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EFFECT OF MATERNAL AFLATOXIN EXPOSURE THROUGH DIET ON GROWTH OF INFANTS 0 - 3 MONTHS IN KISUMU COUNTY, KENYA

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ABSTRACT

Aflatoxins are naturally occurring carcinogenic toxins associated with poor growth outcomes in young children. Although evidence supports mother-to-infant exposure during pregnancy and breastfeeding, evidence of its effect on growth is limited to the period after introduction of complementary foods. It is, therefore, unclear whether early maternal exposure to aflatoxins affects infant growth right from birth. Prevalence of aflatoxin levels of 40% has been observed in Nyanza region, Kenya, and 22.7% of children under 5 years are stunted. The purpose of this study was to determine the effect of maternal aflatoxin exposure on growth of infants 0-3 months old in Kisumu County, Kenya. Specific objectives were to: establish association between maternal baseline characteristics and aflatoxin exposure; establish association between infant baseline characteristics at birth and maternal aflatoxin exposure; determine effect of maternal aflatoxin exposure on infant growth indicators at 3 months of age. Out of 553 pregnant women who were screened for aflatoxin exposure, 137 exposed and 137 non-exposed women, matched for age and household income, participated in an 8-month cohort study. The women were followed up to delivery and their infants up to 3 months after delivery. Infant length and weight data was collected monthly. Length-for-age (LAZ), weight-for-length (WLZ)) and weight-for-age (WAZ) z-scores were generated. Aflatoxin levels were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) in parts per billion (ppb). Effects of aflatoxin on infant growth outcomes were assessed using multi-variate linear and logistic regression. Effect of maternal aflatoxin exposure on infant length, weight, LAZ, WLZ, WAZ was determined using Cox regression with constant time at risk. Infants of exposed women had lower weight (95% CI:-0.85,-0.53), length (95% CI: -4.08, -3.36), LAZ (95% CI: -1.93, -1.16) and WAZ (95% CI:-1.03, -0.54) at 3 months of age, but there was no difference in WLZ (95% CI:-0.03, 0.74). Risk for stunting was higher in infants of exposed women (RR=4.08; 95% CI: 1.35, 12.29). There was no difference in the risk for underweight (RR=6.61; 95% CI: 0.80-54.33) and wasting (RR=0.37; 95% CI: 0.40, 3.39, P=0.38).These results underpin the need to reduce aflatoxin exposure in infants and young children who are very vulnerable.

Key words: Aflatoxin, maternal, infant, growth, z-scores, ELISA, stunting, underweight

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INTRODUCTION

Aflatoxins are naturally occurring carcinogenic food toxins produced by species of fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. The toxins are associated with poor growth outcomes, especially stunting in young children [1, 2], as well as liver and other cancers in humans and other animals [3]. It is indicated that about 25% of the world's food could be contaminated with aflatoxins [4] and about 4.5 billion people globally exposed to aflatoxins through contaminated foods [3], giving rise to concerns that if not checked, aflatoxin contamination could impair food security and pose a great health risk to consumers, especially in developing countries [5]. Prior to the review by Williams and colleagues [3], aflatoxin was not accorded much attention, yet adverse effects may result from long term exposure even to low levels of the toxin in the food supply chain [6].

Studies carried out in Kenya reveal that more than 40% of diets in both rural and urban communities are likely to be contaminated by the aflatoxin [7]. This suggests that a big proportion of the Kenyan population risks exposure to aflatoxin and the associated health risks. High levels of aflatoxins in maize have been reported in Eastern Region of Kenya [8]. Cases of aflatoxin contamination of staple foods have also been reported in Nyanza [7, 9], and western regions of Kenya [10]. In an earlier study, consistently higher levels of aflatoxins were found in sorghum compared to other foods [11]. This points to widespread contamination of foods in other parts of the Kenya apart from eastern region and in other food chains.

Pregnant women may be exposed to aflatoxin through consumption of aflatoxin contaminated foods, posing a health risk not only to the women but also to the unborn fetus and the young child [12]. However, previous studies have used aflatoxin serum albumin adducts to determine aflatoxin exposure in pregnant women. Dietary intake, although a less sensitive indicator, has been recommended as one of the most practical ways of determining aflatoxin exposure [3], and was used to determine aflatoxin exposure in pregnant women in the current study. Actual information on aflatoxin intakes through diet has been shown in West Africa [1, 12]. In spite of aflatoxicosis outbreak in Kenya [8], information on aflatoxin intake that would enable estimation of exposure of pregnant women through diet in Kisumu County is lacking.

Globally, malnutrition is the underlying contributing factor in about 45% of the deaths of millions of children under the age of five years [13], and more than 50% of deaths in this group occur in developing countries. Data by Kenya Demographic and Health Survey [KDHS] 2008-2009 show stunting rates of 26.9% for Nyanza region [14], suggesting the need for more intervention to address factors contributing to malnutrition. Maternal aflatoxin exposure has been implicated in poor growth outcomes in infants, yet the focus has been on the period after complimentary feeding [1]. Further, most studies addressing exposure of infants to aflatoxin prior to complementary feeding have mainly assessed exposure to aflatoxin in women and infants using serum aflatoxin albumin adducts [15], and associations of aflatoxin with growth status in cross sectional studies [16], but not in appropriately designed longitudinal studies that are necessary for inference of causation. Conclusive evidence of the role of aflatoxin on growth of infants prior to complementary feeding is, therefore, lacking up to the time of writing this manuscript.

This study aimed to determine the effect of maternal aflatoxin exposure on the growth of infants 0-three months old in Kisumu County, Kenya. Certain characteristics make Kisumu County prone to aflatoxin contamination, such as prevailing climatic conditions including frequent floods, drought, erratic rainfall (1200mm and 1300mm), high temperatures (20ºC and 35ºC) and high humidity (40 % - 89%) [17], which provide a favorable environment for the growth of moulds and production of aflatoxins. Kisumu is, therefore, an appropriate area where the question of effect of aflatoxins on infant growth may be investigated, but such information would also be relevant to other aflatoxin-prone areas.

MATERIAL AND METHODS

This study was conducted in Kisumu East and Nyando Sub-Counties, Kisumu County, Kenya. Kisumu County has seven Sub-Counties (Kisumu East, Kisumu West, Kisumu Central, Seme, Nyando, Muhoroni and Nyakach), with an area of 2,085.9 km^2 and a population of 968,909 (Males, 48.9 % and Females, 51.1 %), 160,609 children 0-4 years (50.1% males, 49.9% females), 226,719 households and a population density of 465 people per square kilometer [17].

The study used a prospective cohort design to assess the effect of aflatoxin exposure through maternal dietary intake on growth of infants of exposed and non-exposed women in the first three months of life. All pregnant women attending antenatal care clinics at Kisumu County Referral and Ahero County Hospitals, Kisumu County, were recruited and screened for inclusion in the study between May and August 2013. Pregnant women were eligible for inclusion in the study if they were registered attendees at Kisumu County Referral Hospital or Ahero County Hospitals, and consented to participate in the study; were up to 8 months pregnant as confirmed by the records in the mother-child health booklet; were residing in Kisumu East and Nyando Sub-Counties, Kisumu County, for the entire study period; were willing to deliver at designated health facilities; had no history of chronic illness such as diabetes and HIV and AIDS as indicated in mother/child-health booklet; were not on regular medication; and had no pregnancy complications. The 'up to 8 months' pregnancy stage was chosen to give ample time for collection of meal samples and analysis in order to identify exposed and non-exposed women to participate in the study.

Sample size calculation was based on the minimum sample size required to detect a difference in growth of exposed and non-exposed infants. Based on the ability to detect a mean difference of 0.6 cm in length [18] with 90% power, significance level of 5% (α =0.05); two-sided test; a standard deviation (SD) of 1.5 and an estimated loss to follow-up of 10% [19], 132 mother/infant pairs were required (146 after inflating to cater for loss to follow-up), as shown in the formula below:

$$n = \frac{(u+v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

$\mu_1 - \mu_0 =$ Difference between the means
σ_1, σ_0 = Standard deviations of exposed and non-exposed; 1.5
u = Two sided percent point of the normal distribution corresponding to 100% minus the power for example if power = 90%, u is 1.28 (for all cases)
v = Percentage point of normal distribution corresponding to the (two-sided) significance level (95% confidence interval); if significance level = 5%, v = 1.96

$$n = \frac{(1.28 + 1.96)^2 (1.5^2 + 1.5^2)}{(0.6)^2}$$

$$n = \frac{(1.28 + 1.96)^2 (1.5^2 + 1.5^2)}{0.36} = \frac{47.2329}{0.36} = 132 \text{ minimum sample size per group}$$

Assuming an estimated loss to follow-up of 10% (10% of 132 =14), 146 women was required per group (a total of 146 x 2 = 292 women).

To estimate the number of women needed to be screened to achieve the required sample size, it was assumed that 40% of the population could be exposed to aflatoxin. This was based on research findings on prevalence of aflatoxin exposure in the study area, which indicate that 40% of households in Nyanza Region are likely to be exposed to aflatoxin [7, 10]. It was, therefore, assumed that at most 40% of participants could express indicators of aflatoxin exposure. A minimum of 365 women needed to be screened to identify 146 exposed women, using the following formula:

$$\frac{100 \times 146}{40} = 365 \text{ women needed to be screened.}$$

However, because of the uncertainty about the 40% that would be exposed, double the minimum number required (365 x 2= 730) of pregnant women were screened. A total of 553 women were finally enrolled to participate in the study and of these, 137 were exposed and 416 were non-exposed. This was above the minimum required sample size of 132 for exposed and non-exposed. Ahero County Hospital had a higher number of pregnant women attending antenatal clinic compared to Kisumu County Referral Hospital.

In the Kenyan healthcare system, Community Health Volunteers (CHVs) aid in health promotion and collection of health-related data from communities this network was tapped into. Community Health Volunteers assisted to screen 730 pregnant women of upto the eighth month of pregnancy attending antenatal clinics and 553 were eligible to participate in the study. A sample of 185 pregnant women was required out of the target population of 1372 in Kisumu County Referral Hospital and 368 pregnant women out of the target population of 2728 from Ahero County Hospital. All pregnant women attending antenatal clinic at Kisumu County Referral Hospital and Ahero County Hospital between May and August, 2013, were screened until the required sample size for each health facility was achieved. Aliquot samples of 10% of the foods eaten over the past 24-hours (breakfast, lunch, supper and snacks) by each of the 553 pregnant women were collected, packaged in plastic containers and taken to Kenya

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Agricultural and Livestock Research Organization (KALRO) Kitale for aflatoxin analysis. The women were followed up to delivery and infants of both exposed and non-exposed women were followed up for three months after birth.

Anthropometric measurements of the infants were taken monthly for 3 months postpartum. Three weight measurements and three length measurements of the infant were taken and recorded on the questionnaire sheet at each antenatal clinic visit, and the average calculated. At three months of age, 137 infants from exposed mothers and 137 infants from non-exposed mothers, matched for maternal age and monthly household income using McNemar's test for proportions, were randomly selected for analysis of cohort study data. Data on women's socio-demographic factors, food frequency, 24-hour dietary recall, as well as infants' food and milk intake, and anthropometry were obtained through a questionnaire.

Community Health Volunteers were trained on all procedures to be used in recruitment of participants and data collection using a developed field manual. The women recruited into the study were required to attend post-natal clinic at Kisumu County Referral and Ahero County Hospitals for subsequent follow-ups. Those who attended post-natal clinic at other clinics closer to their residence were asked to indicate the clinic and were followed up there. Questionnaires on women's socio-demographic factors (including residence, maternal age, education level, source of income, employment status, number of children); food frequency, 24-hour dietary recall; as well as infants' data on sex, birth weight, sickness episodes, feeding history and food frequency were used to collect study data. Weighed food records for 20 participants were taken to establish quantity of food consumed in one day. Aliquot samples of the women's one day meal (breakfast, lunch, supper or snacks) were collected and analyzed for aflatoxin levels. The women were followed up to delivery and postpartum period; infants of exposed and non-exposed women were followed up for three months. Anthropometric measurements of the infants were taken monthly for 3 months postpartum. Weights of infants were recorded using calibrated SECA infant weighing scales, model No. 7251021009 and lengths were determined using SECA 0 Child Length Boards. Infants' breast milk intakes were determined by the frequency of breastfeeding and recorded on infant food frequency questionnaire. Infants' food intakes were determined from data obtained from the mothers on other foods fed to infants using infant food frequency questionnaire.

Aflatoxin intake by women through diet was determined by the levels of aflatoxin in the aliquot food samples collected from participants and analyzed at KALRO Kitale. Pregnant women consuming foods with aflatoxin levels >10 parts per billion (ppb) were classified as exposed and those consuming foods with aflatoxin levels ≤ 10 ppb as non-exposed. Maternal aflatoxin exposure was determined by the levels of aflatoxin in women's diets and by the proportion of women whose intake was above 10 ppb.

Infant weight was measured as average of three weight measurements and length as average of three length measurements; lengths for age Z-scores (LAZ), weight for length Z-scores (WLZ) and weight for age Z-scores (WAZ) were computed using WHO Anthro 2005, w3.2.2 software programme [20]. The computed indices were used as indicators of stunting, underweight, and wasting, based on -2SD cut-off point. Stunting was measured by a child's length-for-age being 2 standard deviations below the WHO growth reference (LAZ ≤- 2); underweight was measured by a child's weight-for-age being 2 standard deviations below the WHO growth reference (WAZ ≤ - 2); and wasting was measured by child's weight-for-length being 2 standard deviations below the WHO growth reference (WLZ ≤- 2) [20].

The variable on exclusive breastfeeding was generated using Statistical Package for Social Sciences (SPSS) software (IBM SPPS Statistics®) version 20. Variables for those who consumed selected foods in a day, week and month (porridge, cow milk, infant formula, water and fruit juice) were generated, with 0 and 1 denoting "did not consume" and "consumed" the specified food, respectively. This gave the total number of infants who were exclusively breastfed and those who consumed other foods apart from breast milk. Amount of other food consumed by the infant was determined from the food intake data collected using the infant food frequency questionnaire.

Levels of aflatoxin in cooked foods consumed by pregnant women and proportion of women exposed to aflatoxin levels > 10 ppb were used to determine infant aflatoxin exposure status. Infants of exposed and non-exposed mothers were matched by maternal age and household income and logistic regression was used to compare differences between proportions of matched infant-mother pairs. Independent student T-tests were used to determine mean difference in infant weight and length at birth and logistic regression used to generate Odds Ratios (OR) of aflatoxin exposure at birth. Mean differences in infant length, weight, WLZ, LAZ and WAZ of exposed and non-exposed infants at 3 months after birth were assessed using Cox regression with constant time at risk. Relative Risks (RR) were calculated to determine the predictors of infant nutritional status: wasting, underweight and stunting using Cox regression. P-values <0.05 were considered statistically significant results. The adjusted analysis generated relative risk ratios and 95% confidence intervals.

The research proposal went through the School of Graduate Studies, Maseno University approval process, where also permission to conduct the study was sought. Ethical clearance was granted by the Maseno University Ethical Review Committee (MUERC). Ethical guidelines of anonymity, privacy and confidentiality of information gathered through the study were adhered to so the identity of each participant was not disclosed to any unauthorized persons or to anybody for any purpose. Informed written consent was obtained from all the study participants. Participation in the study was voluntary and there were no direct benefits to participants. Participants benefited from education on aflatoxin awareness. All the necessary protocols were observed during entry into the study site.

RESULTS AND DISCUSSION

Of the 553 participants recruited from two Sub-Counties, 185 participants (33.5%) were from Kisumu East Sub-County and 368 participants (66.5%) from Nyando Sub-County. Majority of the participants (37.8%) were aged between 20 and 24 years, with a mean age of 24.6 ± 5.4 years. Most households (91.1%) comprised one to six persons.

About 81 % of the women were married and 92.8% had either primary (55.4%) or secondary (37.4%) level of education. The major sources of income were business (49.7%) and husband (23.3%), with most of the households (56.1%) having a monthly earning of Kshs. 2001- 5000 (Table 1).

The participants were matched for aflatoxin exposure by maternal age and monthly household income and their baseline characteristics determined by exposure (Table 2). Of the exposed women, a bigger proportion (81%) was from Nyando Sub-County, compared to Kisumu East (19%). Of the non-exposed women, 31.4 % were from Kisumu East Sub-County, 68.6 % from Nyando Sub-County. Of the exposed women, 56.2% had household income ranging from Kshs. 2001-5000 (Table 2).

Results of this study indicate that women recruited from Ahero County Hospital in Nyando Sub-County were almost three times more likely to be exposed to aflatoxin, compared to women recruited from Kisumu County Referral Hospital in Kisumu East Sub-County. Climatic and environmental conditions in Nyando Sub-County favor growth of aflatoxin producing moulds [17]. Much of Nyando Sub-County is rural and contamination of food supplies by aflatoxin is reported to be greater in rural communities of developing countries with low monthly income [21]. Therefore, based on the findings of this study, Nyando Sub-County could be at a higher risk of aflatoxin exposure compared to Kisumu East Sub-County because of the prevailing weather conditions and the rural location.

The participants were matched for aflatoxin exposure by maternal age and monthly household income and their baseline characteristics determined by exposure (Table 3). The odds of being exposed were 71%[1] lower in women aged 30-34 years (OR=0.29, 95% CI: 0.11, 0.76, p=0.012) compared to women of age category 15- 19 years; 2.64 times higher in women from Ahero County Hospital (OR=2.64, 95% CI: 1.65, 4.23, p>0.001) compared to women from Kisumu County Referral Hospital; and 86% lower in women from households earning >10000 (OR=0.14, 95% CI:0.05, 0.41, p=0.001) compared to women in households with monthly income < 2000 [20 USD] (Table 3). [1] Calculated as (Odds Ratio – 1) x 100.

The Kenya Bureau of Standards (KBS) recommends 10 ppb as maximum permissible levels of aflatoxin in foods for human consumption [22], Codex Alimentarius recommends aflatoxin allowable limit of 9 ppb [23], and the European Commission (EC), 2ppb [24]. This implied that a big proportion of women and their infants were exposed to varying levels of aflatoxin through dietary intake. In a study carried out in the Gambia, a strong correlation was established between serum levels of AF-albumin and dietary aflatoxin [25]. The Gambian study supports dietary intake as indicator of aflatoxin exposure, further giving strength to our study.

The results of this study also indicate that more than half of the exposed women were from households with low monthly income. Higher monthly household income was associated with reduced prevalence of aflatoxin exposure. These results are consistent with findings of a study carried out in Kumasi, Ghana, which found that mean aflatoxin, as well as the percentage of pregnant women having high aflatoxin levels were inversely associated with indices of higher socioeconomic status [15]. This could be attributed to the fact that women in the lower socio-economic status do not have the financial capacity to enable them purchase quality foods, and may, therefore, only afford food of lower quality which may be contaminated by aflatoxins and other food contaminants. The findings of this study indicate that households with low monthly income and mean age of 30-34 were more likely to consume aflatoxin contaminated foods than their higher income counterparts. These women should be targeted for any interventions on aflatoxin management and information on effect on maternal aflatoxin exposure should be incorporated in nutrition information given during antenatal clinics. Data from literature review on relationship between maternal age and aflatoxin exposure were lacking.

The amount of aflatoxin consumed through diet might not have been determined with a high degree of accuracy in the current study due to variations in individual daily food intake. Individuals differ in their daily food intake and food preferences may also vary on different days, whereas data for this study were collected in one day for each participant. In addition, food samples were collected between June and August, 2013. This is normally the period when food harvesting is taking place in the Kisumu County. Aflatoxin contamination has been reported to increase with storage time [26]. This could have contributed to the levels of aflatoxin observed in the current study. Groundnut, one of the foods consumed in Kisumu County, is normally planted during the short rains in the months of August to October and harvested in December. This commodity was scarce in the market during the time of data collection, implying low consumption at household level during the study period. In a study carried out in the Gambian children, it was observed that aflatoxin albumin adduct concentration, an indicator of aflatoxin exposure, was strongly influenced by the month of sampling [27]. Further, in a follow-up cohort study carried out to assess levels of aflatoxin exposure among nursing Egyptian mothers and their children, the most dominant factor affecting the presence of Aflatoxin M_1 (AFM_1) in breast milk was the seasonal effect [28]. Seasonality is an important factor in determining aflatoxin levels in foods. Given that this study was carried out in a season when aflatoxin levels could have been low, levels presented could have been lower than usual, hence the need to assess seasonal variations in Kisumu County. This study was conducted at one point in time, making it difficult to reflect seasonal variations in aflatoxin exposure. For conclusive information relevant to policy, data on seasonal variation are relevant and should be generated in a suitably designed study.

The proportion of pregnant women consuming aflatoxin levels above 10 ppb was determined using data on aflatoxin level in food samples consumed by the women in one day (Figure 1), supported by data from food frequency questionnaire, 24-hour recall and weighed food records from a sample of twenty participants. Of the cooked food sampled from pregnant women who participated in the study, 24.8% had aflatoxin levels above 10 ppb (Figure 1).

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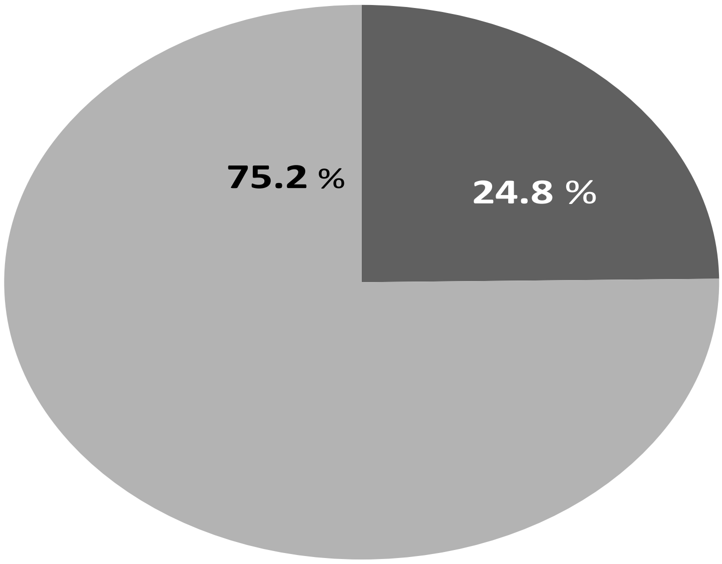

Figure 1: Proportion of Pregnant Women Exposed to Aflatoxin Levels >10 ppb

The results of this study indicate that about 25% of the pregnant women in Kisumu County are exposed to aflatoxin levels above the recommended regulatory limit of 10 ppb. This is of concern because of the cumulative effect of such exposure on the health of consumers over time, given that aflatoxin in the blood has a half-life of 30 to 60 days [31] and both low and high levels of exposure are associated with negative effect on human health. The Kenya Bureau of Standards recommends 10 ppb as maximum permissible levels of aflatoxin in foods for human consumption [22]. This means that pregnant women in Kisumu County consume higher amounts of aflatoxin than recommended limits, which may have negative effect on the health of the unborn fetuses.

Given that the premise of this study was to determine the effect of aflatoxin intake by the mother on infant growth, the possibility that other sources of food apart from breast milk could have been consumed by the infant, hence introducing sources of aflatoxin other than breast milk, was first assessed (Table 4). All infants who consumed foods other than breast milk in the first three months were considered as not having been exclusively breastfed.

Data on breastfeeding frequency were collected to determine how many times an infant was breastfed in a day, because this could translate into amount of milk consumed in a day and also amount of aflatoxin exposure through breast milk. Of the 274 infants matched for mother's marital age and household income, 86% were breastfed more than five times in a day; 12% of the infants were breastfed five times a day, while 1.6% were breastfed four times a day and 0.4% were breastfed three times a day (Figure 2).

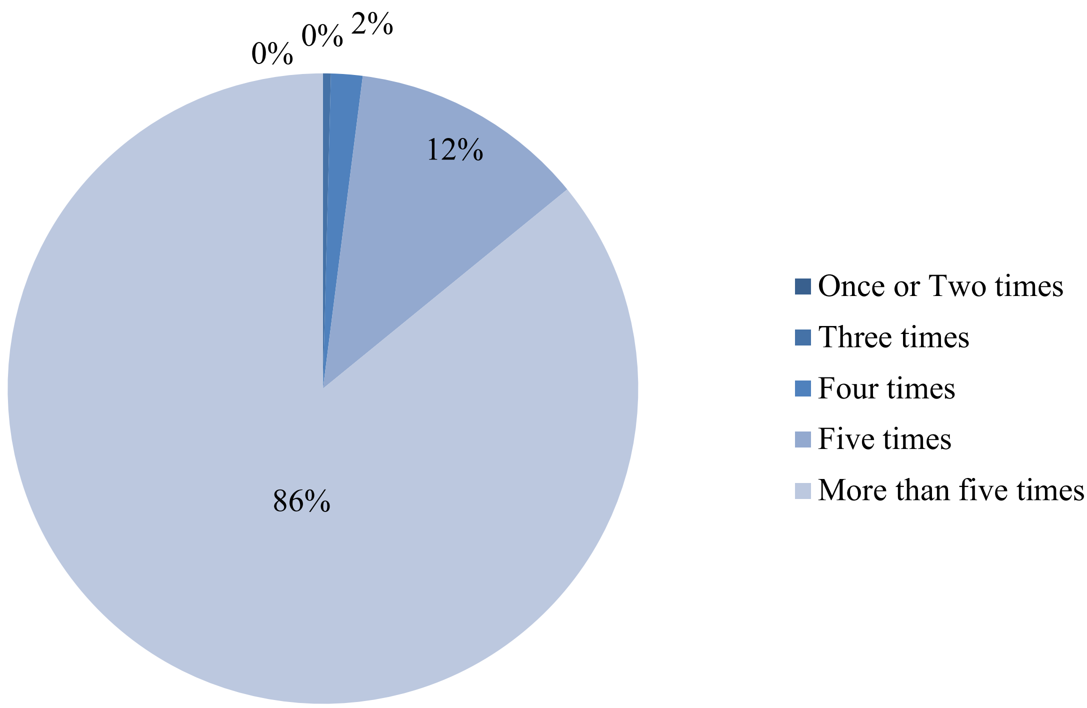

Figure 2: Breast Feeding Frequency per Day (%)

The KDHS 2008-2009 report indicated that about 85% of breastfeeding children under 6 months in Nyanza Region were breastfed six or more times daily, hence our data aptly reflected the situation in Kisumu County [14]. However, the KDHS 2014 did not provide information on breastfeeding frequencies [30]. Data from this study supported exposure of infants 0 to three months to aflatoxin being more likely to be through maternal intake than through influence of other foods, confirming that infants may be exposed to aflatoxins through breast milk.

Infant characteristics that could influence growth in exposed and non-exposed infants were assessed at birth to identify factors that could influence growth at birth, for adjustment in the effect analyses. Being a cohort study, such balance was determined by assessing mean differences between groups or odds ratios where appropriate (Table 4).
The average birth weight was 0.54 kg lower in infants of exposed women than in infants of non-exposed women (95% CI: -0.61, -0.46, p< 0.001). The average birth length was 2.99 cm lower in infants of exposed women than in infants of non-exposed women (95% CI:-3.71, -2.28; p< 0.001). The odds of wasting was 50% lower in infants of exposed women compared to infants of non-exposed women (95% CI:-3.71, -2.28; p<0.001). The odds of stunting was 6.88 times higher in infants of exposed women compared to infants of non-exposed women (95% CI: 2.56, 18.46; p<0.001). The odds of being underweight was 8.43 times higher in infants of exposed women than in infants of non-exposed women (95% CI: 1.04, 68.39; p<0.001). Of the 234 exclusively breastfed infants, equal numbers of 117 (85.4%) were from both exposed and non-

exposed women. The odds of lower birth weight was 10.71 times higher in infants of exposed women than in infants of non-exposed women (95% CI: 1.35, 84.85; p=0.05), Table 5.

Results from this study indicated that infants of women exposed to aflatoxin weighed less, and were shorter than infants of non-exposed women. Further, exposed infants were at higher risk of stunting in the first 3 months of life. Because any differences at birth were adjusted for, these findings suggested that maternal exposure to aflatoxin adversely affected growth of their infants during this period. Both length and weight appeared to increase over the study period in both exposed and non-exposed groups with significant differences between the exposed and non-exposed groups at each of the four time points.

Association between weight and length and aflatoxin exposure has been shown in other studies: a cross-sectional hospital-based survey conducted in Ghana found that mothers with the highest aflatoxin B_1(AFB_1)-albumin quartile were more likely to have low birth- weight babies, with a trend of increasing risk for low weight, compared to participants in the lowest quartile [15]; a longitudinal study conducted in Benin, found a strong negative correlation between serum aflatoxin albumins adducts and length increase [1]. Data on infant baseline characteristics by aflatoxin exposure at birth from this study concurred with these studies; that maternal aflatoxin exposure was a significant predictor of infant weight and length. However, cross-sectional data can only infer associations and not causation, which can only be determined in longitudinal studies, a strength of the current study. There was no difference in exclusive breastfeeding between infants of exposed and non-exposed women. The KDHS 2014 data further indicated that Nyanza Region had a median duration of exclusive breastfeeding of 3.4 months in the first 6 months of life.

Maternal aflatoxin exposure was assessed to determine the effect on infant length and weight at months one, two, and three in exposed and non-exposed infants. Mean length of infants of exposed women was consistently lower than mean length of non-exposed women at birth, one month, two months and three months. The results of this study further showed that although mean weight of all infants increased over the study period, mean weights of infants of women exposed to aflatoxin were consistently lower than those of infants of non-exposed women at all time points of the study. Difference in weight and length between exposed and non-exposed infants at birth, one, two and three months was determined using t-tests to establish existence of potential trends in growth over the study period (Table 6; Figure 3; Figure 4).

Trend analysis for weight and length from birth to three months showed that both length and weight appeared to increase over the study period in both exposed and non-exposed groups, with significant differences between the exposed and non-exposed groups at each of the four-time points.

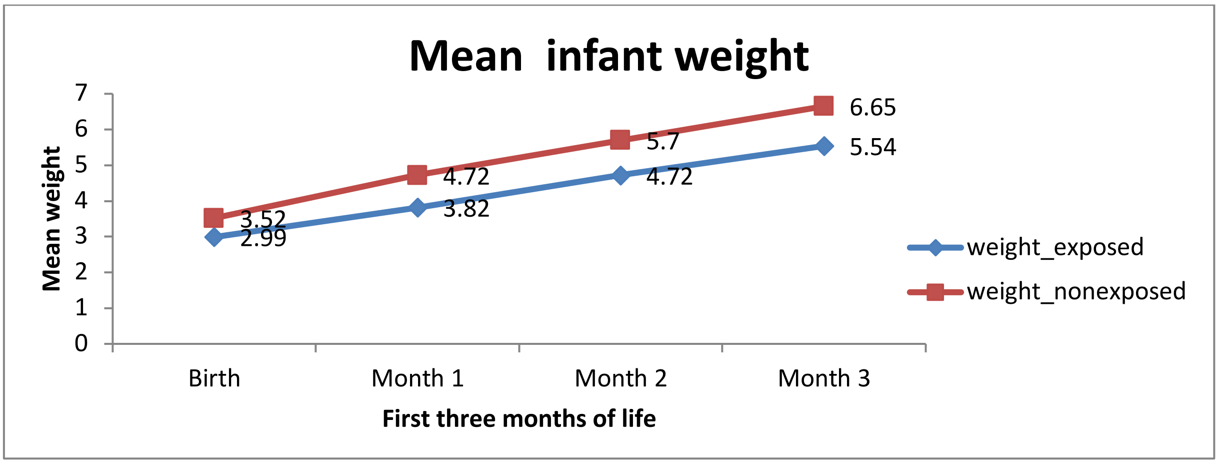

Figure 3: Mean Weight of Infants at the First Three Months

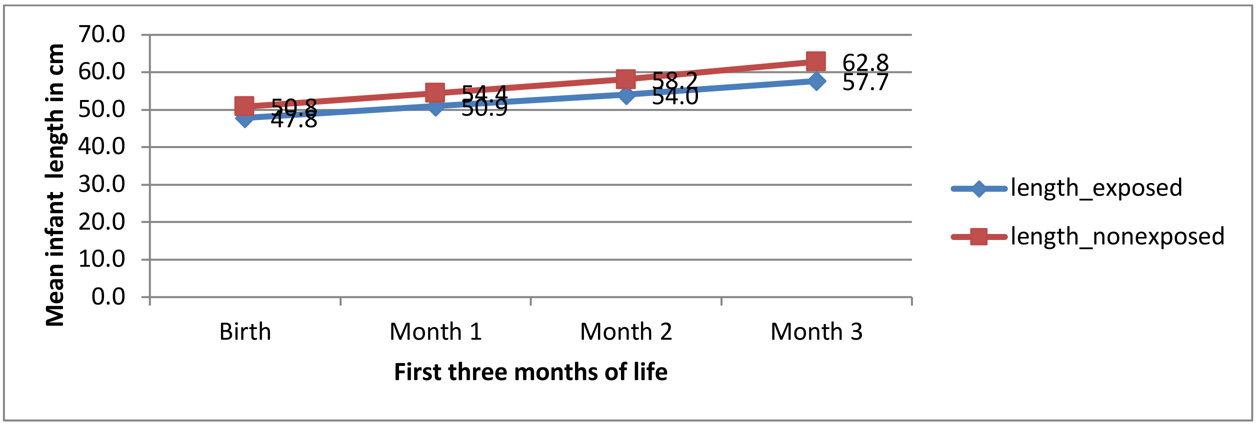

Figure 4: Mean Length of Infants at the First three Months

Infants of exposed women had lower birth weight and lower birth length than infants of non-exposed women; and these variables were adjusted for in the analyses of the effect of aflatoxin exposure on infant growth. Association between weight and length and aflatoxin exposure has been shown in a cross-sectional hospital-based survey conducted in Ghana [15]. The results of this study were also consistent with those of a previous study which had reported a negative relationship between aflatoxin exposure and infant length [26]. Data on infant baseline characteristics by aflatoxin exposure at birth from this study concurred with these studies; that maternal aflatoxin exposure was a significant predictor of infant weight and length. However, cross-sectional data can only infer associations and not causation which can only be determined in longitudinal studies, a strength of the current study.

Crude and adjusted analyses were carried out to determine whether aflatoxin exposure had an effect on infant anthropometric indices including weight, length, WLZ, LAZ and WAZ. Results in Table 7 show the effect of maternal aflatoxin exposure on infant LAZ and WAZ at 3 months of age. Infants of pregnant women exposed to aflatoxin levels above 10 ppb had lower mean weight, length, LAZ and WAZ, at three months of age compared to infants of women who were not exposed p<0.001. However, there was no evidence of any difference in mean WLZ between infants of exposed and infants of non-exposed women (p = 0.071). This study, like two previous studies [1, 18], found no evidence of a relationship between maternal aflatoxin exposure and infant WLZ.

The findings of this study on effect of aflatoxin exposure on infant weight were consistent with results of a longitudinal study conducted in the Gambia, examining, in which AF-albumin in maternal blood was a strong inverse predictor of infant weight gain over the first year, with lower gains in those with higher exposure [2]. Similarly, study conducted by Turner and colleagues [2] showed that AF-albumin in maternal blood was a strong inverse predictor of length gain of the infant over the first year, with lower gains in those with higher exposure. Whereas the study by Turner and colleagues covered a period of one year, indicating that it accommodated exposure of aflatoxin from breast milk and other sources, this study focused on exposure from breast milk alone, hence isolated the effect of breast milk alone; and to determine whether or not women's exposure to aflatoxin could affect growth of their infants in early infancy.

This study, like two previous studies [1, 16], found no evidence of a relationship between maternal aflatoxin exposure and infant WLZ. The findings of this study, therefore, indicated that aflatoxin consumed by an infant through breast milk alone could result in reduced length and stunting. If not checked, aflatoxin exposure could hinder efforts by the Kenyan Government, as well as Kisumu County to achieve the Eight Global Nutrition Targets, among them, achieving a 40% reduction in children under five who are stunted and achieving 30% reduction in low birth weight [31]. This study confirmed that maternal aflatoxin exposure led to reduced infant growth (length, weight, and stunting); however, it could not provide information on whether or not there was a threshold above which such an effect occurred. This would require measurement of effect at different levels of maternal exposure.

Relative risk for wasting, underweight and stunting was assessed using Cox regression with constant time at risk to determine the predictors of infant nutritional status; wasting, underweight and stunting (Table 4.10). Risk of stunting and underweight in infants of women who were exposed to aflatoxin levels above 10 ppb was 4.07 times higher (95% CI: 1.35, 12.29; p=0.013) and 6.61 times higher (95% CI: 0.80, 54.33; p=0.079), respectively, than in infants who were not exposed. There was no evidence of a difference in risk of wasting between infants of exposed and non-exposed women (RR 0.38, 95% CI: 0.040, 3.39; p=0.377), Table 8.

The findings of this study supported aflatoxin effect on infant length, weight and stunting, but not on underweight or wasting. However, given the inability of this study to give a conclusive statement on a possible effect on underweight, further study of ample sample size should be conducted to give a conclusive statement. A limitation of this study was that dietary intake was used, a proxy indicator, which could not be sensitive, to determine exposure. However, because the results were consistent with those of other authors who used sound study designs and a sensitive indicator of aflatoxin exposure, the results of this study reflected an effect of aflatoxin exposure on infant growth. This implied that measuring maternal aflatoxin exposure during a period of exclusive breastfeeding could serve as a proxy indicator of infant exposure to aflatoxin.

In conclusion, almost a quarter of pregnant women in Kisumu County are exposed to aflatoxin intakes above the regulatory limit of 10 ppb through their diet, and, therefore, risk exposing their infants to the toxin. Maternal exposure to aflatoxin increases risk of reduced weight, length and risk of stunting in infants, but not risk of wasting. To quantify the exposure of children through breast milk in Kenya, prevalence studies to establish aflatoxin M_1 levels in maternal breast milk is recommended.

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Table 1: Maternal Socio-demographic Data

Variable Description	% (n)*
Health Facility:	
Kisumu District Hospital (KDH)	33.5 (185)
Ahero District Hospital (ADH)	66.5 (368)
Sub-County of residence	
Kisumu East	33.5 (185)
Nyando	66.5 (368)
Woman's age in Years	
15-19	17.3 (96)
20-24	37.8 (209)
25-29	28.3 (156)
30-34	11.6 (64)
35-39	4.2 (23)
40-44	0.9 (5)
Mean (SD)	24.6 ± 5.2
Participant's marital status	
Single	16.8 (93)
Married	81 (448)
Separated	0.9 (5)
Divorced	0.4 (2)
Widowed	0.9 (5)
Participant's education level	
None	0.4 (1)
Primary	55.4 (304)
Secondary	37.4 (207)
College	5.2 (29)
University	1.8 (10)
Participant's religion	
Christian	99.6 (551)
Muslim	0.4 (2)
No of people in the household	
1 – 6	91.1 (504)
7 – 12	8.9 (49)
Participant's source of income	
Farming	9.2 (51)
Employed	8.9 (49)
Business	49.7 (275)
Husband	23.3 (129)
Parents	7.6 (42)
Sibling	1.3 (7)
Household's monthly earnings	
<=2000	16.8 (93)
2001 – 5000	56.1 (310)
5001 – 10000	17.4 (96)
>10000	(54)

* Descriptive statistics were used to produce frequency distributions and proportions

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Table 2: Maternal Baseline Characteristics by Aflatoxin Exposure for Matched Women

Characteristic	Exposed (n=137) n (%)	Non-exposed (n=137) n (%)	All (n=274) n (%)
Sub-County of residence			
Kisumu East	26 (19)	43 (31.4)	69 (25.2)
Nyando	111 (81.0)	94 (68.6)	205 (74.8)
Household Monthly Earnings (Kshs.)			
≤2000	39 (28.5)	39 (28.5)	78 (28.5)
2001-5000	77 (56.2)	77 (56.2)	154 (56.2)
5001- 10000	16 (11.7)	16 (11.7)	32 (11.7)
>10000	5 (3.6)	5 (3.6)	10 (3.6)
Woman’s age*	23.6 (5.0)	24.1 (5.4)	(5.2)

*Descriptive statistics were used to generate Mean (SD), frequency distribution (n) and proportions (%)

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Table 3: Association between aflatoxin exposure and maternal socio-demographic factors at Baseline

Variables	N	Exposed n (%)	Not exposed n (%)	Crude Odds Ratio* [95%CI]	p-value
Age categories					
15-19	96	25(26.0)	71(74.0)	Ref	
20-24	209	63(30.1)	146(69.9)	1.22(0.71, 2.11)	0.463
25-29	156	35(22.4)	121(77.6)	0.82(0.45, 0.48)	0.514
30-34	64	6(9.4)	58(90.6)	0.29 (0.11, 0.76)	**0.012**
35-39	23	7(30.4)	16(69.6)	1.24 (0.46, 3.37)	0.670
40-44	5	1(20.0)	4(80.0)	0.71(0.08, 6.67)	0.764
Health facility					
Kisumu County Hospital	185	26 (14.1%)	169 (85.9)	Ref	
Ahero County Hospital	368	111(30.2%)	257 (69.8)	2.64 (1.65, 4.23)	**0.001**
Marital status					
Single	93	22 (23.7%)	71 (76.3)	Ref	
Married	448	113 (25.1%)	335 (74.8)	1.10 (0.60, 1.80)	0.750
Separated	5	1 (20.0%)	4 (80.0)	0.80 (0.09, 7.60)	0.851
Divorced	2	1 (50.0%)	1 (50.0)	Excluded	
Educational level					
None	2	1 (50.0%)	1 (50.0)	Ref	
Primary	306	82 (26.8%)	224 (73.2)	0.37 (0.02, 5.92)	0.48
Secondary	207	47 (22.7%)	160 (77.3)	0.29 (0.18, 4.79)	0.390
College	28	5 (17.9%)	23 (82.1)	0.22 (0.12, 4.09)	0.308
University	10	2 (20.0%)	8 (80.0)	0.25 (0.10, 5.99)	0.392
Occupation					
Housewife	153	39 (25.5%)	114 (74.5)	Ref	
Self employed	300	76 (25.3%)	224 (74.7)	0.99 (0.63, 1.55)	0.971
Employed	39	8 (20.5%)	71 (79.5)	075 (0.32, 1.78)	0.520
Other	61	14 (23.8%)	47 (77.0)	0.87 (0.43, 1.75)	0.698
Religion					
Christian	551	136 (24.7%)	415 (75.3)	Ref	0.434
Muslim	2	1 (50.0%)	1 (50.0)	0.33 (0.20-5.28)	0.431
Source of income					
Farming	57	14 (27.5%)	37 (72.5)	Ref	
Employed	48	10 (20.8)	38 (79.2)	0.70 (0.28, 1.76)	0.444
Business	276	70 (25.4%)	206 (74.6)	0.90 (0.46, 1.76)	0.754
Husband	129	31 (24.0%)	98 (76.0)	0.84 (0.40, 1.75)	0.633
Parents	42	12 (28.6%)	30 (71.4)	1.00 (0.43, 2.62)	0.905
Siblings	7	0 (0.0%)	7 (100.0)	Exclude	
Household monthly earnings Kshs)					
≤2000	93	39 (41.9%)	54 (58.1)	Ref (1.0)	
2001-5000	310	77 (24.8%)	233 (75.2)	0.46 (0.28, 0.74)	**0.002**
5001-10000	97	16 (16.5%)	81 (83.5)	0.27 (0.14, 0.54)	**0.001**
>10000	53	5 (9.4%)	48 (90.6)	0.14 (0.05, 0.41)	**0.001**

*Odds ratio and 95% CI were generated using binary logistic regression

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Table 4: Infant Food Intake (n=553)

Food Item and Frequency of Consumption	Quantity	n (%)*
Porridge (ml) consumed by infant in a day	0	540 (97.6)
	50 – 300	13 (2.4)
Cow's milk (ml) consumed in a day	0	517 (93.5)
	50 – 300	16 (2.9)
NAN(g) consumed in a day	0	552 (99.8)
	20 – 300	1 (0.2)
Water consumed in a day	0	520 (94)
	5 – 300	33 (6)
Water consumed in a day	0	547 (99)
	20 – 140	6 (1)
Exclusively breastfed	-	333 (85.4)

0 implies not consumed
*Descriptive statistics were used to generate frequency distribution (n) and proportions

Table 5: Infant Baseline Characteristics by Aflatoxin Exposure at Birth

	All Infants n=274	Not Exposed n=137	Exposed n=137	Group Comparison (95% CI)	Pvalue
				Mean difference*	
Birth mean weight[1](Kg)	3.26 ± 0.49	3.53 ± 0.43	2.99 ± 0.37	-0.54 (-0.61, -0.46)	<0.001
Birth meam length[1](cm)	49.45 ± 3.93	51.14 ± 3.49	47.84 ± 3.64	-2.99 (-3.71, -2.28)	<0.001
				Odds Ratios**	
Wasting (%)	18.8 (	23.5	13.3	0.500 (0.25, 1.01)	0.05
Stunting (%)	13.8	4.2	23.0	6.88 (2.56, 18.45)	<0.00
Underweight(%)	3.3	0.7	5.8	8.43 (1.04, 68.39)	<0.00
Exclusive Breastfeeding (%)	85.4 (234)	85.4 (117)	85.4 (117)	1.00 (0.51, 1.96)	=1.00
Low birth weight (%)	4	0.7	7.3	10.71 (1.35, 84.85)	=0.03

[1]Mean±SD; *Generated using t-test; **Generated using logistic regression

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Table 6: Mean Weight and Length of Infants by Aflatoxin Exposure

Time (Months)	Mean Weight (kg)*				Mean Length (cm)*			
	Birth	**1**	**2**	**3**	**Birth**	**1**	**2**	**3**
Exposed	2.99±0.37	3.82±0.65	4.72±0.60	5.54±0.54	47.84±3.64	50.93±3.24	53.98±2.58	57.67±2.39
Not Exposed	3.52±0.41	4.72±0.64	5.70±0.73	6.65±0.74	50.84±3.40	54.43±3.22	58.15±3.27	62.83±3.59
P-value**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Overall	3.39±0.46	4.50±0.75	5.46±0.82	6.39±0.84	53.56±3.56	53.56±3.56	57.13±3.60	61.56±4.00

* Mean±SD generated using descriptive statistics
**Based on results of difference between means of exposed and non-exposed children determined by t-test

Table 7: Effect of Aflatoxin Exposure on Infant Growth at Three Months (Matched Analysis)

Variable	Crude Analysis		Adjusted Analysis	
	Mean (95% CI)	p-value Test	Mean (95% CI)	p-value*
Weight (kg)	-1.06 (-1.21, -0.92)	**<0.001**	-0.69 (-0.85, -0.53)§	**<0.001**
Length (cm)	-4.88 (-5.57, -4.20)	**<0.001**	-4.08 (-4.08, -3.36)◊	**<0.001**
WLZ	0.36 (0.04, 0.75)	**=0.022**	0.35 (-0.03, 0.74)†	=0.071
LAZ	-1.92 (-2.29, -1.55)	**<0.001**	- 1.54 (-1.93, -1.16)‡	**<0.001**
WAZ	-1.23 (-1.48, -0.99)	**<0.001**	- 0.79 (- 1.03, -0.54)^	**<0.001**

*Cox regression with constant time at risk was used to determine effect of aflatoxin exposure on infant growth
§Adjusted for maternal age and infant weight at birth; ◊Adjusted for maternal age and infant length at birth; †Adjusted for maternal age and infant WLZ at birth; ‡Adjusted for maternal age and infant LAZ at birth; ^Adjusted for maternal age and infant WAZ at birth

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Table 8: Risk of Aflatoxin Exposure on Wasting, Underweight and Stunting at Three Months in Matched Infants

Nutritional status	RR*	95% CI		Pvalue
		Lower	Upper	
Wasting (LAZ <-2)	0.37	0.04	3.39	0.38
Underweight (WAZ <-2)	6.61	0.80	54.33	0.08
Stunting (LAZ <-2)	4.07	1.35	12.29	**0.01**

*Cox regression analysis used to generate RR

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