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

Paphani Chalashika, Christine Essex, Duane Mellor, Judy Swift, Simon Langley-Evans

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Predictors of malnutrition in Botswanan infants during their first 1000 days; the contribution of birthweight, infant feeding practices and HIV-exposure

Authors:

1. Paphani Chalashika: PhD student
2. Christine Essex: Technical Specialist
3. Duane Mellor: Senior Lecturer in Human Nutrition
4. Judy A. Swift: Associate Professor of Behavioural Nutrition
5. Simon Langley-Evans: Head of School of Biosciences and Professor of Human Nutrition

1,2,4,5: University of Nottingham, Division of Nutritional Sciences, School of Biosciences, Sutton Bonington, Loughborough, LE12 5RD, United Kingdom.
3: Coventry University, School of Life Sciences, Coventry, CV1 5FB, United Kingdom

Corresponding author:
Paphani Chalashika: (Paphani.Chalashika@nottingham.ac.uk or paphanic@gmail.com)

Authors’ Roles:

PC, SLE, JAS and DM were responsible for the study design and protocol and tools used in the study. PC was responsible for data collection. CE was responsible for nutritional analysis of the cereal samples consumed by study participants. Data analysis and review was conducted by PC, JAS and SLE. All authors were responsible for completing the manuscript.

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 interventions 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. 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
259 HUU infants. Data from 154 HEU infants and 259 HUU infants living in selected districts in 260 Botswana demonstrated that HEU infants had higher prevalence of underweight and stunting. HEU 261 infants were also more likely to formula feed in their first 12 months of life whereas HUU infants 262 were more likely to breastfeed. Low birthweight was the strongest predictor of undernutrition in 263 addition to HIV exposure, birth length, mother/care giver’s education level, high Apgar score and 264 residing in Kweneng East.

265 Prevalence of undernutrition in this study was higher in HEU infants compared to HUU infants during 266 their first 1000 days. This is consistent with findings from a number of studies conducted in Zambia, 267 Kenya, South-Africa, Uganda and Tanzania which have demonstrated that HEU infants have poor 268 growth compared to HUU infants (9, 12, 20, 38-39). A study in Kenyan infants found that HEU infants had 269 poor nutritional outcomes especially very high levels of stunting by 24 months (12). Prevalence of 270 stunting in our study between HEU and HUU infants was similar to one found in a study of Ugandan 271 infants enrolled in the PMTCT program (20). Our bivariate analysis of the prevalence of stunting and 272 underweight between HEU and HUU infants is therefore consistent with the larger body of literature. 273 However, other studies conducted in sub-Saharan Africa did not find any differences in nutritional 274 outcomes between HEU and HUU infants (19, 40-41). It was found that HEU infants though born slightly 275 smaller compared to HUU infants, were able to quickly catch up in weight and length (19, 41-42). This 276 lack of difference in growth patterns was attributed to higher levels of breastfeeding and/or effective 277 counselling for feeding choices in HEU infants (19, 41). In the current study HEU infants were more 278 likely to be formula fed than breastfed compared to HUU infants. This may have contributed to their 279 poor growth compared to HUU infants, since poor growth is linked to no or sub-optimal breastfeeding 280 (38, 43). It is important to note that our regression modelling indicated that mode of feeding in the first 281 year of life, was not a statistically significant predictor of undernutrition. However, these studies were 282 conducted before ART was widely available to HIV positive women, therefore this may have resulted in 283 no difference in growth between HEU and HUU infants (19, 41-42). Other feeding practices such as 284 age of introduction of complimentary feeding (weaning), average energy and protein intake and 285 dietary diversity were not significantly different between HEU and HUU infants. Dietary diversity 286 was poor in both groups of infants because majority of infants did not consume a variety of foods in 287 the 24 hours preceding the study. Dietary diversity is an important indicator of the quality of the diet 288 as opposed to the quantity of the food served (26, 33).

289 HEU infants in this study were vulnerable to poorer nutritional outcomes, especially stunting because 290 even after adjusting for other variables, HIV-exposure remained a strong predictor for stunting. This 291 finding is consistent with results from a number of studies (18, 44-45). A study, conducted in Tanzania 292 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. This may affect the future potential development of these infants, especially if stunting is not reversed within the first 1000 days. 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. In studies where poor growth was associated with HIV-exposure it was found that HEU infants had lower birthweight compared to HUU infants. 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. 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. 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.

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. 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. These caring practices may especially be relevant in settings where HEU infants tend to formula feed. 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. 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. 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.

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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.

References


