Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants

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Main Document

Title

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Key words: child undernutrition, malnutrition, HIV, infant feeding practices, 1000 days, Botswana

Abstract

Background: A better understanding of the nutritional status of infants who are HIV-Exposed-Uninfected (HEU) and HIV-Unexposed-Uninfected (HUU) during their first 1000 days is a key to improving population health, particularly in sub-Saharan Africa.

Methods: A cross-sectional study compared nutritional status, feeding practices and determinants of nutritional status of HEU and HUU infants residing in representative selected districts in Botswana during their first 1000 days of life. Four hundred and thirteen infants (37.3% HIV-exposed), aged 6-24 months attending routine child health clinics were recruited. Anthropometric, 24-hour dietary intake and socio-demographic data was collected. Anthropometric *z*-scores were calculated using 2006 WHO growth standards. Modelling of the determinants of malnutrition was undertaken using logistic regression.

Results: Overall, prevalence of stunting, wasting and underweight were 10.4%, 11.9% and 10.2% respectively. HEU infants were more likely to be underweight (15.6% vs. 6.9%), ($p<0.01$) and stunted (15.6% vs. 7.3%), ($p<0.05$) but not wasted ($p= 0.14$) than HUU infants. HEU infants tended to be formula fed (89.4%) whereas HUU infants tended to breastfeed (89.6%) for the first six months ($p<0.001$). Significant predictors of nutritional status were HIV exposure, birthweight, birth length, Apgar score and mother/caregiver's education with little influence of socioeconomic status.

Conclusions: HEU infants aged 6-24 months had worse nutritional status compared to HUU infants. Low birthweight was the main predictor of undernutrition in this population. Optimisation of infants' nutritional status should focus on improving birthweight. In addition, specific inteverntions should target HEU infants in order to eliminate growth disparity between HEU and HUU infants.

Introduction

Globally, under-five mortality declined from 90 to 43 deaths per 1000 live births between 1990 and 2015 [(1)]. However, in sub-Saharan Africa under-five mortality still remains higher at 86 deaths per 1000 live births [(1)]. Mortality in children aged less than five years is mainly attributed to undernutrition, with 45% of these deaths being preventable through optimal nutrition, especially in the first 1000 days (the period from conception to the child's second birthday) [(2, 3-4)].

Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is still a major health challenge in Botswana [(5-6)]. Strategies including, prevention of mother-to-child transmission (PMTCT) of HIV have been highly successful in Botswana, reducing mother-to-child transmission rates to approximately 2.6% [(7-8)]. Without PMTCT strategies, HIV transmission from mother to child could be as high as 25% [(8)]. However, this success has resulted in the increase in the population of HIV-exposed but uninfected (HEU) infants. [(9-10)]. Health and/or nutritional issues unique to HEU infants will have major population health implications as their numbers increase [(11-12)]. Currently, the health and nutritional consequences of HIV-exposure are largely under study [(10, 12)]. However, a higher risk for mortality in HEU compared to HIV-unexposed uninfected (HUU) infants has been previously reported [(13-16)]. Risk of mortality can be modified by optimising nutritional status of infants, this requires a good understanding of context specific patterns and determinants of undernutrition in this group [(17)].

Studies conducted in other African countries comparing the nutritional status of HEU and HUU infants show large variations in the levels of undernutrition [(12, 18-20)]. Majority of these studies were conducted before ART was widely available to mothers and infants [(12, 18-20)]. In contrast, ART is available to approximately 92% of pregnant women in Botswana [(8)]. Monitoring and management of infant health and nutrition is intensive and widely accessible [(21)]. The same conditions are often not present in other sub-Saharan African countries with high HIV prevalence. However, the level of mortality in HEU infants in Botswana is comparable or higher than in other sub-Saharan African countries [(13)]. Furthermore, the feeding policy adopted for HEU infants in Botswana is unique, as it has inadvertently undermined breastfeeding levels through provision of free formula [(22)]. Currently, the nutritional status of HEU and HUU in Botswana have not been well documented. Therefore, understanding nutritional status and its determinants between HEU and HUU infants in Botswana is important for informing policies and interventions which can be used to achieve comparable growth between these infants if such differences exist. Thus helping reduce the risk of mortality in HEU infants. This study sought to investigate the patterns of undernutrition per HIV-exposure within context of feeding practices in infants aged 6- 24 months in selected districts in Botswana. In addition, this study, also aimed to identify determinants of nutritional status in these infants.

Methods

Study participants and population

The study was conducted in Botswana using a comparative cross-sectional study design between December 2014 and February 2015 in 19 different government health facilities of varying sizes (hospital, primary hospital, clinics and/or health posts) located across the four districts (Kweneng-East, Kgatleng, Selebi Phikwe and Francistown). Health facilities in districts with high HIV prevalence in the adult population were selected in order to obtain an adequate number of HEU infants. Prevalence of HIV in these districts ranged from 26.3% in Kweneng East to 39.6% in Selebi Phikwe They were selected as having higher HIV prevalence than the national average, in order to ensure an appropriate sample of HEU infants. These four districts were selected to represent urban, semi-urban and rural areas. Kweneng-East is mainly rural with some semi-urban locations. Kgatleng is mainly rural, Selebi Phikwe is semi-urban while Francistown is mostly urban. These locations span the eastern hardveld where at least 80% of the population of Botswana live [(23-24).] All caregivers from the general population with infants aged 6-24 months attending their monthly growth monitoring in a health facility, were invited to participate in the study. Eligible caregivers had to be citizens of Botswana, aged over 18 years and were the infant's parent and/or legal guardian. There were no other exclusion criteria. The participants were approached as they arrived at the health facility. Children in Botswana, aged 0-59 months attend routine monthly growth monitoring in government health facilities across the country. When more participants than required showed interest in the study, simple randomisation was used to select participants by allocating each participant a number.

Sample size

A representative sample of infants in selected districts was stratified according to the population of the infants aged under five years in each district based upon data supplied by the Ministry of Health and Wellness in Botswana (Nutrition and Food Control division). Therefore, a district with a higher number of under-fives had a larger representation within the sample. In addition, the composition of the sample within each district was selected such that it represented the proportions of infants attending each type of health facility (hospital, primary hospital, clinics and/or health posts) within that district. Therefore, a type of health facility receiving a higher number of infants would have a higher share of the sample within each district.

To facilitate a logistic regression analysis, an adequate sample size assuming a medium size relationship between the dependant variables (underweight, stunting and wasting) and independent variables and, $\alpha = 0.05$ and $\beta = 0.20$ was taken to be $N \geq 50 + 8m$ (where m is the number of independent variables) [25]. In total, 44 potential independent variables were identified *a priori* to the data collection; resulting in a minimum sample size of 402 caregiver-infant pairs (see Table S1). In addition, oversampling by 10% was also employed to counter missing data. Independent variables identified *a priori* and known to affect undernutrition in infants such as birthweight, sex, and maternal age, care giver education level and socio economic factors were included ([26-28]). These variables were derived from data collection (anthropometry, dietary recall, interview of caregivers) and review of the child health card. However, due to the cross-sectional nature of the study, maternal nutrition and health variables prior to the study, such as during pregnancy were not available. HIV-exposure was maintained in all analysis as it was a variable of interest.

Procedures

Participants were recruited during their infant's free monthly routine health check-up at a health facility. In total 419 participants were approached to take part in the study. Five infants with an undocumented HIV status and/or missing PCR DNA/rapid HIV tests were not enrolled into the study. Of all the participants approached, only one declined to take part in the study. The final sample size was, therefore, 413 infants.

Data were collected by the lead author and two trained assistants using a structured interview with the caregiver and review of each child's health card. All caregivers in Botswana are given and keep a health card for their infant at birth. This card contains details such as birthweight and length, vaccinations, monthly weight and feeding practices. HIV-exposure was determined from the child's health card as per the latest DNA/PCR or rapid test result. HIV negative mothers were tested every three months for HIV during antenatal care, with the latest test at 36 weeks documented in the child's health card. Socio-demographic characteristics, feeding practices and health history as potential independent variables were collected from the caregiver and the health card. Anthropometric measures of length/height and weight were measured in duplicate from all the infants as per WHO standard procedure [29] using standardised equipment. Weight was measured to the nearest 0.05g using calibrated Seca® Scales 385 and 875 (Seca gmbh & co, Hamburg, Germany) and length/height was measured to the nearest 1 mm using Seca® measuring board 417 (Seca gmbh & co, Hamburg, Germany) and Seca® stadiometre, Seca 217 (Seca gmbh & co, Hamburg, Germany). Length for age *z*-scores (*LAZ*), weight for age *z*-scores (*WAZ*) and weight for length *z*-scores (*WLZ*) were calculated according to the 2006 WHO child growth standards using the WHO

Anthro 2005 programme, Beta version [30]. Stunting, underweight and wasting was determined at *z* score < -2 SD based on LAZ, WAZ and WLZ respectively.

A modified USDA five step multiple Pass 24-hour dietary recall protocol [31] was used to measure infant's current nutritional intake as recalled by the caregiver. A similar multiple pass 24-hour dietary recall was validated in Ugandan children and was found to be valid in assessing dietary intake of infants residing in communities with similar diets [32]. Dietary diversity was calculated by allocating a score for consumption of food from one of the seven food groups (Grains, roots and tubers: Legumes & nuts: Dairy products: Flesh foods: Eggs: vitamin A rich fruits and vegetables: other fruits and vegetables) in the preceding 24 hours [33]. Therefore, resulting in a maximum possible score of 7, an infant's diet scoring 4 or more is considered diverse [33]. In addition, to dietary diversity [33], Nutritics© software [34], was used to derive the energy and protein intake of each infant. Nutritional information of foods consumed was derived from packaging, data from South African Composition Database [35] and McCance and Widdowson's composition of foods databases [36]. Cereals such as sorghum and fortified sorghum were consumed by majority of infants but nutritional content was not available. Therefore, cooked samples of these were weighed, frozen then freeze dried and analysed in the laboratory for protein per 100 grams using the Flash EA1112 nitrogen elemental analyser (Soeks FL 33334, USA). Energy per 100 grams was analysed using Parr 6300 Oxygen bomb calorimetre (Parr Instrument Co., Moline, Illinois, USA).

Data was entered into SPSS version 22 software [37] for analysis and 10% of this data was randomly selected using a computer number generator and then screened for accuracy by the co-authors.

Ethics

Ethical approval was received both from the University of Nottingham's Medical School Research Ethics Committee and the Health Research and Development Committee in Botswana. Informed consent was obtained from all caregivers. The two assistants were trained in seeking informed consent. When inappropriate feeding and/or malnutrition were identified the caregiver was briefly counselled by the lead author, who is also a registered dietitian. The caregiver was then referred to the health facility for further follow-up and this was documented in the child's health card to ensure continuity of care.

Statistical methods

Data was analysed using Statistical Package for Social Sciences, SPSS version 22 [37]. A case-control analysis approach was employed where HEU and HUU infants were compared for outcomes of interest. Baseline data is described as per HIV exposure. Chi square, was used to test for proportions between the two groups (HEU and HUU infants) to determine prevalence of underweight, wasting and stunting. Continuous variables were analysed using Kolgorov-Smirnov test to determine whether the distribution was Gaussian or not. Independent samples t-test or Mann-Whitney U test were used to test for differences between the two groups for parametric and non-parametric variables respectively. Variation of the mean was presented as standard deviation. Forward logistic regression was performed to determine predictors of stunting, underweight and wasting. The threshold for introducing the variables into the logistic regression model was set at $p < 0.1$. Cases with missing values for some of the independent variables were excluded. On this basis 86.2% of cases with no missing values were included in the analysis for each of the three dependent variables (stunting, underweight and wasting). Variables with missing data included feeding method at < 6 months (2.6%), feeding method at 6-12 months (6.1 %), birthweight (4.1%), Apgar score (2.9%), and age at which complementary feeds were introduced (2.4%). One of the co-authors (JAS) had the overall oversight of the statistical methods and analysis. Statistical significance was taken at $p < 0.05$ in all analysis.

Results

Characteristics of participants

A total of 413 participants were recruited, of which 154 were HEU (37.3%) and 259 were HUU (62.7%). Table 1 shows the characteristics of participants by HIV exposure. No significant differences were found between HEU and HUU infants in terms of age, proportions of sex, birthweight or length nor birthweight classification. However, HEU infants had significantly more siblings compared to HUU infants ($p<0.001$). In addition, HEU infants were more likely to have had a sibling who died compared to HUU infants ($p < 0.05$).

As shown in Table 1, HIV positive mothers tended to be older at the time of the infant's birth ($p<0.001$). In addition, the primary caregivers of HEU infants had significantly lower education levels ($p<0.001$). No significant differences were found in other mother/caregiver and household characteristics between the two groups.

Feeding practices

Table 2 shows feeding practices of infants per HIV-exposure from birth to age at time of data collection. These feeding practices were self-reported by the caregiver and corroborated using data from each child's health card, where possible. HEU infants were more likely to be formula fed from birth and at 6-12 months compared to HUU infants ($p<0.001$). The remainder of the infants (n=11) not breastfeeding or formula feeding in the first twelve months were taking cow's milk. Of those infants aged more than 12 months, it was found that HUU infants were more likely to be breastfed compared to their HEU counterparts ($p<0.001$). Overall the energy and protein intake for male and female HEU and HUU infants were higher than recommended nutrient intakes (RNI for infants aged 1-3 years). Average energy and protein intake was found to be higher in HEU compared to HUU infants for females and *vice versa* for males. However, both these differences did not reach statistical significance. In addition, there were no significant differences between HEU and HUU infants in age at which the infant was introduced to complementary feeds. Dietary diversity was low for all infants, and there was no significant difference between HEU and HUU infants.

Nutritional outcomes

The prevalence of underweight was higher in HEU infants (Table 3; $p<0.01$). In addition, HEU infants also had significantly higher prevalence of stunting compared to HUU infants (15.6% vs. 7.3 %, $p<0.05$). Wasting prevalence was higher in HEU infants; however this did not reach statistical significance ($p=0.14$).

Determinants of nutritional status

The results of logistic regression to identify the determinants of underweight, stunting and wasting are shown in Tables 4, 5 and 6. Table 4 shows the determinants of underweight. The analysis revealed that infants living in homes where a child had previously died were over three times more likely to be underweight (adjusted OR 3.205, 95% CI 1.097- 9.362). However, a higher birthweight or birth length was negatively associated with underweight ($p<0.001$, $p=0.03$ respectively). Each kilogram higher weight reduced risk of underweight by 82% (OR 0.182, 95% CI 0.073 -0.450). Similarly, a 1cm increase in birth length reduced risk by 10% (OR 0.899, 95% CI 0.818 -0.988). Importantly, HIV exposure, infant nutrient intakes, maternal and household factors were not associated with risk of underweight. Predictors for stunting as shown in Table 5, were consistent with the simple chi square analysis of prevalence. HEU infants were found to be more than twice as likely to be stunted compared to HUU infants (adjusted OR 2.361, 95% CI 1.105 -5.046). In addition, a lower level of mother/caregiver's education, and lower birthweight was associated with stunting. Again, nutrient intakes and other maternal and household factors were not significantly associated with risk of stunting. Wasting was more likely in infants with a high Apgar score, however residing in Kweneng East district (rural/semi urban) and having a higher birthweight was negatively associated with wasting. Each kilogram extra weight at birth reduced risk of wasting by 58% (adjusted OR 0.423, 95%CI 0.205-0.872). HIV exposure, infant nutrient intake and other household and maternal factors were not significantly associated with risk of wasting.

Discussion

Our study has demonstrated that HEU infants aged 6-24 months have poor nutritional outcomes compared to HUU infants. This has implications for policy and programming because currently prevention of mother-to-child transmission of HIV in HEU infants is prioritised over achieving optimal nutritional status. This has inadvertently resulted in inequitable growth between HEU and HUU infants. Data from 154 HEU infants and 259 HUU infants living in selected districts in Botswana demonstrated that HEU infants had higher prevalence of underweight and stunting. HEU infants were also more likely to formula feed in their first 12 months of life whereas HUU infants were more likely to breastfeed. Low birthweight was the strongest predictor of undernutrition in addition to HIV exposure, birth length, mother/care giver's education level, high Apgar score and residing in Kweneng East.

Prevalence of undernutrition in this study was higher in HEU infants compared to HUU infants during their first 1000 days. This is consistent with findings from a number of studies conducted in Zambia, Kenya, South-Africa, Uganda and Tanzania which have demonstrated that HEU infants have poor growth compared to HUU infants [9, 12, 20, 38-39]. A study in Kenyan infants found that HEU infants had poor nutritional outcomes especially very high levels of stunting by 24 months [12]. Prevalence of stunting in our study between HEU and HUU infants was similar to one found in a study of Ugandan infants enrolled in the PMTCT program [20]. Our bivariate analysis of the prevalence of stunting and underweight between HEU and HUU infants is therefore consistent with the larger body of literature. However, other studies conducted in sub-Saharan Africa did not find any differences in nutritional outcomes between HEU and HUU infants [19, 40-41]. It was found that HEU infants though born slightly smaller compared to HUU infants, were able to quickly catch up in weight and length [19, 41-42]. This lack of difference in growth patterns was attributed to higher levels of breastfeeding and/or effective counselling for feeding choices in HEU infants [19, 41]. In the current study HEU infants were more likely to be formula fed than breastfed compared to HUU infants. This may have contributed to their poor growth compared to HUU infants, since poor growth is linked to no or sub-optimal breastfeeding [38, 43]. It is important to note that our regression modelling indicated that mode of feeding in the first year of life, was not a statistically significant predictor of undernutrition. However, these studies were conducted before ART was widely available to HIV positive women, therefore this may have resulted in no difference in growth between HEU and HUU infants [19, 41-42]. Other feeding practices such as age of introduction of complimentary feeding (weaning), average energy and protein intake and dietary diversity were not significantly different between HEU and HUU infants. Dietary diversity was poor in both groups of infants because majority of infants did not consume a variety of foods in the 24 hours preceding the study. Dietary diversity is an important indicator of the quality of the diet as opposed to the quantity of the food served [26, 33].

HEU infants in this study were vulnerable to poorer nutritional outcomes, especially stunting because even after adjusting for other variables, HIV-exposure remained a strong predictor for stunting. This finding is consistent with results from a number of studies [18, 44-45]. A study, conducted in Tanzania found a lower length for age in HEU compared to HUU infants at three and six months [44]. A higher risk of stunting in HEU compared to HUU infants has serious implications because stunting is associated with poorer psychomotor and mental development in HEU infants [45]. This may affect the future potential development of these infants, especially if stunting is not reversed within the first 1000 days [46-48]. Factors such as exposure to ART during pregnancy, poor sanitation and infections in infants especially diarrhoea may account for the increased risk of stunting in HEU compared to HUU infants [26, 46]. In studies where poor growth was associated with HIV-exposure it was found that HEU infants had lower birthweight compared to HUU infants [14, 18, 40]. In the current study, HEU infants had lower birthweight compared to HUU infants, however this did not reach statistical significance. This is in contrast with a number of studies where HEU infants are more likely to be smaller at birth compared to HUU infants [49, 11, 44, 46]. Interestingly, low birthweight was a strong and consistent predictor for poor nutritional status (underweight, stunting and wasting). Infants with low birthweight tend to be more vulnerable to poor nutrition and/or diseases effect [14,18]. The findings of the current study show that birthweight is a more powerful predictor of later nutritional status than nutrient intakes from complementary feeds, breastmilk versus formula feeding, household and environmental factors including number of people living in a household, primary water source and income level. Even though birth length was not significantly lower in HEU compared to HUU infants, birth length remained a predictor for underweight, indicating that a lower birth length increased the risk of underweight in these infants. This is consistent with findings from some studies where birth length is a significant intermediary of growth in infants [44, 49-50].

Consistent with a number of studies it was found that mother/care giver's education level was a predictor for stunting after adjustment for other variables [26, 12, 18, 51]. In addition, HIV positive mothers were significantly older than HIV negative mothers. Younger age and higher education level are associated with better nutritional outcomes because these caregivers tend to have more knowledge about optimal feeding, hygiene and child caring practices [12, 18, 51-52]. These caring practices may especially be relevant in settings where HEU infants tend to formula feed [18]. It was also found that HEU infants had significantly more siblings than HUU infants. A higher number of siblings is associated with poor nutritional outcomes in children [53]. Although growing up in a household where another child had died was a significant predictor of the risk of underweight in univariate analysis, after adjusting for potential confounding factors there was no relationship between the number of deceased siblings and risk of stunting, wasting or underweight.

Other determinants of nutritional outcomes in these infants included residing in Kweneng East district and Apgar score. Infants who resided in Kweneng East had a lower risk of wasting compared to those in other districts. It is should be noted that Kweneng East district was the only district where growth and health monitoring services were still offered in the main and primary hospital. Other districts have moved these services to smaller clinics and/or health posts. Therefore, infants in Kweneng East district may have benefited from having close access to a multidisciplinary team of health professionals such as paediatricians and dietitians. These health-care workers are not typically accessible in smaller clinics. Accessibility to specialised care is highly relevant to wasting because wasting is an acute form of undernutrition, characterised by rapid weight loss due to acute inadequate intake and/or disease [(54)]. Therefore, infants in Kweneng East district were more likely to have accessed swift and specialised care upon being diagnosed with wasting compared to other districts. A higher Apgar score increased the risk of wasting in these infants almost two-fold. This was not expected because a higher Apgar score is associated with better nutritional outcomes [(55)]. However, a study in Asian Indian infants found that Apgar is a poor prognosis for growth and development in infants [(56)].

It is important to note the following limitations about the current study. We have only considered the impact of HIV exposure, infant feeding, maternal and household factors upon nutritional status using the extreme outcome measures of stunting, wasting and underweight as determined by anthropometry and reference to WHO cut-offs for *z* scores. Indices such as micronutrient deficiencies were not included and we also did not focus on lower variance from cutoffs in terms of growth. Contribution of HIV-exposure may be greater at these subclinical levels and thus the *z* scores may be lower in HEU compared to HUU infants. Due to the cross-sectional study design, we did not have access to maternal nutrition and health indicators variables such as weight, height, CD4 count and use of ART pre-and post-natally. There is also a possibility, albeit a limited one, that some of the infants who were classified as HEU may have been HIV-infected after 6 weeks, since testing of HIV in these infants in Botswana is done at 6 weeks, post weaning if the mother was breastfeeding (6 months) and at 18 months. Some of the infants in our study were not yet 18 months, at the time of data collection. However, a majority of these infants were formula feeding, therefore it was highly unlikely that they would have seroconverted. The parity of the mother was not considered in logistic regression. In addition, we have to acknowledge the cross-sectional nature of this study especially in regards to HIV-exposure and nutritional outcomes. Longitudinal studies are therefore required to elicit more data which will allow us to disentangle feeding modalities from HIV-exposure and also to derive more information on maternal nutrition and health during pregnancy.

PMTCT strategies in Botswana needs to be refined, so that optimal nutritional outcomes in HEU infants are prioritised in addition to prevention of MTCT of HIV. This can be achieved by integrating nutrition-specific and -sensitive interventions into this program. This will ensure equitable and optimal growth in HEU and HUU infants during their first 1000 days. Botswana as a country in terms of its health care system infrastructure, PMTCT strategies and growth surveillance for infants is in a good position to effect these significant changes, and thus improve population health.

In Botswana, HEU infants aged 6-24 months have poor nutritional status compared to HUU infants. Although mode of feeding was not a statistically significant factor determining risk of undernutrition, HEU infants tended to formula feed while HUU infants tended to breastfeed for the first twelve months of life. Therefore, HEU infants are missing out on the well documented benefits of breastfeeding. In order to increase breastfeeding levels in HEU infants there is need to review the current Botswana government's infant feeding policy in order to align with the new 2016 recommendations by WHO. Furthermore, this study demonstrated that the strongest predictor of nutritional outcomes is birthweight, therefore strategies designed to optimise infants' nutritional status in the first 1000 days should aim to improve birthweight.

Running title

HIV-exposure and nutritional status in infants.

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Transparency Declaration

The lead author confirms that the manuscript is an honest, accurate and transparent account of the study being reported and that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with STROBE guidelines.

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Table 1: Characteristics of HEU and HUU infants from selected districts in Botswana (N= 413)

Characteristic	Total (N= 413)	HEU infants (n= 154)	HUU infants (n=259)	*P- value*
Infant's characteristics				
Age in months [Median,(IQR)] [1,3]	14.00 (9.00)	14.00 (9.00)	14.00 (9.00)	0.96
Sex [%] [2]				
Females	52.3	50.6	53.3	0.68
Males	47.7	49.4	46.7	
Birthweight in Kg [Mean ± SD] [3]	3.01 ± 0.47	2.96 ± 0.50	3.03 ± 0.46	0.15
Birth Length in cm [Mean ± SD] [3]	50.01 ± 3.87	49.71 ± 3.96	50.19 ± 3.80	0.23
Birthweight classification [%] [2]				
Low: < 2.5Kg	2.1	12.3	12.1	1.00
Normal: ≥ 2.5 kg	87.4	87.7	87.9	
Number of siblings [Median,(IQR)] [3]	1.00 (2.00)	2.00 (2.00)	1.00 (1.00)	**<0.001**
Siblings who have died? [%] [2]				
Yes	7.7	11.7	5.4	**0.03**
No	92.3	88.3	94.6	
Mother/care-giver's characteristics				
Mother's age at birth [Median,(IQR)] [3]	26.00 (9.00)	30.00 (8.00)	25.00 (7.00)	**<0.001**
Primary care-giver's education level [%] [2]				
0-7 years	9.9	17.5	5.4	
≥ 8 years	90.1	82.5	94.6	**<0.001**
Primary care-giver's marital status [%] [2]				
Single/widowed/divorced/ other	79.2	76.0 %	81.1	0.27
Married/lives with partner	20.8	24.0 %	18.9	

Primary care-giver's Employment status [%][2]				
Not employed	75.8	77.3	74.9	
Self-employed	3.9	4.5	3.5	0.63
Formally employed	20.3	18.2	21.6	
Primary care-giver's monthly income [%][2]				
0-599 BWP[4]	69.0	68.8	69	
600-999 BWP	5.1	5.8	5.1	0.86
1000+ BWP	25.9	25.3	25.9	
Characteristics of the household				
Number of people in the household [Median,(IQR)][3]	6.00 (4.00)	6.00 (4.00)	6.00 (4.00)	0.88
Primary water source [%][2]				
Piped	99.5	99.4	99.6	
Not piped	0.5	0.6	0.4	0.71
Toilet type in homestead [%][2]				
Flush	25.6	21.6	28	
Pit latrine	74.4	78.4	72	0.18

1. Age at the time of data collection
2. Chi square was used to test difference in proportions [%] between HEU and HUU infants for various variables.
3. Mann-Whitney U test/ t-test was used to test for differences between HEU and HUU infants for non- parametric and parametric variables respectively.
4. $1 = 10.30 BWP (Botswana Pula) at the time of data collection
5. **Abbreviations:** HEU:HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected; SD: Standard Deviation; IQR: Inter Quartile Range

Table 2: Feeding practices of infants by HIV exposure from selected districts in Botswana.

Feeding practice	Total	HEU infants (n= 154)	HUU infants (n=259)	*P- value*
Feeding method at < 6 months [%][1]				
Breastfeeding [BF]	n= 260 [63.0]	n= 27 [17.5]	n= 233 [94.0]	**<0.001**
Formula Feeding [FF]	n= 142 [34.3]	n= 127 [82.5]	n= 15 [6.0]	
Feeding method between 6-12 months [%][1]				
Breastfeeding [BF]	n= 186 [45.0]	n= 0 [0]	n= 186 [82.3]	**<0.001**
Formula Feeding [FF]	n= 188 [45.5]	n= 148 [100]	n= 40 [17.7]	
Feeding method at 12+ months [%][1]				
Breastfeeding [BF]	n= 29 [19.0]	n= 0 [0]	n= 29 [30.2]	**<0.001**
Formula Feeding [FF]	n= 12 [7.9]	n= 7 [12.5]	n= 5 [5.2]	
Cow's milk	n= 111 [73.0]	n= 49 [87.5]	n= 62 [64.6]	
Age introduced complementary feeds in months[2]				
[Median,(IQR)]	n= 413 6.00 (0.00)	n= 154 6.00 (0.00)	n= 259 6.00 (0.00)	0.92
Average Energy Intake in Kcal [Mean ± SD][3]				
Females	n= 216 [1684.9 ± 867.7]	n= 78 [1778.5 ± 855.4]	n= 138 [1632.0 ± 878.7]	0.23
Males	n= 197 [1810.3 ± 830.6]	n= 76 [1747.7 ± 716.8]	n= 121 [1849.6 ± 895.4]	0.38
Average Protein Intake in grams [Mean ± SD][3]				
Females	n= 216 [53.2 ± 27.5]	n= 78 [56.5 ± 26.9]	n= 138 [51.3 ± 27.7]	0.18
Males	n= 197 [57.5 ± 28.0]	n= 76 [56.4 ± 26.8]	n= 121 [58.2 ± 28.9]	0.65
Dietary Diversity [%][4]	n= 413	n= 154	n= 259	
Diet diverse	[16.8]	[20.1]	[14.8]	0.17
Diet not diverse	[83.2]	[79.9]	[85.2]	

1. Chi square was used to test for differences in proportions of feeding method between HEU and HUU infants.
2. Mann-Whitney U test was used to test for differences in age introduced complementary feeds.
3. Independent-samples t-test was used to test for differences in energy and protein intake between HEU and HUU infants per gender.
4. **Dietary diversity:** Proportion of children 6-23 months of age who receive foods from 4 or more food groups (Food groups: Grains, roots & tubers, Legumes & nuts, Dairy products, Flesh foods, Eggs, Vitamin A rich fruits and vegetables, Other fruits and vegetables).

5. **Abbreviations:** HEU:HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected; SD: Standard Deviation; IQR: Inter Quartile Range

Table 3: Prevalence of undernutrition in HEU and HUU infants from selected districts in Botswana.

Nutritional status	Total (N=413)	HEU infants (n= 154)	HUU infants (n=259)	*P- value*
Underweight [%][1]				
Yes	10.2	15.6	6.9	**< 0.01**
No	89.8	84.4	93.1	
Stunting [%][1]				
Yes	10.4	15.6	7.3	**< 0.05**
No	89.6	84.4	92.7	
Wasting [%][1]				
Yes	11.9	14.9	10	0.14
No	88.1	85.1	90	

1. Chi square was used to test for difference in proportions [%] of nutritional status between HEU and HUU infants.
2. **Abbreviations:** HEU: HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected

Table 4: Logistic regression model of predictors of underweight in infants aged 6-24 months in selected districts in Botswana (n= 356)

							95% C.I.for odds ratio	
		B	S.E.	Wald	*P-value*	Odds ratio	Lower	Upper
Step 1	Birthweight	-2.091	.410	25.981	.000	.124	.055	.276
	Constant	3.836	1.127	11.589	.001	46.346		
Step 2	Primary water source	-2.696	1.468	3.375	.066	.067	.004	1.198
	Birthweight	-2.144	.416	26.510	.000	.117	.052	.265
	Constant	6.646	1.939	11.749	.001	769.770		
Step 3	Primary water source	-2.689	1.456	3.412	.065	.068	.004	1.179
	Birthweight	-1.694	.460	13.567	.000	.184	.075	.453
	Birth Length	-.104	.048	4.811	.028	.901	.821	.989
	Constant	10.475	2.661	15.498	.000	35411.123		
Step 4	Primary water source	-2.821	1.459	3.738	.053	.060	.003	1.040
	Infant lives in home where a child has died	1.165	.547	4.537	.033	3.205	1.097	9.362
	Birthweight	-1.706	.463	13.550	.000	.182	.073	.450
	Birth Length	-.106	.048	4.902	.027	.899	.818	.988
	Constant	10.610	2.665	15.852	.000	40537.131		

Model 1: R^2 = 16.3%, X^2=30.25, *df*= 1, p< 0.001: Model 2: R^2 = 17.8 %, X^2=33.03, *df*= 2, p< 0.001: Model 3: R^2 = 20.0 %, X^2=37.46 *df*= 3, p< 0.001: Model 4: R^2 = 22.0 %, X^2=41.48, *df*= 4, p< 0.001

Other independent variables entered into the model are: HIV exposure, gender, feeding method at < 6 months, feeding method at 6-12 months, Infant primary care giver, is mother alive?, mother/caregivers education, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, consumption of at least one source of iron rich food?, dietary diversity, age in months , APGAR score, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

Abbreviations: C.I: Confidence Interval, WAZ: Weight for Age; APGAR (Appearance, Pulse, Grimace, Activity and Respiration). APGAR test is usually given to a baby twice, a minute after birth and then five minutes after birth.

Table 5: Logistic regression model of predictors of stunting in infants aged 6-24 months in selected districts in Botswana (n= 356)

		B	S.E.	Wald	*P-value*	Odds ratio	95% C.I.for Odds ratio	
							Lower	Upper
Step 1	Birthweight	-1.519	.393	14.945	.000	.219	.101	.473
	Constant	2.142	1.099	3.800	.051	8.516		
Step 2	Mother/care giver education	-1.429	.478	8.954	.003	.239	.094	.611
	Birthweight	-1.588	.400	15.740	.000	.204	.093	.448
	Constant	3.563	1.228	8.417	.004	35.276		
Step 3	Mother/care giver education	-1.475	.481	9.410	.002	.229	.089	.587
	Primary water source	-2.845	1.457	3.814	.051	.058	.003	1.010
	Birthweight	-1.637	.406	16.295	.000	.194	.088	.431
	Constant	6.552	2.006	10.661	.001	700.311		
Step 4	HIV exposure	.859	.388	4.913	.027	2.361	1.105	5.046
	Mother/care giver education	-1.257	.498	6.376	.012	.284	.107	.755
	Primary water source	-2.797	1.441	3.769	.052	.061	.004	1.027
	Birthweight	-1.637	.413	15.728	.000	.195	.087	.437
	Constant	5.902	2.025	8.499	.004	365.789		

Model 1: R^2 = 9.1 %, X^2=15.64, *df*= 1, p< 0.001: Model 2: R^2 = 13.4 %, X^2=23.40, *df*= 2, p< 0.001: Model 3: R^2 = 15.1 %, X^2=26.45 *df*= 3, p< 0.001: Model 4: R^2 = 17.8 %, X^2=31.44 *df*= 4, p< 0.001

Other independent variables entered into the model are: gender, feeding method at < 6 months, feeding method at 6-12 months, Infant primary care giver, is mother alive?, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, does infant live in environment where a child has died?, consumption of at least one source of iron rich food?, dietary diversity, age in months, birth length, APGAR score, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

Abbreviations: C.I: Confidence Interval, LAZ: Length for Age; APGAR (Appearance, Pulse, Grimace, Activity and Respiration). APGAR test is usually given to a baby twice, a minute after birth and then five minutes after birth.

Table 6: Logistic regression model of predictors of wasting in infants aged 6-24 months in selected districts in Botswana (n= 356)

		B	S.E.	Wald	*P-value*	Odds ratio	95% C.I.for Odds ratio	
							Lower	Upper
Step 1	Residing in Kweneng East district	-.908	.349	6.750	.009	.404	.203	.800
	Constant	-1.596	.197	65.697	.000	.203		
Step 2	Residing in Kweneng East district	-.924	.352	6.890	.009	.397	.199	.791
	Birthweight	-.807	.354	5.188	.023	.446	.223	.894
	Constant	.775	1.043	.553	.457	2.172		
Step 3	Primary water source	-2.734	1.454	3.536	.060	.065	.004	1.123
	Residing in Kweneng East district	-1.002	.361	7.679	.006	.367	.181	.746
	Birthweight	-.834	.358	5.435	.020	.434	.215	.876
	Constant	3.588	1.848	3.768	.052	36.152		
Step 4	Primary water source	-2.517	1.456	2.989	.084	.081	.005	1.400
	Residing in Kweneng East district	-1.053	.365	8.332	.004	.349	.171	.713
	Birthweight	-.860	.369	5.437	.020	.423	.205	.872
	Apgar score at birth	.582	.283	4.247	.039	1.790	1.029	3.115
	Constant	-2.106	3.330	.400	.527	.122		

Model 1: $R^2 = 3.9\%$, $X^2=7.30$, *df*= 1, p< 0.001: Model 2: $R^2 = 6.6\ \%$, $X^2=12.50$, *df*= 2, p< 0.001: Model 3: $R^2 = 8.0\ \%$, $X^2=15.36$ *df*= 3, p< 0.001: Model 4: $R^2 = 10.7\ \%$, $X^2=20.61$, *df*= 4, p< 0.001

Other independent variables entered into the model are: HIV exposure, gender, feeding method at < 6 months, feeding method at 6-12 months, Infant primary care giver, is mother alive?, mother/caregivers education, mother/caregiver's marital status, mother/caregiver's employment status, mother/caregiver's income per month, toilet type in homestead, health facility type, district, does child live in environment where a child has died?, consumption of at least one source of iron rich food?, dietary diversity, age in months, birthweight, birth length, age introduced complementary feeds, number of servings of tsabana per week, number of consultations with diarrhoea, number of feeds given yesterday, energy intake, protein intake, mother's age at birth, number of people in household and number of siblings/relatives living with infant aged <5 years.

Abbreviations: C.I: Confidence Interval, WLZ: Weight for Length; APGAR (Appearance, Pulse, Grimace, Activity and Respiration). APGAR test is usually given to a baby twice, a minute after birth and then five minutes after birth.

Supplementary Table 1: Independent variables used in logistic regression model

Variable	Definition	Variable type	Data Source
Dependant variables			
Wasting	Yes: WLZ < -2 No: WLZ ≥ -2	Categorical	Anthropometric data
Stunting	Yes: LAZ < -2 No: LAZ ≥ -2	Categorical	Anthropometric data
Underweight	Yes: WAZ < -2 No: WAZ ≥ -2	Categorical	Anthropometric data
Independent variables			
1. HIV exposure	Yes: HEU No: HUU	Categorical	Health card
2. Gender	0= Females 1=Males	Categorical	Health card/interview of caregiver
3. Feeding method at < 6 months	0= Formula feeding 1= Breast Feeding	Categorical	Health card/interview of caregiver
4. Feeding method 6- 12 months	0= Formula feeding 1= Breast Feeding	Categorical	Health card/interview of caregiver
5. Predominant feeding method	0= Formula feeding 1= Breast Feeding	Categorical	Health card/interview of caregiver
6. Child ever breastfed	0=No 1= Yes	Categorical	Health card/interview of caregiver
7. Type of mixed feeding	0= Formula feeding/other milk 1= Breast Feeding/other milk	Categorical	Health card/interview of caregiver
8. Formula feeding frequency appropriate	0=No 1= Yes	Categorical	24 hour dietary recall
9. Provision of formula	0= Poor 1= Good	Categorical	Health card/interview of caregiver
10. Consumption of at least one source of iron?	0= No	Categorical	24 hour dietary recall

	1= Yes		
11. Diet diverse?	0= No 1= Yes	Categorical	24 hour dietary recall
12. Minimum acceptable diet	0= No 1= Yes	Categorical	24 hour dietary recall
13. Gross development delay at assessment	0= No 1= Yes	Categorical	Assessment/ interview of caregiver
14. Does the child live in an environment where a child has died?	0= No 1= Yes	Categorical	Interview of caregiver
15. Infant Primary care giver	Parent (1) vs. Otherwise (0) Grandmother (1) vs. Otherwise (0)	Categorical	Interview of caregiver
16. Is mother alive	0= No 1= Yes	Categorical	Interview of caregiver
17. Caregivers education level	0= 0-7 years 1= ≥ 8 years	Categorical	Interview of caregiver
18. Caregivers marital status	0= single/divorced/widowed 1= married/partnered	Categorical	Interview of caregiver
19. Caregivers employment status	Not employed (1) vs. Otherwise (0) Formally employed vs. Otherwise (0)	Categorical	Interview of caregiver
20. Caregivers income per month	0-599 BWP[1] (1) vs. Otherwise (0) 600-999 BWP[1] (1) VS. Otherwise (0)	Categorical	Interview of caregiver
21. Primary water source	0= Not-Piped 1= Piped	Categorical	Interview of caregiver
22. Toilet type in homestead	0= Pit latrine 1= Flush	Categorical	Interview of caregiver
23. Health facility type	Hospital/primary hospital (1) vs. Otherwise (0) Clinic (1) vs. Otherwise (0)	Categorical	Observation
24. District	Kweneng East (1) vs. Otherwise (0) Francistown (1) vs. Otherwise (0) Selebi Phikwe (1) vs. Otherwise (0)	Categorical	Observation
25. Location type	Rural (1) vs. Otherwise (0) Semi-urban (1) vs. otherwise (0)	Categorical	Observation

	Urban vs. otherwise (0)		
26. Pedal oedema	Presence of pedal oedema on both feet	Categorical	Observation
27. Vaccination update	Assessment of whether vaccinations for each child is updated.	Categorical	Health card
28. Mother HIV status	Mother's current HIV status	Categorical	Health card
29. Age in months at assessment	Age in months at time of data collection	Continuous	Health card/interview of caregiver
30. Birthweight	Weight as recorded in the health card	Continuous	Health card
31. Birth Length	Length as recorded in the health card	Continuous	Health card
32. APGAR Score	Measure of the physical condition of a new-born infant for heart rate, respiratory effort, muscle tone, response to stimulation and skin colour. Max score = 10	Continuous	Health card
33. Age introduced complementary feeds	Age in months first started on other foods besides milk.	Continuous	Health card/interview of caregiver
34. Number of servings of Tsabana[2] per week	Frequency of servings of Tsabana regardless of serving size per week.	Continuous	24 hour dietary recall
35. Number of consultations for diarrhoea (health card)	Frequency of consultation of diarrhoea as recorded in the health card in the previous month. Counted as one consultations per each date.	Continuous	Health card
36. Number of days with episode of diarrhoea (caregiver)	Number of days with diarrhoea as recalled by the caregiver in the previous month.	Continuous	Interview of caregiver
37. Number of feeds given yesterday	Frequency of feeds solid, semi-solid or soft food received during the previous day	Continuous	24 hour dietary recall
38. Energy intake	Energy intake as determined from the 24 hour dietary intake recall.	Continuous	24 hour dietary recall
39. Protein intake	Protein intake as determined from the 24 hour dietary intake recall.	Continuous	24 hour dietary recall

40. Mother's age at birth	The age that mother was when she gave birth to the infant.	Continuous	Health card/interview of caregiver
41. Number of children mother has	The number of children the mother has	Continuous	Interview of caregiver
42. Mother's current age	Mother's current age	Continuous	Health card/interview of caregiver
43. Number of people in the household	All people who resides in the household within which the infant lives and who also share meals as a family unit.	Continuous	Interview of caregiver
44. Number of siblings/relatives living with infant aged < 5 years	All infants aged < 5 years currently living in same home (thus sharing meals) with infant.	Continuous	Interview of caregiver
45. Duration mother given ZDV	As indicated from the health card, only applicable in HIV-positive mothers.	Continuous	Health card

Abbreviations: HEU: HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected; WAZ: Weight for Age; LAZ: Length for Age; WLZ: Weight for Length; APGAR (Appearance, Pulse, Grimace, Activity and Respiration). APGAR test is usually given to a baby twice, a minute after birth and then five minutes after birth.

Tsabana: a sorghum based cereal, with added soya blend and micronutients. Provided to infants aged 6-36 months in governemnt health facilites as a way of improving the nutritional intake of infants during weaning.