked to low socioeconomic status (maternal education) and maternal antibiotic use, a potential indicator of mother's overall health.

Diarrhea incidence in this HEU cohort was slightly lower than the 3.5 episodes/child-year incidence reported from children in Nairobi during this period [52]. The lower rates of infant diarrhea in our study relative to other contemporaneously published estimates may be influenced

by ascertainment of diarrhea at monthly clinic visit rather than more frequent home visits used in other studies. Because of longer periods between data collection, mothers may have only reported more severe diarrhea episodes [53]. Our study documented a peak in infant diarrhea incidence around 9-11 months of age, similar to peak incidence (6-9 months of age) previously reported in HEU [22,54] and other cohorts [55]. Routine HIV treatment, PMTCT, and well child clinic visits around 6-9 months of age may be an opportunity to reinforce infant diarrhea prevention strategies including appropriate complementary feeding, vaccination, and preventative zinc supplementation [56].

Our cohort had similar rates of prolonged/persistent diarrhea and MSD as recent studies [16,52], including a nearly identical incidence rate of MSD to that reported from Western Kenya, a region of high maternal HIV prevalence, of 0.51 episodes/infant-year [3]. While diarrhea burden is generally thought to be declining in the sub-Saharan Africa, the rate of decline of the more severe forms of diarrhea may be stalled in populations with a number of HEU or other vulnerable children.

We evaluated risk factors for three different types of diarrhea to elucidate overlapping and distinct mechanisms. There are limited data comparing risk factors between all reported diarrhea and more severe types of diarrhea (MSD and prolonged/persistent) associated with long-term sequelae, particularly among HEU infants [3,57].

We found that the risk factors for any diarrhea were linked to the likelihood that a child was exposed to an infectious agent through crowding, toilet type, and maternal diarrhea. We found flush toilets to be associated with a reduced risk of diarrhea, supporting the inclusion of improved sanitation components in diarrhea prevention strategies even for children not yet old enough to use the toilets themselves [58,59]. Toilet type in our cohort may also represent lower socioeconomic status and limited access to clean water or other risk factors for diarrhea. Holistic

approaches to families with HIV, including addressing water and sanitation, are likely to yield improved health [60].

Our finding of an association between maternal diarrhea and infant diarrhea is consistent with Zambia and Malawi studies among HEU children [61,62]. The association between maternal diarrhea and infant diarrhea and MSD is likely due to shared environment or person-to-person pathogen transmission. Maternal-infant illnesses are often shared and underscore the importance of concurrently addressing mother and child health to optimize child growth and developments. Interventions in caregivers likely impact child health as evidenced by reductions in infant diarrhea associated with maternal ART and multivitamins [21,22,63].

Similar to all diarrhea, MSD was associated with household and environmental factors (pit latrine and maternal diarrhea). In addition, breastfeeding and low maternal VL were protective against MSD suggesting that these factors influenced the infant's capacity to contain diarrheal infection. International guidelines recommend exclusive breastfeeding for all infants in the first six months of life as a key diarrhea prevention strategy [64,65]. It is possible that breastfeeding prevents exposure to or protects against pathogens causing more severe diarrhea [66,67]. The increased risk of MSD among infants of mothers with high VL may stem from impaired infant immune responses following *in utero* exposure to maternal HIV [68–70]. Although today there is higher coverage of ART for mothers resulting in lower viral loads with an estimated 80% of HIV infected mothers globally being virally suppressed during pregnancy. These data again highlight the importance of a strong maternal immune system for her infants' health [71].

In contrast to all diarrhea and MSD, prolonged/persistent diarrhea was associated with lower maternal education and postpartum maternal antibiotic use. Low mother's education is an established predictor for poor childhood health outcomes, including diarrhea [16,72] and

malnutrition [73], the latter being an established consequence of prolonged/persistent diarrhea [16]. Antibiotics taken by the mother may be transferred to the infant through breastfeeding, disrupting the infant's microbiome, and increasing their risk of antibiotic-associated diarrhea [74]. However, such a reason would not explain maternal antibiotic's unique association with prolonged/persistent diarrhea and not all diarrhea or MSD. Maternal antibiotic use during the postpartum period may be an indicator of declining health and immunity in the mother. Given the infant immune system is especially implicated in prolonged/persistent diarrhea, it could be that the association between maternal antibiotic use and infant prolonged/persistent diarrhea is explained by a parallel decline in both mother and child health. There is increasing evidence suggesting exposure to maternal antibiotic *in utero* may affect infant microbiome and immune development, potentially increasing susceptibility and severity of childhood infections [75,76]. However, while we found an association with postpartum maternal antibiotic use and infant prolonged/persistent diarrhea, we did not find associations with prepartum maternal antibiotic use.

There are several limitations of this historic cohort study. The data were collected prior to widespread maternal ART use, HEU cotrimoxazole prophylaxis, and childhood rotavirus vaccination, three interventions that may reduce infant diarrhea. However, this historic cohort provides a unique opportunity to define how maternal diarrhea contributes to infant diarrhea in the absence of such interventions thereby providing a natural history. Also, maternal diarrhea was common in this cohort, likely because of the lack of these interventions, increasing the power to detect mother/infant associations. Maternal CD4 count was not associated with infant diarrhea in the present cohort, and associations between maternal morbidity and infant diarrhea may persist despite ART-associated improvements in maternal immunity. Secondly, the cohort was not initially designed to focus on diarrhea and diarrhea may have been under ascertained because reports were collected monthly. We accepted diagnosis or report of diarrhea even if it was not the primary diagnosis for a sick child visit, likely capturing some diarrhea that was secondary to other

illness. The study may also suffer from non-random loss to follow-up. For example, formula fed infants were more likely to die, and to do so early in follow-up, than breastfeeding infants in this cohort. This may partially explain failure to detect a protective relationship between breastfeeding and all infant diarrhea [66,77].

In summary, we found household and environmental factors both predicted all diarrhea and MSD, while breastfeeding and low maternal VL were uniquely protective for MSD, and maternal education and postpartum maternal antibiotic use appeared important for prolonged/persistent diarrhea. Differing risk factors and consequences between all diarrhea and more severe types of infant diarrhea may represent distinct mechanisms for these diarrhea pathologies. Identification of maternal diarrhea and antibiotic use may be an opportunity to identify high risk infants and deliver interventions to prevent or treat infant diarrhea. Targeted delivery of interventions to HIV-infected caregivers during regular clinic visits, including education on signs of severe, child illness, encouraging care seeking behavior, breastfeeding support, and oral rehydration salts and zinc may result in substantial reductions in diarrhea morbidity and mortality among HEU infants.

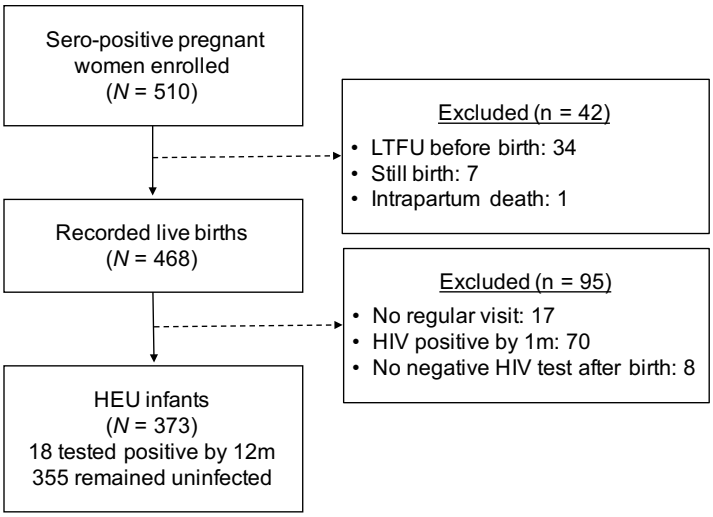


Figure 1: Participant flow chart

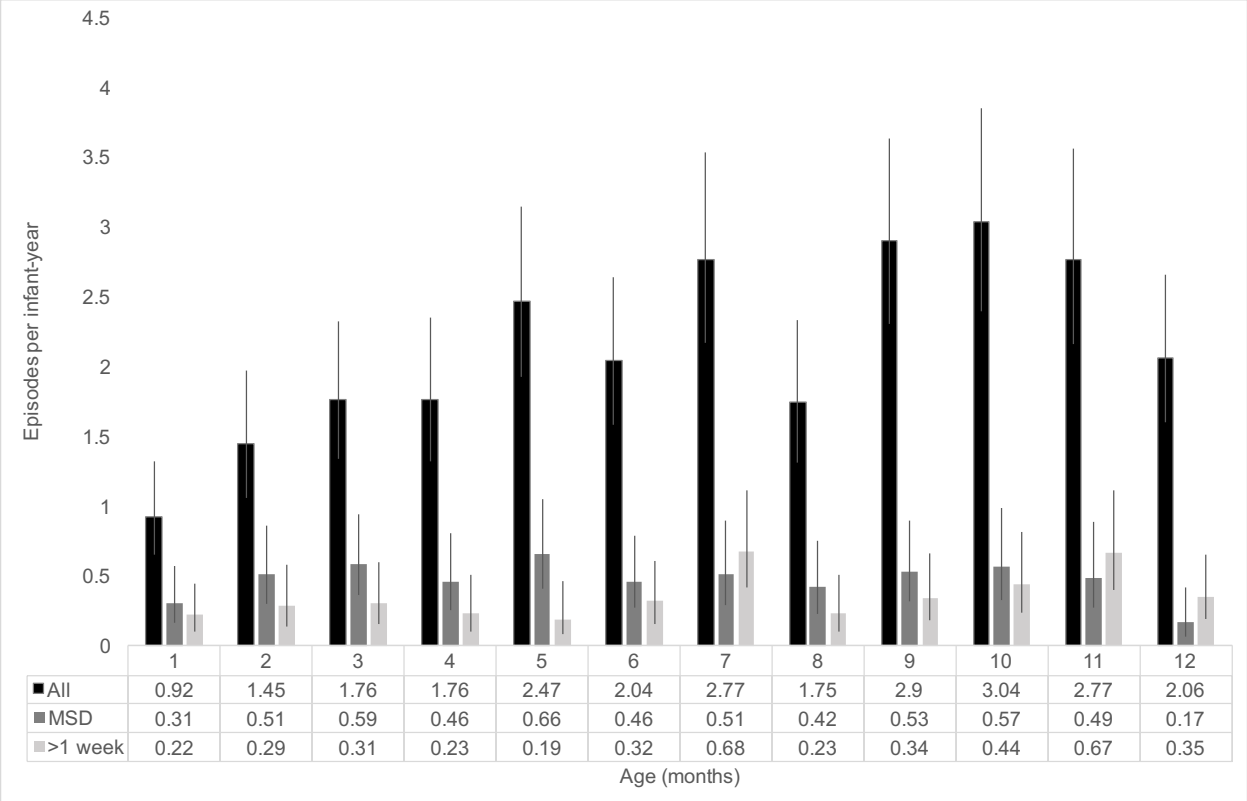
Table 1: Baseline and follow-up characteristics among 373 HEU infants and their mothers.

Covariates	N (%)[1]
TOTAL	**373**
Home environment factors	
Pit latrine	193 (52)
Flush toilet	180 (48)
Shared toilet	340 (91)
Household toilet	32 (9)
≥ 2 persons/room in house	315 (84)
< 2 persons/room in house	55 (15)
Maternal factors	
> primary education	155 (42)
≤ primary education	214 (57)
Anthropometry at 32-weeks gestational age	
MUAC < 23.5	51 (14)
MUAC ≥ 23.5	244 (65)
Maternal HIV at 32-weeks gestational age	
CD4 count < 200 cells/μl	28 (8)
CD4 count 200-499 cells/μl	190 (51)
CD4 count ≥ 500 cells/μl	147 (39)
Log VL ≥ 4	267 (72)
Log VL < 4	67 (18)
Pregnancy health	
Diarrhea	21 (6)
No diarrhea	352 (94)
Antibiotic use	75 (20)
No antibiotic use	298 (80)
Postpartum health	
Ever diarrhea	82 (22)
Never diarrhea	247 (66)
Ever antibiotic use	290 (78)
Never antibiotic use	83 (22)
Infant factors	
Birthweight < 2500g	21 (6)
Birthweight ≥ 2500g	343 (92)
Ever breastfed	279 (75)
Never breastfed	94 (25)

[1]Percents may not add to 100% due to missing data

Table 2: Descriptive diarrhea episodes experience by 373 HEU infants, N=666.

Characteristic	n (%)	Incidence per i-years
Duration		
≥2 days	413 (62%)	1.29
>1 week	110 (17%)	0.34
Moderate-to-severe	149 (22%)	0.47
Dysentery	25 (4%)	0.08
Dehydration	118 (18%)	0.37
Hospitalization	16 (2%)	0.05



	1	2	3	4	5	6	7	8	9	10	11	12
All	0.92	1.45	1.76	1.76	2.47	2.04	2.77	1.75	2.9	3.04	2.77	2.06
MSD	0.31	0.51	0.59	0.46	0.66	0.46	0.51	0.42	0.53	0.57	0.49	0.17
>1 week	0.22	0.29	0.31	0.23	0.19	0.32	0.68	0.23	0.34	0.44	0.67	0.35

Figure 2: Incidence rate of all, moderate-to-severe (MSD), and prolonged/persistent infant diarrhea by infant age**.**

Table 3: Correlates of all, moderate-to-severe, and prolonged/persistent diarrhea in HEU infants in Kenya N=373.

Covariates	All Diarrhea		Moderate-to-severe diarrhea		Prolonged/persistent diarrhea	
	Incidence i-yr (95% CI)	Final HR (95% CI)	Incidence i-yr (95% CI)	Final HR (95% CI)	Incidence i-yr (95% CI)	Final HR (95% CI)
Total	2.09 (1.93, 2.25)	--	0.47 (0.40, 0.55)	--	0.34 (0.29, 0.42)	--
Home environment factors						
Pit latrine	2.44 (2.22, 2.69)	**1.44 (1.19, 1.74)**	0.55 (0.45, 0.68)	**1.49 (1.04, 2.14)**	0.36 (0.28, 0.46)	1.07 (0.67, 1.73)
Flush toilet	1.69 (1.49, 1.91)	Ref	0.37 (0.28, 0.48)	Ref	0.33 (0.25, 0.44)	Ref
Shared toilet	2.15 (1.99, 2.33)	1.30 (0.88, 1.91)[1]	0.48 (0.40, 0.56)	1.68 (0.92, 3.04)[3]	0.36 (0.29, 0.43)	1.73 (0.62, 4.81)[1,3]
Household toilet	1.54 (1.16, 2.04)	Ref	0.38 (0.22, 0.68)	Ref	0.26 (0.13, 0.51)	Ref
≥ 2 persons/room in house	2.16 (2.00, 2.35)	**1.35 (1.04, 1.76)**	0.46 (0.39, 0.55)	1.07 (0.68, 1.68)	0.37 (0.3, 0.45)	1.38 (0.63, 3.03)[3]
< 2 persons/room in house	1.62 (1.3, 2.01)	Ref	0.43 (0.29, 0.66)	Ref	0.24 (0.13, 0.42)	Ref
Maternal factors						
> primary education	1.89 (1.67, 2.14)	0.86 (0.71, 1.04)	0.42 (0.32, 0.54)	0.84 (0.58, 1.20)	0.22 (0.15, 0.31)	**0.42 (0.22, 0.80)[3]**
≤ primary education	2.19 (1.99, 2.42)	Ref	0.50 (0.40, 0.61)	Ref	0.43 (0.34, 0.54)	Ref
Anthropometry at 32-weeks gestational age						
MUAC < 23.5	2.03 (1.64, 2.52)	0.98 (0.73, 1.30)	0.58 (0.39, 0.87)	1.23 (0.75, 2.02)	0.48 (0.31, 0.75)	1.86 (0.91, 3.83)
MUAC ≥ 23.5	2.09 (1.90, 2.29)	Ref	0.47 (0.39, 0.57)	Ref	0.29 (0.22, 0.37)	Ref
Maternal HIV at 32-weeks gestational age						
CD4 count < 200 cells/μl	1.62 (1.16, 2.26)	0.76 (0.55, 1.07)	0.37 (0.19, 0.74)	0.79 (0.36, 1.72)	0.09 (0.02, 0.37)	0.32 (0.08, 1.27)[3]
CD4 count 200-499 cells/μl	2.08 (1.88, 2.32)	0.97 (0.80, 1.18)	0.48 (0.38, 0.59)	1.01 (0.70, 1.45)	0.35 (0.27, 0.45)	0.87 (0.49, 1.55)[3]
CD4 count ≥ 500 cells/μl	2.15 (1.91, 2.42)	Ref	0.47 (0.36, 0.61)	Ref	0.36 (0.27, 0.48)	Ref
Log viral load ≥ 4	2.04 (1.86, 2.23)	**1.04 (0.82, 1.32)**	0.51 (0.42, 0.61)	**1.87 (1.04, 3.33**)	0.33 (0.26, 0.41)	0.86 (0.41, 1.83)[3]
Log viral load < 4	1.99 (1.66, 2.38)	Ref	0.27 (0.17, 0.44)	Ref	0.34 (0.22, 0.53)	Ref
Pregnancy health						
Diarrhea	2.69 (2.03, 3.56)	1.33 (0.99, 1.79)	0.60 (0.33, 1.09)	1.33 (0.84, 2.11)	0.49 (0.26, 0.95)	1.28 (0.56, 2.94)[3]
No reported diarrhea	2.05 (1.89, 2.22)	Ref	0.46 (0.39, 0.54)	Ref	0.34 (0.28, 0.41)	Ref
Antibiotic use	2.15 (1.82, 2.53)	1.06 (0.84, 1.33)	0.51 (0.36, 0.71)	1.11 (0.71, 1.74)	0.34 (0.22, 0.51)	1.01 (0.55, 1.85)[2,3]
No reported antibiotic use	2.07 (1.90, 2.25)	Ref	0.46 (0.38, 0.55)	Ref	0.35 (0.28, 0.43)	Ref

Covariates	All Diarrhea		Moderate-to-severe diarrhea		Prolonged/persistent diarrhea	
	Incidence i-yr (95% CI)	**Final HR (95% CI)**	**Incidence i-yr (95% CI)**	**Final HR (95% CI)**	**Incidence i-yr (95% CI)**	**Final HR (95% CI)**
Postpartum health at study visit						
Diarrhea	4.41 (3.21, 6.06)	**2.09 (1.43, 3.06)**	0.70 (0.31, 1.55)	**2.89 (1.10, 7.59)**[4]	0.58 (0.24, 1.4)	0.83 (0.21, 3.26)
No reported Diarrhea	1.86 (1.71, 2.03)	Ref	0.24 (0.19, 0.30)	Ref	0.32 (0.26, 0.4)	Ref
Antibiotic use	2.09 (1.75, 2.48)	1.09 (0.87, 1.36)	0.08 (0.03, 0.20)	0.46 (0.14, 1.53)[4]	0.46 (0.32, 0.67)	**1.63 (1.04, 2.55)**
No reported antibiotic use	1.82 (1.65, 2.01)	Ref	0.18 (0.13, 0.25)	Ref	0.27 (0.21, 0.35)	Ref
Infant factors						
Birthweight < 2500g	2.30 (1.66, 3.19)	1.13 (0.74, 1.71)	0.58 (0.30, 1.11)	3.02 (0.78, 11.66)[4]	0.51 (0.26, 1.02)	0.82 (0.34, 1.98)[2]
Birthweight ≥ 2500g	2.07 (1.92, 2.24)	Ref	0.46 (0.39, 0.55)	Ref	0.34 (0.28, 0.41)	Ref
Ever breastfed	2.09 (1.92, 2.28)	1.02 (0.81, 1.28)	0.42 (0.34, 0.51)	0.69 (0.47, 1.00)[5]	0.38 (0.31, 0.47)	1.41 (0.65, 3.05)[3]
Never breastfed	2.07 (1.78, 2.40)	Ref	0.61 (0.46, 0.80)	Ref	0.24 (0.16, 0.38)	Ref
Currently breastfeeding	1.89 (1.70, 2.11)	0.98 (0.81, 1.19)	0.35 (0.27, 0.45)	**0.58 (0.39, 0.86)**	0.34 (0.26, 0.43)	1.3 (0.69, 2.42)[3]
Not currently breastfeeding	2.32 (2.09, 2.59)	Ref	0.60 (0.49, 0.74)	Ref	0.36 (0.27, 0.47)	Ref

Adjusted for [1] crowding, [2]32-week gestation CD4 percent, or [3]32-week maternal undernutrition (MUAC < 23.5), or [4]all 3.
[5]p-value=0.051
BOLD pvalue <0.05 * pvalue<.001
Abbreviations: i-yr: infant year; MUAC: Mid-upper arm circumference

Chapter 2: Neonatal pneumonia associated with linear growth faltering among HIV-exposed uninfected infants in Kenya

INTRODUCTION

Chronic malnutrition is prevalent in sub-Saharan Africa, where approximately 36% of children under-five are stunted (length-for-age z-score [LAZ] <-2) [23]. Poor linear growth contributes to nearly a half of all childhood deaths globally [23,78]. Linear growth faltering, an abnormally slow rate of height gain (measured as decline in LAZ), in the first two years of life is also associated with cognitive delays, poor school achievement, and reductions in adult income earning potential [5–11].

The rapid expansion of effective strategies to prevent mother-to-child transmission (PMTCT) has resulted in a growing population of HIV-exposed infants who are born uninfected (HEU) and remain HIV-uninfected [24–26]. In 2016 alone, more than one million HEU infants were born [79]. These HEU infants are born with lower mean birthweight and length as compared to HIV-unexposed, uninfected infants [32,36,80–84]. HEU infants also experience higher rates of stunting within the first five years of life than HIV-unexposed uninfected (HUU) children [80,81,85–87].

Linear growth during infancy is influenced by a multitude of factors. Both maternal and neonatal factors influence linear growth during infancy [88,89], with up to 20% of stunting at one year of age originating in the fetal period [23]. However, while the relationship between childhood infections and malnutrition is clear, studies evaluating the long-term effects of early life diarrhea and pneumonia, on childhood linear growth are not clearly defined [23,88–94]. Further assessment of prenatal and early life risk factors potentially associated with linear growth faltering is needed to identify interventions to optimize child growth.

Few studies have examined risk factors for poor linear growth among HEU children. HEU children may have immune consequences due to HIV exposure in utero and during breastfeeding. In addition, HEU children may be more likely to be exposed to infectious diseases as a consequence of living with one or more HIV-infected individuals. We examined prenatal and early infant predictors of linear growth during the first year of life among HEU children.

METHODS

Study design

The parent cohort study has been described elsewhere [49,50,95]. Briefly, HIV-infected pregnant women living in Nairobi, Kenya between 1999 and 2002 were enrolled at ≥28 weeks' gestation and followed with their infants for 12 months postpartum to assess immunologic mechanisms of protection from breastmilk HIV transmission.

Clinical procedures

During pregnancy, all women were counseled regarding the risk of HIV-transmission via breastmilk and they were supported in their choice to breastfeed or formula feed their infant, including provision of free formula. According to national guidelines during the study period, mothers received short-course zidovudine for PMTCT, and those severely immunocompromised mothers with CD4 count <200 cells/μl received cotrimoxazole prophylaxis (per Kenyan National Guidelines at that time). Mothers did not receive antiretroviral therapy during breastfeeding.

Sociodemographic and current and past health information was collected at enrollment with a standardized questionnaire. All participants underwent a comprehensive clinical exam that included anthropometric measurements and blood samples for CD4 count and HIV RNA viral load tests. Mothers and infants were seen at two weeks postpartum and monthly from birth to 12 months of age. At each visit, infants underwent a physical examination by study physicians; trained staff measured the infant's recumbent length and weight. Mothers were asked about

breastfeeding and infant illness in the past month including diarrhea, (including diarrhea, pneumonia, and hospitalizations) using standardized questionnaires. To assess infant HIV status, dried blood spots (DBS) were collected at birth and all subsequent study visits for HIV DNA detection using polymerase chain reaction (PCR) for HIV DNA *gag* sequences [96].

Current analysis

The current analysis included singleton or firstborn twins with documentation of sex, at least one negative HIV PCR confirmatory test, and at least two recorded length measurements. Infants were excluded from the analysis if they tested positive for HIV at or before one month of age due to likely perinatal HIV infection. Infants remained in the analysis until the end of follow up (age 12 months) or their last HIV-negative test for those who died, tested HIV PCR positive, or were lost to follow up.

To reduce potential bias due to missing values, multivariable Normal regression models using a Markov chain Monte Carlo method were used to impute missing values (m=10 imputations) [97]. All correlates in addition to 12-month HIV status, infant sex, infant mortality, and maternal mortality were included in the imputation models. For comparison, we also conducted a complete case analysis. LAZ was calculated using the WHO Anthro software package based on the 2006 WHO Child Growth Standards [98]. Neonatal illness included diagnosis or maternal report of pneumonia, diarrhea, or hospitalization at or before (since the last visit) the week two or month one study visit.

Loess curves were used to plot growth over time. We estimated the average LAZ trajectories by environmental, maternal, and infant characteristics using linear spline mixed-effects models with knot points at four and eight months. Random effects for the intercept and slope as well as an autocorrelation structure for residual errors were included to account for within-person correlation in the variance estimation. Models included the correlate of interest, spline terms for infant age,

and interaction terms between each correlate and age. The multivariable model included correlates associated with LAZ in univariate models (p-value <0.05). We tested for collinearity using standard error assessments. Predicted LAZ from the univariate mixed-effects models were used to plot growth profiles for correlates included in the multivariable model. We conducted sensitivity analyses to evaluate the influence of error in length measurements. If the difference in length between two consecutive visits indicated a loss in length, the length measure from the previous visit was carried forward to replace the smaller length measurement and the main analysis was replicated using an edited dataset.

The parent cohort study received ethical approval from the Kenyatta National Hospital Ethics and Research Committee and the University of Washington Institutional Review Board. The current analysis was ruled exempt from ethics review by the University of Washington, as a secondary analysis of de-identified data. All analyses were conducted in Stata 14 (StataCorp, College Station, Texas) and p<0.05 was considered statistically significant.

RESULTS

Study population

Overall, 372 singleton or first twins (6 twins) born to HIV-positive women in the parent cohort were included in the present analysis (Figure 1). At enrollment, median maternal age was 25 years (IQR 22-28) and 42% of mothers had more than a primary education. At 32 weeks' gestation (enrollment), 13% of mothers were undernourished (mid-upper arm circumference [MUAC] <23.5), 8% were severely immunocompromised (CD4 count <200 /μl), and 30% had high viral load (viral load >$\log_{10}$ 5 copies/ml). At enrollment, 52% of participants' homes had a pit latrine, 91% a shared versus household toilet, and 84% of households were considered crowded (defined as ≥2 people per room).

Six percent of infants were born with low birthweight (<2500 g; LBW) and 14% were stunted at birth; fifteen (4%) infants had both LBW and stunting at birth. The majority of infants (75%) were breastfed with 29% exclusively breastfed for at least three months. In the neonatal period, 4% of infants experienced diarrhea, 4% were diagnosed with pneumonia, and 7% were hospitalized.

Growth patterns in the first year of life

Of the 372 infants, 18 acquired HIV and 29 died before the end of 12-month follow-up. These children were censored at their last negative HIV test, a median of 92 (interquartile range [IQR]: 30-183) and 90 (IQR: 32-180) days, respectively. On average, infants experienced a deterioration of LAZ during the first year of life, from -0.33 (standard deviation [SD]: 1.18) at birth to -0.96 (SD: 1.23) at 12 months, with a mean change in LAZ of -0.41 (95% Confidence Interval [CI]: -0.55, -0.27). By one year of age, 17% (39) of children were stunted, of whom 28% (11) were stunted at birth. Among the 34 infants stunted at birth with available 12-month LAZ, 32% (11) remained stunted at one year, while 68% (23) were not stunted at 12 months of age.

Early-life correlates of infant linear growth

Socioeconomic status and sanitation were associated with change in LAZ from birth to 12 months. Infants in homes with pit latrines (p=0.010), shared toilets (p=0.031), or crowded living conditions (p=0.007) experienced a greater deterioration of LAZ (Table 1), despite having similar LAZ at birth (Figure 3). Of the household sanitation factors, crowding and use of a pit latrine remained significantly associated with greater declines in LAZ in the multivariable analysis (p=0.034, p=0.028, respectively).

Mothers with more than primary education had infants with significantly less deterioration of LAZ (p<0.001) compared to mothers with less education, even after adjusting for infant birth size, household toilet type, and neonatal infant pneumonia. Greater maternal height (p=0.017) was associated with gains in LAZ, while maternal undernutrition (as defined by MUAC) (p=0.117) and

pregnancy weight (p=0.148) were not associated with infant LAZ. The significant association between infant growth and maternal height also held after adjustment for LBW, birth stunting, and other correlates (Table 1). Maternal CD4 count in pregnancy (CD4 <200: p=0.702; CD4 200-499: p=0.481) and viral load (p=0.422) were not associated with infant LAZ.

Infants with LBW or stunting at birth had lower LAZ scores in the first year of life, but experienced gains in linear growth by 12 months while non- LBW and non-stunted at birth infants experienced loss or no change (Figure 3; p<0.001). Despite experiencing gains, infants with low birth weight and born stunted had residual length deficits at 12 months. The mean LAZ at 12 months was lower for LBW infants relative to of non-LBW infants (-1.31; SD: 1.26 versus -0.92; SE: 1.22, respectively, p=0.258), and similarly lower for infants stunted at birth compared to infants not stunted at birth (1.57; SD: 1.34 versus -0.81; SE: -0.97 respectively, p<0.001). The associations remained significant in the multivariable analysis for both variables (p=0.007; p<0.001, respectively). Neither any breastfeeding nor exclusive breastfeeding for the first three months were associated with 0-12 month change in LAZ (p=0.090; p=0.412, respectively). Early life diarrhea and hospitalization were also not associated with changes in LAZ (p=0.508, p=0.243, respectively). Neonatal pneumonia was associated with declines in LAZ (p=0.004), even after adjustment. Infants with neonatal pneumonia had lower birth LAZ and continued to have lower LAZ and greater LAZ decline from birth to 12 months than infants without neonatal pneumonia (Figure 3).

Results from the sensitivity analyses without imputed data and corrections in infant length measurement error (Tables 2 and 3) were consistent with those observed in the univariate analyses. While the main effects in the multivariable analyses were relatively unchanged, pit latrine (vs flush toilet), household crowding, and LBW were no longer significantly associated with infant LAZ in either sensitivity analysis.

DISCUSSION

Stunting results from a combination of biologic, social, economic, and political factors [99]. In this cohort of HEU infants we assessed early life household, maternal, and infant factors associated with linear growth from birth to 12 months of life. HEU infants with LBW or birth stunting experienced modest recovery in linear growth and less linear growth decline as compared to normal birthweight and non-stunted infants, and also experienced some residual length deficits remained at 12 months of age. Most of the HEU infants that were stunted by 12 months of age were not stunted at birth, underscoring the importance of postnatal factors in growth during the first year of life. Neonatal pneumonia was independently associated with persistent declines in length throughout the first year of life, while neonatal diarrhea and all-cause hospitalization was not. Higher maternal education and maternal height protected from linear growth faltering whereas household crowding and lack of access to flush toilets were associated with linear growth declines.

On average, the HEU infants in this analysis experienced a decline of 0.55 LAZ during the first year of life. The mean LAZ at 12 months in this analysis (-0.96) was half that of the reported Kenya national average at the same age (-1.80 SD), the magnitude of the observed decline in this study is lower than other recent cohorts of HEU children in sub-Saharan Africa which have documented changes in z-scores of a -1.00 to -1.50 SD in the first year [89,90,94,100,101]. Kenyan national averages include regions such as Western Kenya, which have higher rates of stunting than Nairobi, in which this study was conducted. It is possible that this cohort had more engagement in health care than the general population due to monthly study clinic visits, including regular counseling on infant nutrition and health, and free infant formula. In addition, rates of stunting in this study may be lower than expected because we did not follow children to 24 months of age which is the peak age of stunting prevalence in the general population [89,90,100].

We noted a strong association between neonatal pneumonia and LAZ decline. This relationship persisted after adjusting for socioeconomic factors, maternal height, LBW, and birth stunting. Stunting and acute malnutrition are both known to increase the risk for acquisition and severity of pneumonia [93,102]. While historical studies have shown an association between pneumonia and other respiratory infections and length gains in children age 9-36 months [103,104], recent studies in older children have not detected an association between respiratory illness and linear growth [89,94]. Neonatal pneumonia may be more likely to occur in HEU infants, due to increased exposure to pathogens from household members with HIV or to reduced quantity and function of passively acquired maternal antibodies [105]. Pneumonia infection in the first month of life may identify infants with a declining linear growth trajectory influenced either by prenatal factors or their postnatal environment.

We expected that diarrhea early in life would be associated with linear growth faltering [12,106–108]. However, unlike pneumonia, we did not observe an association between neonatal diarrhea and poor linear growth. In this analysis, a diagnosis of pneumonia was determined by a health care provider whereas a diarrhea diagnosis was either reported by the caregiver or by provider diagnosis. As a result, pneumonia diagnosis may reflect more severe disease. However, hospitalization, another indicator of non-specific disease severity, was not associated with linear growth declines in this cohort. It is possible that the effects of diarrhea on growth may depend on burden or severity of diarrhea over the first year of life, neither of which were well captured in this cohort. In addition, recent evidence suggests that specific enteric pathogens may be responsible for linear growth faltering independent of symptomatic diarrhea and these pathogens were not evaluated in this study [17,109].

Infants with LBW or stunting at birth had lower LAZ throughout follow up than their counterparts. However, consistent with other studies, LBW and birth stunted infants experienced linear growth catch-up, nearly returning to the levels of their non-LBW counterparts by 12 months of age [94].

LBW has been identified as a mediator of the relationship of HIV exposure and stunting [110] and as much as 20% of stunting in the first year of life has been attributed to fetal growth restriction [23,111]. We found that almost a third (28%) of infants stunted at 12 months were stunted at birth, suggesting an appreciable impact of fetal growth on long-term stunting. There is some evidence that maternal antiretroviral therapy (ART) or specific antiretroviral drugs may increase the risk of preterm birth and early stunting in HEU infants [112–114]. As ART continues to be more widely used and the population of HEU children continues to grow, the relative contribution of ART-associated linear growth faltering in the overall prevalence of stunting may be appreciable. With WHO guidelines recommending lifelong ART among all HIV-infected individuals, including HIV-infected pregnant women, it will be important to identify factors associated with linear growth in the context of longer maternal ART exposure and specific drug regimens.

In this analysis infants born to mothers with more than primary education or of taller stature experienced less linear growth decline compared to those with less educated and shorter mothers. Maternal education is consistently associated with improved childhood growth in sub-Saharan Africa [94,115]. Among HIV-infected mothers, those with more schooling may have greater knowledge regarding personal health care, health care seeking, and adherence to medical advice, each of which may help to protect the health of the mother and her infant. In addition, maternal education may be a proxy for improved health care access. Taller maternal stature suggests that linear growth is in part informed by genetic potential for growth of an infant. This association could also suggest the inter-generational causes of stunting where a mother, stunted since childhood, gives birth to a stunted baby, indicating mother's early life environment and nutrition contributes to the growth trajectory of her future infant [23,92,116].

We noted associations between household toilet type and crowding with decline of LAZ. Pit latrines, shared toilets, and household crowding all represent increased potential for contamination of the environment and exposure to infectious pathogens. They also may represent

low socioeconomic status, limited resources, and agency to access necessary health care and nutrient rich foods to prevent and treat infections and malnutrition. Because of the complex relationships between these contributing factors, childhood malnutrition and stunting has been difficult to prevent and treat. Targeted interventions to improve household or community sanitation and hygiene have not demonstrated consistent improvement in linear growth [117,118]. However, some evidence does suggests that broad multi-sectoral interventions to promote access and use of health care services and improved household financial stability decreases the prevalence of stunting nationwide [119].

There are limitations to this study. First, the study cohort experienced less stunting and had higher 12-month LAZ than anticipated which decreased statistical power to detect modest associations. Second, not all infants had length measurements at each of the 12 follow up visits. While we addressed this missingness by using mixed-effects models that address interval missingness of the outcome variables by evaluation associations up until the time of censoring, those with missing 12-month values may have differed from those with values leading to biased estimates. Thirdly, potential measurement errors of length, which we expect to be non-differential across level of predictors, may have reduced the statistical power and led to attenuations of effects. Finally, the study cohort did not receive the current Option B+ regimens, and therefore was unable to examine effects of specific current ART regimens.

In summary, pneumonia in the neonatal period may be a predictor of infants with future declines in linear growth trajectory. Most LBW and birth stunted infants may experience some linear growth recovery in the first year of life, while almost one-third of infants stunted at 1 year of life had stunting at birth. There are likely distinct mechanisms by which pre-and post-birth factors contribute to linear growth decline or recovery. Interventions to improve linear growth among HEU infants require a multi-faceted approach, including improvement of sanitation, maternal education,

and prevention of early life infant infections, to address variety of mechanisms causing linear growth faltering in vulnerable populations.

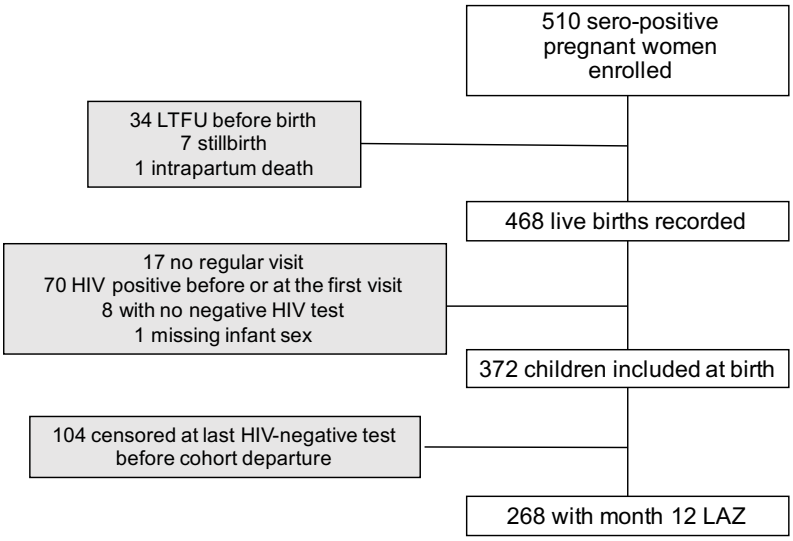


Figure 1: Participant flow chart

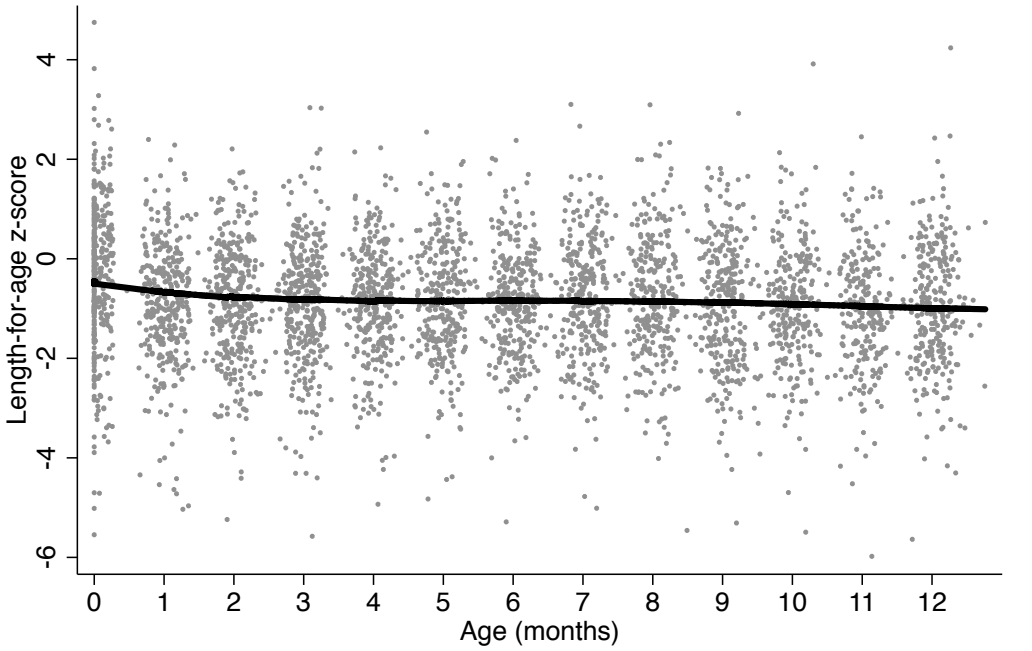


Figure 2: Scatter plot change in LAZ over time by infant age. Loess curve represents modest growth decline over first year.

Table 1: Correlates of 0-12 month change in LAZ scores of 373 Kenyan HEU infants

Correlates	N (%)[1] or median IQR	LAZ scores		
		Change 0-12 months[2]	Univariate Difference in change 0-12 months (95% CI)	Multivariable Difference in change 0-12 months (95% CI)[3]
Total		-0.55 ± 0.08		
Home environment factors				
Pit latrine	193 (52%)	-0.74 ± 0.10	-0.39 (-0.70, -0.09)[4]	-0.30 (-0.57, -0.02)[4]
Flush toilet	179 (48%)	-0.34 ± 0.12	Ref	Ref
Shared toilet	339 (91%)	-0.60 ± 0.08	-0.50 (-0.96, -0.05)[4]	-0.19 (-0.65, 0.26)
Household toilet	32 (9%)	-0.10 ± 0.22	Ref	Ref
≥ 2 persons/room in house	314 (84%)	-0.65 ± 0.08	-0.61 (-1.06, -0.17)[4]	-0.45 (-0.86, -0.05)[4]
< 2 persons/room in house	55 (15%)	-0.04 ± 0.21	Ref	Ref
Maternal factors				
> primary education	155 (42%)	-0.16 ± 0.12	0.68 (0.37, 0.99)[5]	0.44 (0.17, 0.71)[4]
≤ primary education	213 (57%)	-0.84 ± 0.10	Ref	Ref
Primiparous	279 (75%)	-0.59 ± 0.09	-0.17 (-0.51, 0.18)	
Multiparous	90 (24%)	-0.43 ± 0.15	Ref	
Prepartum anthropometry				
Height *(10cm in models)*	160 (157-165)	--	0.29 (0.05, 0.53)[4]	0.22 (0.00, 0.42)[4]
Weight *(10kg in models)*	63 (58-68)	--	0.13 (-0.03, 0.28)	ref
MUAC < 23.5	50 (13%)	-0.86 ± 0.21	-0.36 (-0.81, 0.09)	
MUAC ≥ 23.5	244 (66%)	-0.49 ± 0.09	Ref	
Prepartum Maternal HIV				
CD4 count < 200	28 (8%)	-0.49 ± 0.34	0.13 (-0.53, 0.79)	
CD4 count 200-499	189 (51%)	-0.50 ± 0.17	0.12 (-0.21, 0.45)	
CD4 count > 500	147 (40%)	-0.62 ± 0.13	Ref	
Viral load ≥ 5 $\log_{10}$ copies/ml	110 (30%)	-0.59 ± 0.09	-0.16 (-0.54, 0.23)	
Viral load < 5 $\log_{10}$ copies/ml	223 (60%)	-0.43 ± 0.17	Ref	
Infant factors				
Birthweight < 2500g	21 (6%)	1.36 ± 0.41	2.02 (1.20, 2.83)[5]	0.95 (0.26, 1.63)[4]
Birthweight ≥ 2500g	343 (92%)	-0.65 ± 0.08	Ref	Ref
Birth LAZ < -2 (Stunted)	48 (14%)	0.83 ± 0.22	1.60 (1.15, 2.05)[5]	1.37 (0.96, 1.78)[5]
Birth LAZ ≥ -2 (Not stunted)	295 (86%)	-0.77 ± 0.08	Ref	Ref
Ever breastfed	279 (75%)	-0.64 ± 0.08	-0.34 (-0.73, 0.05)	
Never breastfed	93 (25%)	-0.30 ± 0.18	Ref	
Exclusively breastfed ≥ first 3 m	107 (29%)	-0.65 ± 0.14	-0.14 (-0.47, 0.19)	
Exclusively breastfed < first 3 m	265 (71%)	-0.51 ± 0.10	Ref	
Infant illness in first month of life				
Diarrhea	16 (4%)	-0.24 ± 0.49	0.33 (-0.64, 1.29)	
No Diarrhea	356 (96%)	-0.56 ± 0.08	Ref	
Pneumonia	14 (4%)	-1.84 ± 0.47	-1.33 (-2.27, -0.40)[4]	-1.27 (-2.13, -0.41)[4]
No pneumonia	358 (96%)	-0.50 ± 0.08	Ref	Ref
Hospitalization	26 (7%)	-0.02 ± 0.49	0.57 (-0.39, 1.54)	
No hospitalization	346 (93%)	-0.59 ± 0.08	Ref	

[1]Percents may not add to 100% due to missing data; Missing (n): Shared toilet (1), persons/room (2), maternal education (4), parity (3), maternal height (7), maternal weight (5), maternal MUAC (78), maternal CD4 (8), maternal viral load (39), infant birthweight (8).
[2]Unadjusted mean change ± SE were estimated from linear spline regression models
[3]Adjusted for pit latrine (vs flush toilet), shared toilet (vs household), crowding, maternal education, maternal height, low-birth weight, birth stunting, neonatal pneumonia, and age. 95% CI were calculated using robust variance estimates.
[4]p value < 0.05 [5]p value < 0.001

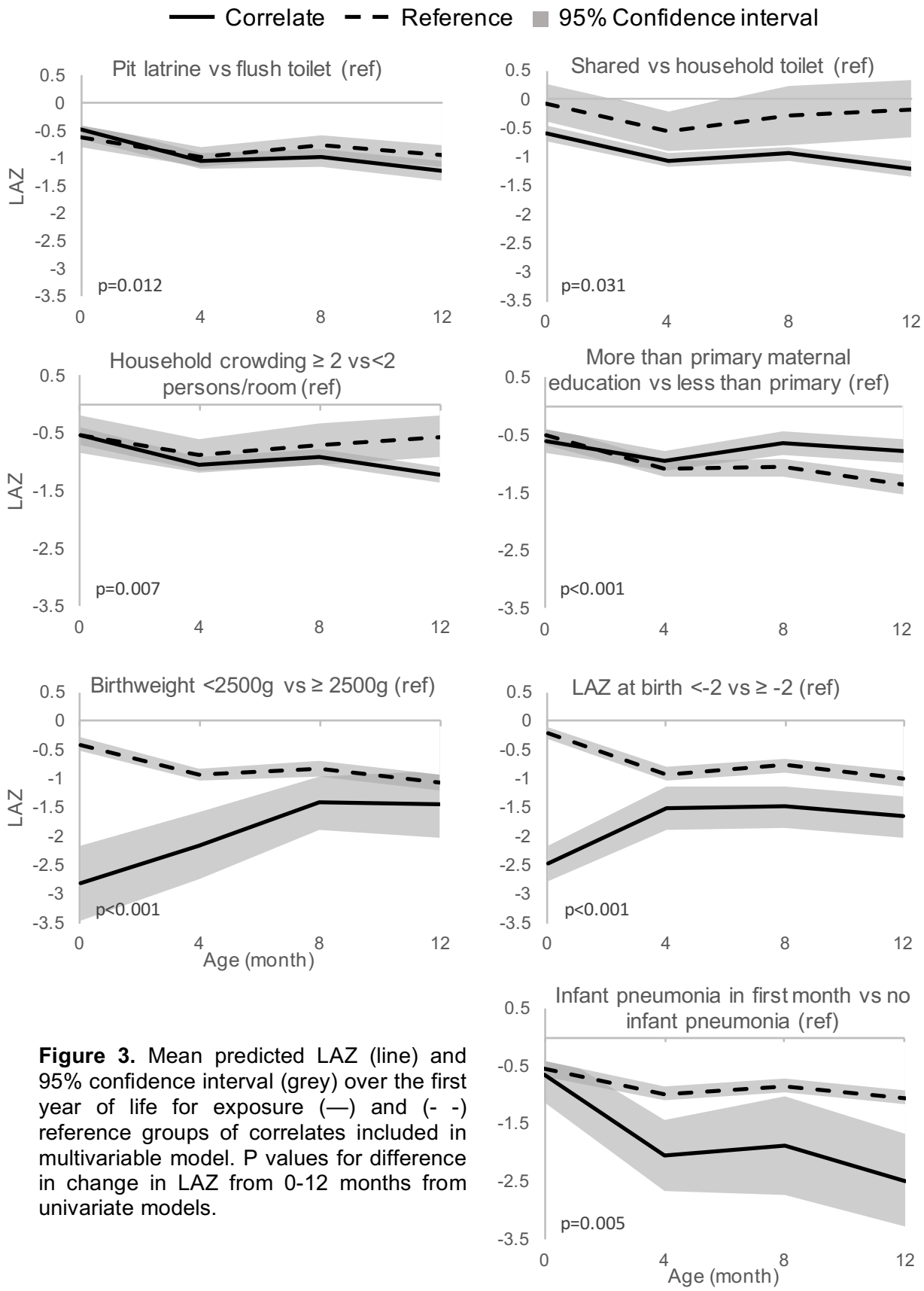


Figure 3. Mean predicted LAZ (line) and 95% confidence interval (grey) over the first year of life for exposure (—) and (- -) reference groups of correlates included in multivariable model. P values for difference in change in LAZ from 0-12 months from univariate models.

Table 2: Correlates of 0-12 month change in LAZ scores of 373 Kenyan HEU infants among those with non-missing correlate values.

Cofactors	Complete Case analysis Univariate Difference in change 0-12 months (95% CI)	Complete Case analysis Multivariable Model Difference in change 0-12 months (95% CI)[1]
Home environment factors		
Pit latrine	-0.4 (-0.71, -0.09)[2]	-0.24 (-0.53, 0.05)
Flush toilet	Ref	Ref
Shared toilet	-0.51 (-0.96, -0.05)[2]	-0.28 (-0.76, 0.19)
Household toilet	Ref	Ref
≥ 2 persons/room in house	-0.65 (-1.09, -0.21)[2]	-0.38 (-0.79, 0.02)
< 2 persons/room in house	Ref	Ref
Maternal factors		
> primary education	0.67 (0.36, 0.98)[3]	0.39 (0.11, 0.67)[2]
≤ primary education	Ref	Ref
Primiparous	-0.14 (-0.49, 0.21)	
Multiparous	Ref	
Prepartum anthropometry		
Height *(10cm in models)*	0.28 (0.05, 0.51)	0.25 (0.04, 0.47)[2]
Weight *(10kg in models)*	0.12 (-0.05, 0.30)	
MUAC < 23.5	-0.45 (-0.92, 0.02)	
MUAC ≥ 23.5	Ref	
Prepartum Maternal HIV		
CD4 count < 200	0.09 (-0.54, 0.71)	
CD4 count 200-499	0.12 (-0.20, 0.45)	
CD4 count > 500	Ref	
Viral Load ≥ 5 $\log_{10}$ copies/ml	-0.19 (-0.58, 0.21)	
Viral Load < 5 $\log_{10}$ copies/ml	Ref	
Infant factors		
Birthweight < 2500g	2.06 (1.27, 2.85)[3]	0.44 (-0.14, 1.03)
Birthweight ≥ 2500g	Ref	Ref
Birth LAZ <-2 (Stunted)	1.66 (1.21, 2.12)[3]	1.46 (1.05, 1.88)[3]
Birth LAZ ≥ -2 (Not stunted)	Ref	Ref
Ever breastfed	-0.34 (-0.74, 0.05)	
Never breastfed	Ref	
Exclusively breastfed ≥ 3 mon	-0.14 (-0.46, 0.19)	
Exclusively breastfed < 3 mon	Ref	
Infant illness in first month of life		
Diarrhea	0.30 (-0.68, 1.28)	
No Diarrhea	Ref	
Pneumonia	-1.35 (-2.30, -0.40)[2]	-1.48 (-2.42, -0.53)[2]
No pneumonia	Ref	Ref
Hospitalization	0.57 (-0.41, 1.54)	
No hospitalization	Ref	

[1]From multivariable linear spline models adjusted for pit latrine (vs flush toilet), shared toilet (vs household), crowding, maternal education, maternal height, low-birth weight, birth stunting, pneumonia in first month, age. 95% CI were calculated using robust variance estimates.
[2]p value < 0.05 [3]p value < 0.001

Table 3: Correlates of 0-12 month change in LAZ scores of 373 Kenyan HEU infants among those with no-decreasing length measurements.

Cofactors	Univariate Difference in change 0-12 months (95% CI)	Multivariable Model Difference in change 0-12 months (95% CI)[1]
Home environment factors		
Pit latrine	-0.35 (-0.67, -0.03)[2]	-0.23 (-0.52, 0.06)
Flush toilet	Ref	Ref
Shared toilet	-0.48 (-0.93, -0.02)[2]	-0.25 (-0.73, 0.22)
Household toilet	Ref	Ref
≥ 2 persons/room in house	-0.52 (-0.97, -0.08)[2]	-0.39 (-0.81, 0.03)
< 2 persons/room in house	Ref	Ref
Maternal factors		
> primary education	0.60 (0.28, 0.93)[3]	0.37 (0.09, 0.66)[2]
≤ primary education	Ref	Ref
Primiparous	-0.25 (-0.60, 0.10)	
Multiparous	Ref	
Prepartum anthropometry		
Height *(10cm in models)*	0.36 (0.11, 0.61)[2]	0.29 (0.08, 0.50)[2]
Weight *(10kg in models)*	0.13 (-0.05, 0.31)	
MUAC < 23.5	-0.29 (-0.74, 0.16)	
MUAC ≥ 23.5	Ref	
Prepartum Maternal HIV		
CD4 count <200	-0.04 (-0.69, 0.60)	
CD4 count 200-499	0.11 (-0.24, 0.45)	
CD4 count >500	Ref	
Viral Load ≥ 5 $\log_{10}$ copies/ml*)*	-0.13 (-0.55, 0.28)	
Viral Load < 5 $\log_{10}$ copies/ml	Ref	
Infant factors		
Birthweight < 2500g	1.84 (0.93, 2.76)[3]	0.64 (-0.11, 1.39)
Birthweight ≥ 2500g	Ref	Ref
Birth LAZ <-2 (Stunted)	1.82 (1.36, 2.28)[3]	1.68 (1.23, 2.12)[3]
Birth LAZ ≥ -2 (Not stunted)	Ref	
Ever breastfed	-0.28 (-0.69, 0.13)	
Never breastfed	Ref	
Exclusively breastfed ≥ 3 months	-0.07 (-0.41, 0.26)	
Exclusively breastfed < 3 months	Ref	
Infant illness in first month of life		
Diarrhea	0.34 (-0.67, 1.35)	
No Diarrhea	Ref	
Pneumonia	-1.10 (-2.17, -0.02)[2]	-1.03 (-2.04, -0.02)[2]
No pneumonia	Ref	Ref
Hospitalization	0.61 (-0.41, 1.63)	
No hospitalization	Ref	

[1]From multivariable linear spline models including multiple imputation for correlate missing values and adjusted for pit latrine (vs flush toilet), shared toilet (vs household), crowding, maternal education, maternal height, low-birth weight, birth stunting, neonatal pneumonia, age. 95% CI were calculated using robust variance estimates.
[2]p value < 0.05 [3]p value < 0.001

Chapter 3: Relationship between timing, severity, and burden of diarrhea and linear growth in the first year among HIV exposed, uninfected infants in Kenya

INTRODUCTION

Linear growth faltering is associated with poor health and economic consequences later in life including increased infectious disease mortality, cognitive delays, lower school achievement, and reduced adult work capacity [5–11,78,120]. Childhood stunting, a commonly used cross-sectional indicator of linear growth faltering, is most common in sub-Saharan Africa and South Asia where the prevalence is estimated to be 36% and 27%, respectively, among children under five years of age [23]. Childhood stunting risk is associated with insults that occur during the fetal period and with inadequate nutrition and recurrent episodes of illnesses, particularly diarrhea, early in life [23,88,90–92].

Diarrheal disease has long been investigated as a potential cause of stunting in early childhood with evidence that diarrhea leads to weight loss and eventually linear growth faltering in the absence of sufficient illness free periods with adequate nutrition to support catch-up growth [16,19,108,121–123]. However, interventional studies testing treatment and prevention of diarrhea to improve linear growth have had mixed results [124–127], and the recent large diarrhea etiology study (the MAL-ED study) found no relationship between diarrheal burden and linear growth, other than a modest association in children with diarrhea not treated with antibiotics [17]. Diarrhea severity [16], treatment [17], burden [12,18], and timing of diarrhea [3,19], may explain differences in the diarrhea and linear growth relationship. Understanding these sources of heterogeneity may provide insights into how to best target interventions to prevent linear growth faltering, and stunting, in young children.

In sub-Saharan Africa, children born to HIV positive mothers, but whom themselves are uninfected (HEU) represent a growing vulnerable population at risk for linear growth faltering. HEU children are at increased risk for infection with enteric pathogens, experience more frequent bouts of diarrhea than their unexposed counterparts, and are at higher risk of developing more severe diarrhea. These vulnerabilities may be a consequence of in-utero HIV-exposure, increased postnatal pathogen exposure from their HIV-infected mother [24,28,82], or may be a function of socio-demographic challenges [27,28,35,128]. As a result, this population may serve as a model for understanding relationships between diarrheal illness and linear growth. Utilizing data from a historical birth cohort of HEU infants from Nairobi, Kenya, we aimed to determine the effect of diarrhea severity, treatment, burden, and timing on linear growth in this vulnerable and growing population.

METHODS

Study design

This secondary analysis utilized data collected from a previously accrued cohort of HIV-infected women and their infants described in detail elsewhere [49,50,95]. In brief, from 1999-2002 HIV-infected women recruited during pregnancy were followed with their infants for one year after birth. Mothers received short-course zidovudine during labor to prevent maternal-to-child transmission of HIV according to Kenyan National guidelines at the time of the study. In addition, and women with severe immunosuppression (CD4 count <200 cells/μl) received cotrimoxazole prophylaxis. Mothers did not receive antiretroviral therapy (ART) during breastfeeding, as was standard at the time of the study.

Data collection

Infant recumbent length measurements were collected during routine study visits at two and four weeks after birth and then monthly to 12 months of age. Length-for-age z-scores (LAZ) were

calculated using the WHO Anthro macro developed for Stata and based on the 2006 WHO Child Growth Standard [98]. Child morbidities, including diarrhea, and antibiotic treatment were documented at routine study visits through a clinical exam or when mothers were asked about infant illness, treatment, and breastfeeding in the past month. Specific types of antibiotic treatment for illness reported in the last month were not recorded. Mothers were encouraged to bring their infant to the study clinic at any point during follow up if the child was sick, where morbidity diagnoses, including diarrhea, and antibiotic treatment data were collected.

Statistical analysis

Singleton or firstborn twin infants with documentation of sex, at least one negative HIV PCR test, and at least two recorded length measurements were included in the present analysis. Infants with an HIV positive test at or before one month of age were considered perinatally infected and excluded from the analysis. Infants were censored from the analysis cohort at their last HIV-negative test at the end of study follow up (12 months), death, HIV seroconversion, or if they were lost to follow-up.

Diarrhea was defined as any diarrhea episode reported by the mother since the last study visit or a clinician diagnosis of diarrhea at any study visit (routine or sick visit). Diarrhea episodes separated by 14 days were counted as independent episodes to conservatively avoid double counting of diarrhea episodes. Clinician diagnosis of diarrhea at sick visits was considered diarrhea even if diarrhea was not the primary diagnosis. An episode was classified as moderate-to-severe diarrhea (MSD) when diarrhea occurred with dysentery or dehydration, or there was a diarrhea associated hospitalization [3].

To reduce potential bias in estimates of cumulative diarrhea due to missing visits, we used multivariable Normal regression models with Markov chain Monte Carlo method to impute missing

values (m=10 imputations) of the number of diarrhea episodes for missed visits [97]. Cumulative count of diarrhea episodes in previous months up until one month prior to the anthropometric measurement were used in imputed data sets. Length measurements were not imputed and thus visits remained missed in the final analysis. An infant was considered breastfed if the mother reported the infant receiving any breastmilk in the last 24 hours at a routine study visit. Incidence of diarrhea and average breastfeeding duration were calculated using interval censoring methods to censor person-time for missed visits and 7 days before and after a diarrhea episode.

We determined the effect of any diarrheal episode in a month (short-term effect), cumulative burden, and timing of the episode on LAZ in the following month, throughout the first year of an infant's life. To determine the specific relationship of MSD and diarrhea not coincident with antibiotics with LAZ, we conducted three sets of analyses for each assessment of short-term effect, cumulative burden and timing subsetting to children with MSD and diarrhea without coincident antibiotic. Therefore, separate models were created to compare: any diarrhea versus none, MSD versus no diarrhea, any diarrhea without coincident antibiotics versus no diarrhea without antibiotics for another indication, and MSD without coincident antibiotics versus no diarrhea without antibiotics for another indication.

All models used linear mixed-effects methods with random effects for intercept and slope and an autocorrelation structure to adjust for within-person correlation of repeated measurements. Linear splines with knots at four and eight months modeled the change of continuous LAZ over time. Model 1, for short-term effect, cumulative burden and timing included the diarrhea predictor of interest (described below), birth LAZ (continuous), and age as linear splines terms (continuous) to test the association of diarrhea on attained LAZ throughout the first year. The short-term effect models used any (binary) diarrhea or MSD (categorical indicators MSD, non-MSD diarrhea, no diarrhea), and included an indicator for antibiotics (binary) and an interaction term between with

the diarrhea indicator. The cumulative burden model included a continuous term for the cumulative number of diarrhea episodes since birth. The difference between cumulative number of diarrheal episodes and cumulative reports antibiotics represented diarrhea without coincident antibiotics. To assess timing, we additionally included interaction terms between the diarrhea and four month age intervals (1-4, 5-8, and 9-12 months) and spline terms. Models for diarrhea not coincident with antibiotics include a three way interaction between diarrhea, linear spline term, and antibiotics. We estimated the difference in LAZ from start to end of each interval for infants with and without diarrhea from the spline model and used linear combination of parameters to test the differential effect for each interval relative to the 9-12 month age interval. Because there was no evidence of effect modification by time within the first year of life, we did not include interaction terms between diarrhea incidence and infant age in the short term or diarrhea burden models. Diarrhea variables each had a 1-month lag effect to reflect the biologic relationship between diarrhea and linear growth. For example, diarrhea occurring between month one and two were considered exposures relevant to LAZ outcome at 3 months.

Model 2 was a replicate Model 1 and included potential confounding variables identified based on a priori knowledge of common causal influences on both diarrhea and growth. Infant birth size, socioeconomic factors (maternal education and household crowding), and breastfeeding are known strong predictors of LAZ throughout the first year and are risk factors for diarrhea [43,66,88]. Model 2 included birth LAZ (continuous), infant age (continuous), maternal education (≥ primary vs < primary education), household crowding (≥2 vs <2 persons per room) and time varying breastfeeding (yes, no).

The original cohort study was approved by the Kenyatta National Hospital Ethics and Research Committee and the University of Washington Institutional Review Board. The current analysis was exempt from review as a secondary analysis of de-identified data. All statistical tests used 2-sided

p-values and alpha of 0.05 to determine statistical significance. All analyses were conducted in Stata 14 (StataCorp, College Station, Texas).

RESULTS

Among 372 HEU infants, mean birth LAZ was -0.31 (standard deviation [SD]: 1.47) and 12% were stunted. About half (52%) of infants lived in homes with a pit latrine versus a flushed toilet and the majority (84%) of infants lived in crowded households (≥2 persons per room). Most (75%) infants were breastfed, 25% never receiving any breastmilk over the entire follow-up period. On average, infants were exclusively breastfeed for 1.9 months (range 0-7). Nearly half (42%) of mothers had more than a primary education. Prior to delivery, mothers' median CD4 count was 444 cells/μl (Inter Quartile [IQR] Range: 317-618) and median HIV VL was 4.7 $\log_{10}$ copies/ml (IQR: 4.1-5.1; Table 1).

By one year of age, mean LAZ among the 268 HEU infants with a 12-month length measurement was -0.97 (SD: 1.2). Seventeen percent of infants were stunted at 12 months (Chapter 2). Infants in this cohort, experienced a mean 2.11 episodes of diarrhea and 0.48 episodes of MSD per month during follow up (varying slightly from Chapter 1 due to differences in censoring of person time). Incidence of diarrhea varied over the first year and peaked around 9-11 months of age, while MSD incidence remained stable over time (Figure 1). Of the 671 diarrhea episodes, 55% (369) were not treated with antibiotics. Similarly, of the 149 MSD episodes, 48% (72) were not treated with antibiotics, and although 16 of the MSD episodes were associated with hospitalization, half of which (seven) did not appear to have been treated with antibiotics. Twenty-seven of the MSD episodes included a presentation of dysentery, one third of which (10) were not treated with antibiotics.

Short-term effect

Diarrhea episodes were associated with decreased linear growth (Table 2). Diarrhea was associated with an average loss of 0.07 (95% confidence interval [CI]: -0.14, -0.00) in LAZ. Infants with a MSD episode had a greater decline in LAZ (adjusted difference [AD]: -0.18; 95% CI: -0.31, -0.06) compared to those without any diarrhea (Table 2). Both relationships remained statistically significant after adjustment for age, birth LAZ, household crowding, and maternal education. Infants who had diarrhea without antibiotics had a lower LAZ than those with no diarrhea and no antibiotics (AD: -0.07 95% -0.16, 0.01) with a trend for association, and similarly MSD without antibiotics was associated with decrease in LAZ (AD: -0.22 95% -0.39, -0.04) in the month following the diarrhea episodes.

Cumulative burden

There was a small association between cumulative diarrhea burden and subsequent linear growth in Model 1 (difference: -0.05; 95% CI: -0.09, -0.00), but the effect was not robust to additional adjustment for household crowding, exclusive breastfeeding, and maternal education in Model 2 (AD: -0.03; 95% CI: -0.08, 0.01, Table 2). There were not significant associations of LAZ and cumulative MSD (AD: -0.02; 95% CI: -0.08, 0.04), diarrhea without coincident antibiotics (AD: -0.02; 95% CI: -0.08, 0.04), nor MSD without coincident antibiotics (AD: -0.01; 95% CI: -0.15, 0.12).

Timing

LAZ decreased during 0-4 months, stabilized or increased during the following period (5-8 months), and declined again during the 9-12 month period (Figure 1). The relationship between diarrhea and LAZ was variable in different time periods during the first year (Figure 2). Diarrhea in the first months of life appeared to have the greatest effect on 4 month linear growth for any diarrhea (AD: -0.18; 95% CI: -0.87, 0.43), MSD (AD: -0.40; 95% CI: -1.49, 0.71), and diarrhea without antibiotics (AD: -0.54; 95% CI: -0.39, 1.27) based on the magnitude of effect in this period compared to other periods, albeit not significantly so (Table 3). However, the relationship between

diarrhea and LAZ was not significantly different between periods for either model, nor for any of the other classifications of diarrhea.

DISCUSSION

In this study, diarrhea was associated with linear growth faltering in HEU infants. In addition, a greater degree of growth faltering was observed among infants with diarrhea who did not receive antibiotics. These findings are consistent with a large body of evidence linking diarrhea and growth faltering [14,129]. However, there is mixed evidence regarding the importance of diarrhea severity, treatment, burden, and timing on linear growth faltering [17]. We found minimal effect of burden, but found a significant effect of diarrhea severity on growth. Children with MSD had more substantial growth faltering than was seen with other diarrhea episodes.

The relationship between diarrhea and linear growth faltering may depend on the severity of diarrhea. We found that MSD was associated with a decline of -0.11 z-scores relative to the -0.07 associated with all diarrhea. Other studies have found that diarrhea episode duration [16] and MSD [3] are associated with subsequent linear growth faltering. Diarrhea severity may reflect the underlying cause of the diarrhea with rotavirus, adenovirus 40/41, *Cryptosporidium, Campylobacter,* and *Shigella* being the leading causes of MSD among children under 12 months of age [130]. Diarrhea etiologies appear to have a unique relationship with subsequent LAZ [131] and therefore could explain why we observed MSD to have a stronger relationship with growth than any type of diarrhea. In the absence of having diagnostic tools to determine diarrhea etiology, children with MSD (or the characteristics of MSD such as dysentery, hospitalization, and/or dehydration) might be the appropriate population to target for nutritional interventions. Children with severe diarrhea are also more likely to seek care for diarrhea [53] therefore targeting interventions to health-facility attended diarrhea may result in the greatest improvements in diarrhea-related linear growth faltering.

Our results indicate diarrheal episodes without coincident antibiotics contribute to linear growth faltering, a contribution that was greater among MSD episodes. The MAL-ED study did not find an association between all diarrhea and linear growth, but did show a reduction in linear growth associated with diarrhea without coincident antibiotics [17]. Antibiotic treatment, or lack thereof, may reflect the primary caregivers' care seeking behavior. Caregivers who did not seek care for their child's diarrhea (and therefore did not report antibiotic use) may represent infants who were unable to access necessary health care, possibly due to other social or economic factors which increased their vulnerability to malnutrition and poor linear growth. Alternatively, antibiotics may provide a length benefit when given to children with diarrhea, a hypothesis which is being tested in the WHO-sponsored AntiBiotics for Children with severe Diarrhea (ABCD) trial, findings from which will need to be weighed against rising rates of antibiotic resistance.

Repeated diarrhea infections, in combination with inadequate nutrient intake, systemic inflammation and impaired intestinal absorption are commonly thought to contribute to the cycle of malnutrition and linear growth faltering [19,123]. However, in this study we did not observe such an effect after adjusting for potential confounders. Cumulative burden may only be associated with linear growth past a particular threshold of diarrheal episodes, or the cumulative effect may only become evident after the cessation of breastfeeding and into the second year of life, when the prevalence of stunting peaks [90]. Alternatively, cumulative burden may only have an impact on linear growth if subsequent episodes are clustered together in time. Some evidence also suggests that infants may experience some linear growth decline following a diarrheal episode, but experience linear growth catch-up in the absence of additional diarrheal insults [19]. Therefore, cumulative diarrheal episodes separated by sufficient diarrhea free periods may not have long-term impacts on linear growth.

The infants in this cohort represent a particularly vulnerable group of children. HEU infants may be at increased risk from diarrhea due to in-utero HIV-exposure and subsequent immunosuppression or as a result of increased enteric pathogen exposure from living with HIV-infected household members. Prevention of early diarrhea among HEU infants, through improvements in maternal health, may substantially reduce subsequent linear growth faltering in this vulnerable population [21,22,132].

This study had several notable strengths. The cohort included a large number of HIV exposed infants who were followed prior to the wide-spread availability of ART, allowing for evaluation of the influence of maternal viral load and CD4 count. In addition, data collection was systematic and allowed for analysis of multiple social, demographic and biological exposures. However, there were limitations to this study. First, data was not originally collected to address hypotheses related to diarrhea and linear growth. Monthly ascertainment of diarrhea morbidity and antibiotic use may have contributed to an under count of diarrhea episodes and ascertainment of diarrhea may have been limited by recall bias, particularly for less severe episodes [53]. Hospital and clinic records were not available to review, and data on antibiotic use may have been incomplete, resulting in misclassification. In addition to under ascertainment of diarrhea and antibiotic use, the study population experienced lower rates of linear growth faltering and had higher LAZ throughout the first-year than expected. As a result, we had decreased statistical power, particularly after adjusting for potential confounders, possibly influencing our ability to detect associations within four month intervals of the timing analysis. Lastly, the parent cohort was recruited prior to widespread maternal ART for HIV, cotrimoxazole prophylaxis for HEU infants, and childhood rotavirus vaccination so we could not test the relationship between diarrhea and linear growth for populations adherent to specific standard of care guidelines. However, these results provide important mechanistic insights and may be relevant to HEU children not currently benefiting from available interventions.

In summary, in this HEU cohort, infant diarrhea was associated with linear growth faltering, particularly MSD and diarrhea that was not coincident with antibiotics. Diarrhea severity and antibiotic use may contribute to heterogeneity in the relationship between diarrhea and linear growth, particularly among high risk populations. Prevention of diarrhea in the first year of life, and treatment of more severe diarrhea throughout the first year may protect against linear growth faltering in HEU infants. This population represents a particularly vulnerable and growing population in whom reductions in linear growth faltering could have an impact on the global burden of stunting.

Table 1: Selected characteristics of the 372 mothers and HEU infants included in the analysis.

	N (%)[1] Mean (min, max)
TOTAL	**372**
Home environment factors	
Pit latrine	193 (52)
Flush toilet	179 (48)
≥ 2 persons/room in house	314 (84)
< 2 persons/room in house	55 (15)
Maternal factors at 32 weeks gestational age	
Age	25 (18, 42)
> primary education	155 (42)
≤ primary education	213 (57)
Height (cm)	161 (144, 183)
MUAC < 23.5	51 (14)
MUAC ≥ 23.5	244 (65)
CD4 count < 200 cells/μl	28 (8)
CD4 count 200-499 cells/μl	190 (51)
CD4 count ≥ 500 cells/μl	147 (39)
Log VL ≥ 4	267 (72)
Log VL < 4	67 (18)
Infant factors	
Female	177 (48)
Male	195 (52)
Birth LAZ	-0.32 (-5.49, 4.75)
Exclusively breastfed duration (months)	1.9 (0, 7)

[1]Percents may not add to 100% due to missing data

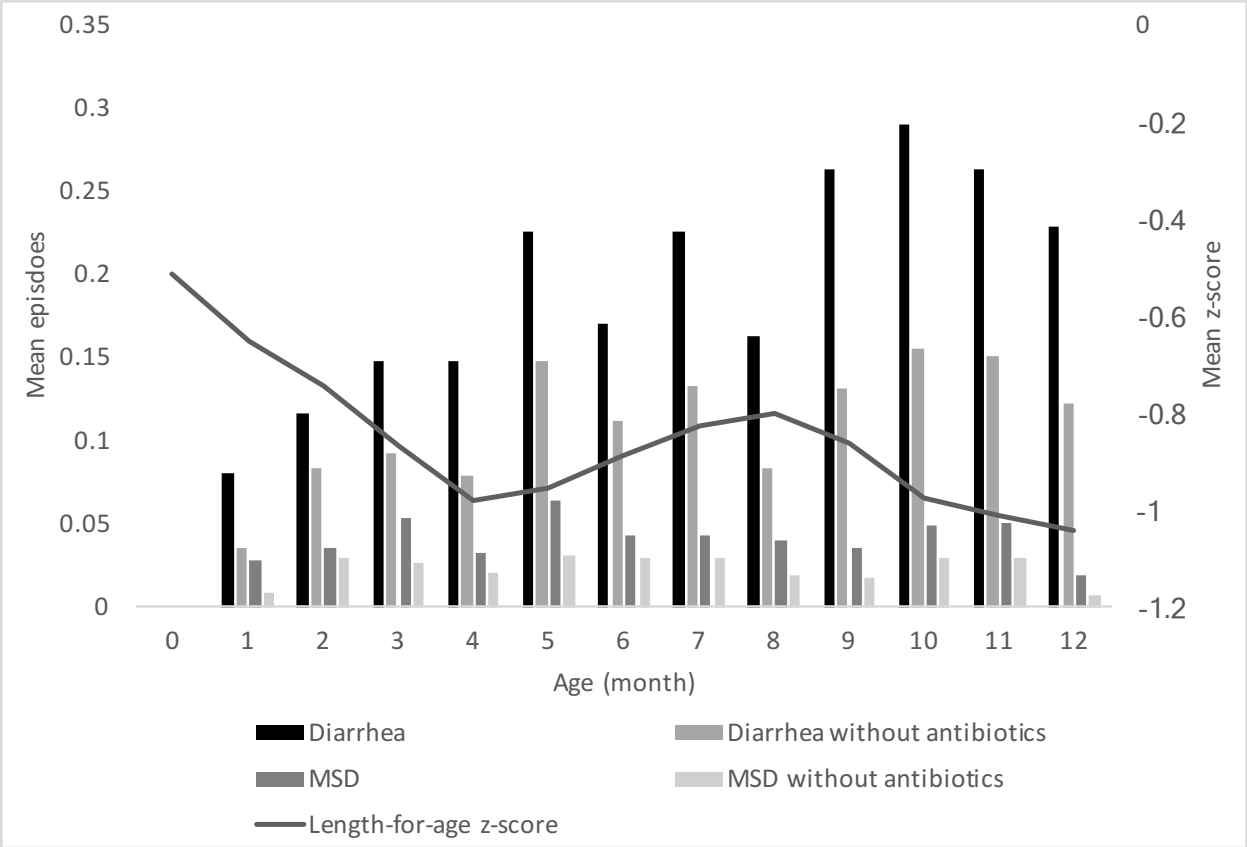


Figure 1: Incidence of diarrhea, diarrhea without antibiotics, moderate to severe diarrhea (MSD), and MSD without antibiotics per month (bar chart) and average length-for-age z-scores (line) across the first year of life among HEU infants.

Table 2: Difference in LAZ by infant diarrhea and cumulative diarrhea burden among 372, HEU infants

	Model 1 Difference in LAZ (95% CI)[1]	P-value	Model 2 Difference in LAZ (95% CI)[2]	P-value
Short-term effect				
Diarrhea	-0.10 (-0.17, -0.03)	0.004	-0.07 (-0.14, -0.00)	0.041
MSD	-0.20 (-0.32, -0.07)	0.002	-0.18 (-0.31, -0.06)	0.005
Diarrhea without antibiotics	-0.10 (-0.19, -0.02)	0.018	-0.07 (-0.16, 0.01)	0.095
MSD without antibiotics	-0.22 (-0.40, -0.05)	0.012	-0.22 (-0.39, -0.04)	0.015
Cumulative burden				
Diarrhea	-0.05 (-0.09, -0.00)	0.048	-0.03 (-0.08, 0.01)	0.172
MSD	-0.08 (-0.17, 0.02)	0.126	-0.05 (-0.15, 0.04)	0.278
Diarrhea without antibiotics	-0.03 (-0.09, 0.03)	0.324	-0.02 (-0.08, 0.04)	0.528
MSD without antibiotics	-0.02 (-0.16, 0.11)	0.757	-0.01 (-0.15, 0.12)	0.851

[1]Model 1 adjusted for age and birth LAZ
[2]Model 2 adjusted for age, birth LAZ, household crowding, exclusive breastfeeding in the last 24 hours, and maternal education

Table 3: Average change in LAZ over intervals among 372 HEU infants by diarrhea type

	Diarrhea Average 4 month change ± SE	No Diarrhea Average change 4 month ± SE	Model 1 Difference in average 4 month change (95% CI)	Comparison between periods P-value	Model 2 Difference in average 4 month change (95% CI)[1]	Comparison between periods P-value
Diarrhea						
0-4m	-0.33 ± 0.37	-0.02 ± 0.11	-0.31 (-1.06, 0.43)	0.451	-0.18 (-0.87, 0.43)	0.768
5-8m	0.16 ± 0.14	0.03 ± 0.05	0.13 (-0.15, 0.41)	0.515	0.08 (-0.15, 0.41)	0.490
9-12m	-0.19 ± 0.12	-0.16 ± 0.06	-0.03 (-0.29, 0.23)	Ref	-0.07 (-0.33, 0.23)	Ref
MSD						
0-4m	-0.37 ± 0.53	-0.02 ± 0.11	-0.35 (-1.41, 0.71)	0.606	-0.40 (-1.49, 0.71)	0.774
5-8m	0.20 ± 0.19	0.03 ± 0.05	0.17 (-0.21, 0.55)	0.243	0.17 (-0.23, 0.55)	0.305
9-12m	-0.37 ± 0.18	-0.16 ± 0.06	-0.21 (-0.58, 0.16)	Ref	-0.19 (-0.55, 0.16)	Ref
Diarrhea without antibiotics						
0-4m	0.06 ± 0.53	-0.14 ± 0.13	0.20 (-0.87, 1.27)	0.505	0.54 (-0.39, 1.27)	0.153
5-8m	0.27 ± 0.21	0.07 ± 0.06	0.19 (-0.22, 0.61)	0.304	0.05 (-0.25, 0.61)	0.466
9-12m	-0.36 ± 0.14	-0.20 ± 0.07	-0.15 (-0.47, 0.16)	Ref	-0.15 (-0.46, 0.16)	Ref
MSD without antibiotics						
0-4m	-0.13 ± 0.69	-0.14 ± 0.13	0.01 (-1.39, 1.40)	0.829	-0.04 (-1.46, 1.40)	0.799
5-8m	0.11 ± 0.22	0.07 ± 0.06	0.04 (-0.41, 0.49)	0.750	0.05 (-0.41, 0.49)	0.794
9-12m	-0.04 ± 0.23	-0.20 ± 0.06	0.17 (-0.29, 0.62)	Ref	0.16 (-0.31, 0.62)	Ref

[1]Model 1 adjusted for age and birth LAZ
[2]Model 2 adjusted for age, birth LAZ, household crowding, exclusive breastfeeding in the last 24 hours, and maternal education
[3]p-value <0.05

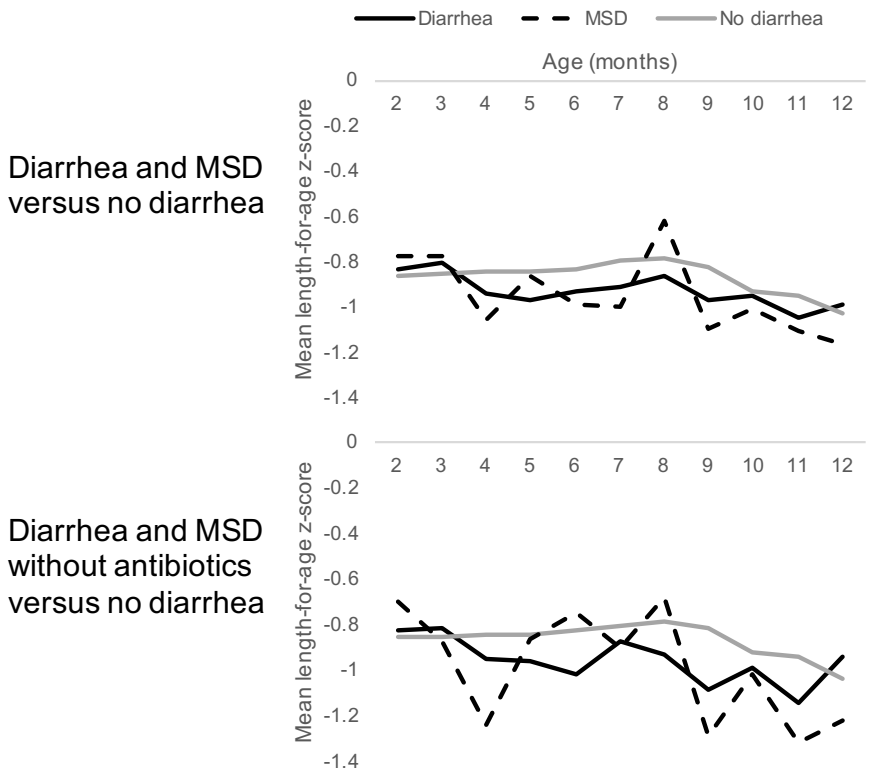


Figure 2: Mean Length-for-age z-score estimated from Model 2 and adjusted for infant age, birth length-for-age z-score, household crowding, maternal education, and excusive breastfeeding for infants with diarrhea (black, —), moderate to severe diarrhea (MSD; - -), and no diarrhea (gray, —).

CONCLUSION

The analyses presented in this dissertation contribute to understanding the burden, risk factors, and relationships between diarrhea and linear growth among HEU children. This work highlights the relationships between maternal health and infants home environment and the risk of diarrhea and linear growth faltering in HEU infants. Maternal pregnancy and postpartum health appear to be strong contributors to infant diarrhea morbidity and linear growth faltering, and may, in part, influence the relationship between diarrhea and linear growth in the first year of life. Additionally, we found overlapping risk factors for both infant diarrhea and linear growth faltering, highlighting increased vulnerabilities among this already high risk group.

Infant health over the first year may be strongly linked to maternal pregnancy and postpartum health. Components of a child's growth trajectory may be determined early in life based on fetal environment and genetics, influenced by a mother's health and nutrition during, and likely before, pregnancy [23,92,116]. In Chapter 2, our findings suggest an appreciable impact of fetal growth on long-term stunting, as 28% of infants stunted at 12 months were already stunted at birth. Additionally, the strongest contributors to infant linear growth were birth size and neonatal pneumonia infection, which are known to be influenced by maternal nutrition and infections during pregnancy [23].

A mother's health can also contribute to an infant's health through person-to-person pathogen transmission and through her influence over their shared environment. In Chapter 1, we found higher diarrhea incidence among infants whose mothers reported that they themselves experienced postpartum diarrhea and took antibiotics. Maternal-infant illnesses are often shared and this underscores the importance of concurrently addressing the health status of both mother and child. This is further illustrated in Chapter 3 where diarrhea in the first few months of life, likely associated with caregiver illness and transmission of illness etiology, to have the strongest

influence on linear growth faltering relative to other time periods in the first year. Maternal health during pregnancy and in the early postpartum period may be particularly important for infant diarrhea in the first few months of life occurring prior to introduction of complementary foods and before the infant is independently mobile in their environment. Nutrition and infection both in the mother and postpartum should be considered as potential intervention targets for the prevention of infant infection and linear growth faltering and to improve infant health and development.

In addition to the maternal health as a risk factor for poor infant health, findings from Chapter 1 and Chapter 2 suggest overlapping risk factors in children experiencing poor health outcomes. Maternal education, household toilet type, and crowding are common risk factors for both infant diarrhea and linear growth faltering. There is a persistent relationship between socioeconomic status, represented here by maternal education and household environment, and health throughout a persons' life. A mother's socioeconomic status may influence an infant's health through direct exposure to pathogens and access to adequate nutrition, or indirectly through social support or heath care seeking behavior. For example, mothers with higher maternal education may have greater agency and knowledge regarding their own personal health, accessing health care services, and adherence to medical advice for both herself and her infant. This dissertation highlights maternal education and household environment as indicators of infants at increased risk for poor health outcomes, even among an already vulnerable population of HEU infants.

In conclusion, diarrheal illness and linear growth faltering remain important causes of childhood morbidity and mortality worldwide. HEU children are at risk for diarrhea and linear growth faltering and among these children, diarrhea contributes to linear growth faltering. Sustained reductions in childhood mortality require attention to vulnerable populations including those with acute illness and malnutrition such as HEU infants. Interventions targeted at improving maternal health during

pregnancy and the postpartum period, alongside continued community and nutritional support, have the potential to substantially reduce multiple childhood morbidities, particularly among HEU children.

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