Aflatoxin exposure among children of age 12–59 Months in Butajira District, South-Central Ethiopia: a community based cross-sectional study

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Abstract

Background: The continued provision of safe food, free of aflatoxin remains a huge challenge in developing countries. Despite several favourable climatic conditions that facilitate aflatoxin contamination in Ethiopia, there is little information showing aflatoxin exposure in children. Therefore, this study assessed aflatoxin exposure among young children in Butajira district, South-Central Ethiopia.

Methods: Community based cross-sectional study stratified by agro-ecology was employed in Health and Demographic Surveillance Site (HDSS) of Butajira. The study included 332 children aged 12–59 months and were selected by simple random sampling technique using the HDSS registration number as a sampling frame. We collected data on dietary practice and aflatoxin exposure. Aflatoxin M1 concentration in urine was measured by Enzyme-Linked Immunosorbent assay (ELISA). The data analysis was carried out using STATA.

Results: Detectable urinary Aflatoxin M1 was found in 62.4% (95% CI: 56.9 – 67.5%) of the children at a level ranging from 0.15 to 0.4 ng/ml. Children living in lowland agro-ecological zone had [AOR = 2.11 (95% CI; 1.15, 3.88)] odds of being exposed to aflatoxin as compared to children living in highland agro-ecological zone. Children at lower socio-economic status [AOR = 0.27 (95% CI; 0.14, 0.50)] and medium socio-economic status [AOR = 0.47 (95% CI; 0.25, 0.87)] had 73% and 53% lower odds of being exposed to aflatoxin as compared to children in the higher socio-economic status, respectively.

Conclusions: Aflatoxin exposure among young children was very high in South-Central Ethiopia. This high aflatoxin exposure might emphasize the need for aflatoxin exposure mitigation strategies in Ethiopia. Especially, raising awareness of the community towards aflatoxin exposure is very crucial. In addition, further research is required to assess long-term aflatoxin exposure and its association with child growth and development.

Keywords: Aflatoxin, Children, Butajira, Ethiopia, Cross-sectional

Background

Aflatoxins are one family of mycotoxins, and are a naturally occurring toxic by-product, named after the genus of fungus that produces it (Aspergillus flavus and Aspergillus parasiticus) [1]. Aflatoxins are largely associated with agriculture commodities produced in the
tropics & subtropics [2]. Aflatoxin may enter the food supply by direct contamination of food products resulting from mould growth on food, or by indirect contamination through the use of contaminated ingredients in processed food or through use of animal products such as milk, milk products, eggs or meat [3]. Pre- and post-harvest crop management has a significant influence on the accumulation of aflatoxin in dietary staples [4]; thus populations highly reliant on these staples and with limited agricultural capacity and storage facilities are most frequently exposed through diets [5]. The use of aflatoxin metabolites as biomarkers has been a common approach to understand individuals’ dietary exposure to these toxins, but also uptake, toxicokinetics and toxicodynamics of these toxins [6]. Aflatoxin M1 (AFM1) has been well established as a biomarker of exposure for the recent (24-72 hours) ingestion of Aflatoxin B1 (AFB1) and is the most frequently detected urinary aflatoxin [7].

In many parts of the developing world, exposure to aflatoxins at high levels remains a significant health burden [8]. It is estimated that approximately 4.5 billion people, predominantly those living in developing countries, are at risk of exposure to aflatoxins [5]. A study done in Northern Ethiopia by A. Ayele et al (2016) among children aged 1 to 4 years, reported that aflatoxin M1 was found in 7% out of 200 urine samples analysed with a mean concentration of 0.064ng/ml [9]. According to Partnership for Aflatoxin Control in Africa (PACA) report in 2014, children under-five remain particularly vulnerable to aflatoxin exposure significantly hindering children’s growth and development while damaging their immunity [10]. In Ethiopia, the prevalence of impaired growth is still worrying high, where the proportion of stunting in children under 5 years is 38% [11]. Moreover, animal studies provide evidence that chronic aflatoxin exposure retards growth and interferes with micronutrient absorption and utilization [12].

Many of Ethiopia’s ecosystems are among the most favourable for aflatoxicogenic fungi and aflatoxin contamination [13]. Features conducive of a possible high contamination of food and feed products in Ethiopia include its climatic conditions, traditional crop production practices, inadequate harvesting, drying and storage practices, limited policy and institutional capacity in assessment and management of fungal contamination in agricultural products, lack of awareness and high reliance on one or two primary crops constituting the main component of the diet [13]. Likely as a result of this, major staple grain crops in the country have been reported to be contaminated with aflatoxin [14–16]. Despite this, there is limited evidence on the levels of aflatoxin exposure in children. Considering the presence of several favourable conditions that facilitate aflatoxin contamination of foods and the high prevalence of impaired child growth in South-Central Ethiopia, this study assessed aflatoxin exposure among children aged 12 to 59 months in Butajira District, Southern Ethiopia. Additionally, we assessed the type of food consumed by the children.

Materials and methods
Study setting and sampling procedure
The study was conducted in Butajira Health and Demographic Surveillance Site (HDSS), which is located 130 km south of Addis Ababa. The HDSS contains 10 kebeles spread through three agro-ecological zones; Highland, Midland and Lowland. Three of the kebeles; Shershero, Yeteker and Werib are located in the Highland agro-ecological zone. Dirama, Misrak Meskan & K04 kebeles are found in the Midland agro-ecological zone and the rest 4 kebeles; Hopie, Dobena, Bati & Mekaklegna jere-demeka are found in the Lowland agro-ecological zone (fig. 1). There is difference in altitude, temperature and precipitation level among the three agro-ecological zones [17]. The HDSS estimated total population in 2018 was 80,369 (taken from HDSS database), from which children 12-59 months of age accounted for 6.3%. Enset (False banana), Teff, maize, millet, barley and legumes are the staple foods in the area [17].

We employed a cross-sectional study, with stratified sampling based on agro-ecological zone. First, we allocated the sample size proportional to the number of children in each agro-ecological zone using the HDSS registration number as a sampling frame. Second, in order to include all the kebeles within each agro-ecological zone, we allocated the sample size proportional to the number of children in each kebele. Finally, we applied a simple random sampling technique to select the households in each kebele. The data collectors used the following steps to select the allocated sample from each kebele: 1) went to the point in the kebele where the population was about equally distributed on all sides; 2) selected a smooth and level spot where one can spin a ballpoint pen; 3) spin the pen; 4) the data collectors determined in which direction the ballpoint of the pen was pointing and went to that direction; 5) the first household in the kebele in that direction became the starting household. Then, households with the target children were consecutively selected until the desired sample size was archived. Only one child between 12 and 59 months living in the house for at least 6 months was recruited from each selected household; whenever more than one eligible child was found, lottery method was applied to select one of them.

Sample size calculation
The sample size required to meet the objective of this study was 306. However this study was a part of another
study (unpublished) which required a higher sample size (n=332). Thus the sample size (n=332) we used for this specific objective provided adequate power.

The sample size for the first objective is calculated using single population proportion formula based on the following assumptions: \( P = \) prevalence of Aflatoxin M1 in urine as 7%, from a study done in Ethiopia in 2017 [9].

\[
D \text{ (margin of error)} = 3% \\
95\% \text{ of confidence interval} \\
N = \text{number of sample} \\
\text{Sample size determination will be as follows} \\
N = \frac{Z^2\alpha/2 \ p \ (1-p)}{d^2} \\
N = 278
\]

Sample size for the second objective is calculated using two population proportion formula in open epi software, based on the following assumption:

\[
P_1 = \text{prevalence of Stunting as 52.5%, from a study done in Butajira in 2017} \ [18] \\
Z\alpha/2 = \text{standard score corresponding 95% confidence interval (1.96)} \\
Z\beta = \text{standard score corresponding 80% power (0.84)} \\
\text{Odds ratio} = 2 \\
r = \text{ratio between group one \& group two as 1}
\]

By using the following assumption, the sample size will be 151 in each group, making a total sample size of 302.

Since, sample size for the second objective is greater than the first, 302 is taken.

Adding a contingency of 10\% for non-respondent

Final sample size is 332

**Data collection approach**

We collected data on socio-demographic characteristics of the households and dietary practices. Data was collected by a team of trained data collectors using structured and pre-tested questionnaires. The questionnaires were translated into Amharic and retranslated back to English to check and maintain its consistency. The data was collected in paper by using Amharic version of the questionnaire.

**Dietary assessment of the index child**

A Food frequency questionnaire (FFQ) was used to identify the types of foods consumed in the three days (72 hours) previous to the day of the visit. The questionnaire collected information on the frequency of consumption (i.e. number of times per day, and per week) and the usual portion size consumed in grams. The food items listed in the FFQ were food items known to be consumed in the study area and known to be vulnerable.

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**Fig. 1** Sampling procedure of children in Butajira HDSS, 2018
to aflatoxin contamination based on previous studies in Ethiopia [10, 14, 16].

**Urine sample collection and handling**
Random urine samples were collected on the day of the visit using a 10ml urine cup by the help of the mother/care giver of the index child. The cup was labelled immediately after urine collection using a sticker with a unique identifier code, and subsequently recorded in the questionnaire. Urine samples were placed in portable freezers. At the end of each data collection day, the collected urine samples were checked for consistency by the field coordinator, then transferred in to 10ml freezing tubes and kept at Butajira Health Center at -20°C until analysis. After the end of the data collection period, all the collected urine samples were transported to Ethiopian Public Health Institute (EPHI) for laboratory analysis.

**Laboratory analysis**

**Urine sample and reagent preparation**
For the determination of urinary concentration of AFM1, a competitive ELISA kit (from Helica Biosystems Inc.Cat. No.991AFLM01U-96) was used and procedures were based on the protocol obtained from the kit manufacturer [19]. Prior to lab analysis, urine samples and reagents were brought at room temperature. Five millilitres of urine sample were aliquoted into centrifuge tubes and centrifuged at 3000rpm for 10 minutes. Phosphate Buffer Saline-Tween packet (PBS with 0.05% Tween20) was reconstituted by washing out the contents with distilled water into 1 litter glass. The PBS was stored refrigerated (2-8°C) when not in use. Standard optimization was carried out before working with the samples, in order to get the same readings in the standards value as stated in the protocol.

**Assay procedure for determination of aflatoxin M1 in urine using Enzyme Linked Immunosorbent Assay (ELISA)**
Nine hundred and fifty microliters (950) of distilled water were pipetted into a 1.5ml micro tube, then each 50µl of standards and urine supernatant were added into the 950µl of distilled water in the tube to make-up a total of 1000µl. It was mixed by vortex mixer for 10 second. Two hundred microliters of the assay-buffer were added into the mixing well, then a 100µl of the diluted standards containing aflatoxin M1 ranging from 0 to 4000 ppt (0.0-4.0ng/ml) and urine samples were added into the mixing well, to make-up 300µl. These were mixed by priming pipette at least 5 times. By using new pipette, a 100µl of the mixture were transferred into the antibody coated micro-wells in duplicate and incubated for 1hr at room temperature. The contents of the antibody coated micro-wells were decanted in to discard basin, then each micro-wells were filled with PBS-Tween packet and decanted into a discard basin for 3 times. A hundred microliters of conjugate were then added into each micro-well and incubated at ambient temperature for 15 minutes. The plate was washed and a hundred microliters of substrate reagent (Tetramethylbenzidine) was added into each well and incubated at room temperature for 15 minutes in the dark. A hundred microliters of stop solution were added into each of the wells using a multichannel pipette. The intensity of the solution colour in the micro-plate was measured optically using an ELISA reader (Bio-Rad) with an absorbance filter of 450nm as soon as stop solution was added. The optical densities (OD's) of the samples were compared to the OD's of the standards and an interpretative result was determined. Aflatoxin concentration is indirectly proportional to the optical density [19].

**Data analysis**
The data were entered in to Epi-data version 4.0 for windows, and then exported to STATA version 14 for analysis.

Principal Component Analysis (PCA) was used to construct the wealth index to classify the households into low, medium and high socio-economic status in STATA using the socio-economic data about the households. All the variables included in PCA were those we thought are appropriate to explain the wealth of the households in the study area. ‘Rule of thumb’ was used to select the variables used to run PCA, where variables with prevalence below 5% or above 95% were excluded from the analysis. Ten variables were included in PCA. The questions for the variables were as follows: ‘house ownership’, options 1= private, 2= government, 3= rent, 4= relatives/others house, 5= other (specify)? We coded ‘other’ into the appropriate categories. Dichotomous response variable was created; 1=yes and 0=no. ‘Does your household has a functioning radio?’ 1= yes and 0= no; ‘Does your household use solar energy?’ 1= yes and 0= no; ‘Does any member of this household own a mobile phone?’ 1= yes and 0= no; ‘How many rooms does your house have?’ 1= three & more rooms and 0= less than three rooms; ‘What kind of toilet facility do members of your household usually use?’ 1= flush or pour flush/pit latrine with slab and 0= pit latrine without slab/no facility/bush/field, ‘Other’ were coded into the appropriate categories; ‘what is the main source of drinking water for members of your household?’ 1= piped inside dwelling or yard/public tab/protected well/protected spring and 0= unprotected well/
pound/lake/river/stream/dam; ‘Does this household own any livestock, herds, other farm animals or poultry?’ 1 = yes and 0 = no; ‘Do you have separate room used as kitchen?’ 1 = yes and 0 = no.

PCA was run with all the above ten variables and the first component (component one) was taken to represent the household’s wealth as it accounts for the largest proportion of the variance. The first, second and third eigenvalues were 2.37, 1.74 and 1.34 respectively. The Kaiser-Meyer-Oiklin value was 0.65, exceeding the recommendation value of 0.6 and the Bartlett’s test of sphericity reached statistical significance (p<0.001). Assuming socio-economic status (SES) to be uniformly distributed, the households were divided into three quintiles; low, medium and high socio-economic status based on the wealth index score by using STATA. Table 1 below shows the mean wealth index score by quintile.

Descriptive statistics such as mean, standard deviation and frequency were used to summarize household characteristics. We employed univariable logistic regression model to identify potential candidate variables to be included in the multivariable logistic regression model. Variables with p-value < 0.25 in the univariable model [20] were included in the multivariable logistic regression model. We calculated odd ratios with their 95% CI and p-value <0.05 in the multivariable logistic regression model determined as a level of significance.

Results
Socio-demographic characteristics of participants
A total of 332 children aged 12 to 59 months were included in the study, of which 98.5% (327/332) provided a urine sample. The mean age of the children in the study was 39 months (SD ± 10.9 months). Distribution of the study subjects by socio demographic and economic characteristics is presented in Table 2. The data on maternal characteristics showed that, 78% were housewives and 51% of them didn't attend any formal education.

Food intake by the children
Based on the three days’ recall, the children consumed maize (in the forms of ‘Kita’; a flat bread) (78.3%) and broad bean (in the form of ‘Shiro Wot’; a stew) (39.3%). In addition, 23.5% of the children had cow milk at least once in the preceding three days (Table 3).

Maize was relatively consumed higher in the Lowland eco-ecological zone in the form of ‘Kita’ (37.9%) and ‘Enjera’ (51.7%). Teff, wheat, pea and cow milk were mainly consumed in the Midland agro-ecological zones (Table 3).

Among the studied households, maize was the most commonly (85.5%) crop stored during the data collection period followed by Teff (13%). In addition, 61% of the households used silos to store their crops and majority (69.7%) of them stored the crops for more than five weeks (Table 4).

Aflatoxin exposure among the study participants
Three-hundred-twenty-seven (327) urine samples from children aged 12-59 month were tested for Aflatoxin M1(AFM1) out of which, detectable urinary AFM1

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**Table 1** Mean wealth index score by quintile in Butajira District, 2018

| Quintile  | Frequency (n = 332) | Mean |
|-----------|---------------------|------|
| Low SES   | 111                 | -1.89|
| Middle SES| 117                 | 0.42 |
| High SES  | 104                 | 1.54 |

**Table 2** Socio-demographic and economic characteristics of children aged 12–59 months in Butajira District, South-Central Ethiopia, 2018

| Characteristics                                   | N  | Percent (%) |
|---------------------------------------------------|----|-------------|
| **Agro-ecology zone**                             |    |             |
| Highland                                         | 93 | 28.0        |
| Midland                                          | 127| 38.3        |
| Lowland                                          | 112| 33.7        |
| **Child age in month**                            |    |             |
| 12–23 months                                     | 25 | 7.5         |
| 24–35 months                                     | 81 | 24.4        |
| 36–47 months                                     | 140| 42.2        |
| 48–59 months                                     | 86 | 25.9        |
| **Sex of the child**                              |    |             |
| Male                                              | 210| 63.0        |
| Female                                            | 122| 36.8        |
| **Maternal occupation**                           |    |             |
| Employed (government/private employee, merchant, daily labourer & farmer) | 63 | 22.0 |
| Not employed/Housewife                           | 269| 78.0        |
| **Maternal educational Status**                   |    |             |
| No formal education                               | 170| 51.0        |
| Primary education                                 | 137| 41.0        |
| Secondary or higher education                     | 25 | 8.0         |
| **Household socio-economic status (SES) categories:** |    |             |
| Low SES                                           | 111| 33.4        |
| Medium SES                                        | 117| 35.2        |
| High SES                                          | 104| 31.3        |

Total children (N) = 332
Factors associated with aflatoxin exposure

In the univariable logistic regression model, except sex of the child all the variables in the model meet the significance level (p-value <0.25) to be included in the multivariable logistic regression model.

In the final multivariable logistic regression model, agro-ecological zone and socio-economic status were associated with aflatoxin exposure (p-value <0.05). Children living in lowland agro-ecological zone had [AOR= 2.11 (95% CI; 1.15, 3.88] odds of being exposed to aflatoxin as compared to children living in highland agro-ecology zone.

Children at lower socio-economic status had [AOR= 0.27 (95% CI; 0.14, 0.50] 73% lower odds of being exposed to aflatoxin as compared to children in the higher socio-economic status. Those children from medium
socio-economic status had \[\text{AOR} = 0.47\ (95\%\ CI: 0.25, 0.87)\] 53\% lower odds of being exposed to aflatoxin than children in the higher socio-economic status (Table 5).

**Discussion**

This study assessed aflatoxin exposure among young children aged 12-59 months by detecting urinary Aflatoxin M1 using ELISA and found high (62.4\%) prevalence of aflatoxin exposure, with detectable level ranging from 0.15 ng/ml to 0.4 ng/ml. The presence of urinary biomarkers of aflatoxin is indicative of acute exposure (i.e. exposure having occurred in the previous 72 hours) [8], so in this study almost two third of children had been exposed to aflatoxins in their diets. As there is no safe threshold for aflatoxin exposure any level of exposure is considered a risk [8].

Our study found higher prevalence and concentration of aflatoxin M1 level than a study done in Northern Ethiopia, were AFM1 was detected in 7\% of the study participants with a range 0.064-0.0070 ng/ml [9]. Other studies done in Cameroon [21] and Nigeria [7] also reported a prevalence of 14\% (range: 0.06-4.7ng/ml) and 14.2\% (mean: 0.3ng/ml; SD: 0.4) of AFM1 in urine respectively. The difference in prevalence of aflatoxin exposure between the above cross-sectional studies and this study could be attributed to two major factors. The studies used LC-MS/MS for detection of aflatoxin in urine, which has high specificity, but made the possibility for trace detection difficult [22]. In addition, the studies have relatively small sample size than this study, where sample size determines the power of detecting the magnitude of aflatoxin exposure. Seasonal difference could be one major reason for the difference in prevalence of aflatoxin exposure in our study and the other from Northern Ethiopia [9]. That particular study was conducted on January 2016 and reported that Teff was the main food item consumed by the study participants. In Ethiopia, Teff is grown in the ‘belg’ rainy season (July to October) and harvested on November & December [23]. Even though aflatoxin contamination could happen during pre-harvesting period, the children might consumed Teff stored for less than one month or not stored at all. In contrast, our data was collected on July 2018 and maize was the main food item consumed by the study participants. Maize is usually grown in ‘Maher’ rainy season (June to September) and harvested on October & November [23] so, the children might consumed stored maize for more than 6 months. The longer the storage time, the greater the possibility of building up environmental conditions conducive to aflatoxigenic mould proliferation and subsequent mycotoxin production [24].

Similar to our study, studies done in Tanzania [25], Kenya [26] and China [27] analysed the presence & level of AFM1 in urine using ELISA and found a prevalence of 86\%, 79.2\% and 84\% respectively. On the other hand, studies done in Ghana in 2010 [28] and 2015 [29] using

| Independent Variables               | Frequency | Crude OR (95\% CI) | Adjusted OR (95\% CI) | P-Value (AOR) |
|------------------------------------|-----------|--------------------|-----------------------|--------------|
| Agro-ecological zone               |           |                    |                       |              |
| Highland                           | 92        | 1                  | 1                     |              |
| Midland                            | 123       | 1.45 (0.84, 2.53)  | 1.62 (0.91, 2.89)     | 0.102        |
| Lowland                            | 112       | 1.77 (1.00, 3.14)  | 2.11 (1.15, 3.88)     | 0.016        |
| Age                                |           |                    |                       |              |
| 12–23 Months                       | 24        | 1                  | 1                     |              |
| 24–35 Months                       | 80        | 0.50 (0.18, 1.39)  | 0.57 (0.19, 1.72)     | 0.318        |
| 36–47 Months                       | 138       | 0.49 (0.18, 1.31)  | 0.49 (0.17, 1.44)     | 0.198        |
| 48–59 Months                       | 85        | 0.64 (0.23, 1.79)  | 0.75 (0.24, 2.27)     | 0.607        |
| Socio-Economic Status (SES)        |           |                    |                       |              |
| High SES                           | 102       | 1                  | 1                     |              |
| Medium SES                         | 116       | 0.44 (0.24, 0.80)  | 0.47 (0.25, 0.87)     | 0.015        |
| Low SES                            | 109       | 0.29 (0.16, 0.54)  | 0.27 (0.14, 0.50)     | 0.000        |
| Maternal educational status        |           |                    |                       |              |
| Secondary or Higher education      | 24        | 1                  | 1                     |              |
| Primary education                  | 136       | 0.57 (0.22, 1.47)  | 0.79 (0.29, 2.15)     | 0.646        |
| No formal education                | 167       | 0.75 (0.29, 1.92)  | 1.22 (0.45, 3.35)     | 0.696        |
A survey by Alemu et al found contamination of maize with aflatoxin B1 (AFB1) in Southern Ethiopia with concentration of 4.1µg/kg [15]. Furthermore, according to a report by USAID in 2011, aflatoxin B1 was detected in 88% of maize samples with a concentration of 4.1µg/kg [14]. A survey by Alemu et al found contamination of maize with aflatoxin B1 (AFB1) in Southern Ethiopia with concentration of 22.72µg/kg [14].

Our study also found that, children in lower socio-economic status store foods for longer duration than low SES households. In subsistence farming households, those households with higher socio-economic status store foods for longer duration than low SES households. In some areas storing food for longer time is considered as an indicator of wealth. This might contribute for this difference; however, it is not precisely clear why we found higher odds of aflatoxin exposure among children from higher SES. There were also studies done in Benin [37] and Ghana [38], which didn’t found a statistically significant correlation between socio-economic status and aflatoxin exposure (p< 0.05).

The strength of this study can be seen in terms of using ELISA to analyse the level of AFM1 in urine, which is a highly sensitive analytical method, simple, rapid, preferred to analyse large samples and made trace detection possible as the excretion rate of aflatoxin M1 through kidneys is very low. However, ELISA has issues with specificity, where compounds with similar chemical groups as AFM1 can also interact with the antibodies. But, again this has been argued by Groopman et al, in that AFM1 is the most common metabolite of AFB1 in urine, so results are unlikely to be distorted [25]. While interpreting the results obtained from this study, recall bias in the case of food frequency questionnaire and the cross-sectional nature of the study need to be taken into consideration.

Conclusion
This study showed that the prevalence of aflatoxin exposure is high among children aged 12 to 59 months. Thus, aflatoxin exposure mitigation strategies might be considered. However, we recommend further research to investigate the impact aflatoxin has on growth and development of children as well as exploring the magnitude of aflatoxin exposure with long-time exposure biomarker like AFB1-albumin adduct with a better analytical method (like LC-MS/MS).

Abbreviations
AFM1: Aflatoxin M1; AFB1: Aflatoxin B1; ELISA: Enzyme-Linked Immunosorbent Assay; EPHI: Ethiopian Public Health Institute; FFQ: Food frequency questionnaire; HDSS: Health and Demographic Surveillance Site; HPLC: High-performance liquid chromatography; ILRI: International Livestock Research Institute; IREC: Institutional Research Ethics Committee; LC–MS/MS: Liquid
chromatography-mass spectrometry; OD’s: Optical densities; PACA: Partnership for Aflatoxin Control in Africa; PBS: Phosphate Buffer Saline; PCA: Principal Component Analysis; SES: Socio-economic status.

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Authors’ contributions
This study was designed by MA, BS, and DH with the intellectual contribution of SA & KH. The field work was conducted by MA with additional support from a team of data collectors. The laboratory work was performed by HS, GT and AA. The statistical analysis and interpretation of results were conducted by MA, with guidance and support from DH, BS and SA. The manuscript was prepared and edited by MA, BS, DH, SA, KH and KT. All authors have read and approved the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was performed in accordance with the ethical standards of the Declaration of Helsinki (1964) and its subsequent amendments. Research ethics approvals were obtained by the Institutional Review Board of Collage of Health Science, Addis Ababa University (Ref. n. 0010) and by the Institutional Research Ethics Committee (IREC) of the International Livestock Research Institute (Ref. n. ILR-IREC2018-09). Informed written consent was obtained from the mothers/caregivers of participant children after necessary explanation on the purpose, procedures, benefits and risks associated with participation in the study. The right of respondents to withdraw from the study any time was assured. The participants were also assured about the confidentiality of the data.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Kensler TW, Roebuck BD, Wogan GN, Groopman JD. Aflatoxin: A 50-year Odyssey of mechanistic and translational toxicology. Toxicol Sci. 2011;120 Suppl 1(Suppl 1):528-48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3043084/.
2. Burch DGS, Rowsell C. The Role of Mycotoxins in Pmws – Fact or Fiction. Pig J. 2001;48:142–7. Available from: https://www.researchgate.net/publication/237542924_THE_ROLE_OF_MYCOTOXINS_IN_PMWS_-_FACT_OR_FICITION.
3. Bankole S, Adebanjo A. Mycotoxins in food in West Africa : current situation and possibilities of controlling it. African J Biotechnol. 2003;209:254–63. Available from: https://www.researchgate.net/publication/228805632.
4. Smith LE, Prendergast AJ, Turner PC, Mbuya MNN, Mutasa K, Kembo G, et al. The potential role of mycotoxins as a contributor to stunting in the SHINE Trial. Clin Infect Dis. 2015;61(Suppl 7):S73–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4657594/pdf/cvi849.pdf.
5. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 2004;80(5):1106–22. Available from: https://academic.oup.com/ajcn/article/80/5/1106/4690142.
6. Njumbe Etlage E, Diana Di Mavungu J, Song S, Wu A, Van Peteghem C, De Saeger S. A direct assessment of mycotoxin biomarkers in human urine samples by liquid chromatography tandem mass spectrometry. Anal Chim Acta. 2012;741:58–69. Available from: https://www.sciencedirect.com/science/article/pii/S000326701200935X?via%3Dihub.
7. Ezekiel CN, Warth B, Ogara IM, Abia WA, Ezekiel VC, Atehnkeng J, et al. Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers. Environ Int. 2014;66:138–45. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24583186.
8. Wild CP, Turner PC. The toxicology of aflatoxin as a basis for public health decisions. Mutagenesis. 2002;17(6):471–81. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12435844.
9. Ayeleign A, Woldegiorgis AZ, Adish A, De Boevre M, Heyndrickx E, De Saeger S. Assessment of aflatoxin exposure among young children in Ethiopia using urinary biomarkers. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2017;34(9):1606–16. Available from: https://doi.org/10.1080/19440497.2017.1350290.
10. Aflatoxin Impacts and Potential Solutions in Agriculture, Trade, and Health 2014 Available from: https://www.un.org/es/a/rdc/fdcd3/wp-conte nt/uploads/sites/2/2015/10/PACA_aflatoxin-impacts-paper1.pdf.
11. Bennett JW, Kich M. Mycotoxins. Clin Microbiol Rev. 2003;80(5):1106–22. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164220/pdf/0050.pdf.
12. Wolde M. Effects of aflatoxin contamination of grains in Ethiopia. Int J Agric Sci. 2017;7(4):1298–308. Available from: https://www.internationalsei horalsjournals.org.
13. Alemu T, Berhanu G, Azerchefe F, Skinner H. Evidence for mycotoxin contamination of maize in Southern Ethiopia: the need for further multidisciplinary research. 3rd Int Symp. 2008;36:337–9.
14. Amare A. Mycotoxins and surface and internal fungi of maize from Ethiopia. 2010;10(9):39. Available from: https://www.researchgate.net/publication/48332181_Mycotoxins_and_surface_and_internal_fungi_of_maze_from_Ethiopia.
15. USAID, DANYA. Aflatoxin: A synthesis of the research in health, agriculture, and trade. 2012.
16. Butajira HDSS, Ethiopia 2013. Available from: http://indepth-network.org/Profiles/Butajira_hdss_2013.php.
17. Dewana Z, Fikadu T, Facha W, Mekonnen N. Prevalence and Predictors of Stunting among Children of Age between 24 to 59 Months in Butajira Town and Surrounding District, Gurage Zone. Southern Ethiopia Heal Sci J. 2017;11(4):1–6.
18. Quantitative Assay for – Aflatoxin M1 in Urine: Helica Biosystems Inc.Cat. No.991FLM01U-96. 2019. Available from: https://www.hygienea.com/wp-content/uploads/2021/02/Helica-Aflatoxin-M1-Urine-ELISA-Kit-Insert.pdf.
20. Austin PC, Tu JV. Automated variable selection methods for logistic regression produced unstable models for predicting acute myocardial infarction mortality. J Clin Epidemiol. 2000;53(11):138–46.

21. Njumbe Ediage E, Diana Di Mavungu J, Song S, Sioen I, Desaeger S. Multimycotoxin analysis in urines to assess infant exposure: a case study in Cameroon. Environ Int. 2013;57–58:50–9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23669720.

22. Turner NW, Bramhmbhatt H, Szabo-Vezse M, Poma A, Coker R, Piletsky SA. Analytical methods for determination of mycotoxins: An update (2009–2014). Anal Chim Acta. 2015;901:12–33. Available from: https://www.sciencedirect.com/science/article/pii/S0003267015012799.

23. GIEWS - Global Information and Early Warning System. Food and Agricultural Organization (FAO) of the United Nations. 2019. Available from: https://www.fao.org/giews/countrybrief/country.jsp?code=ETH.

24. Udoh JM, Cardwell KF, Ikotun T. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. J Stored Prod Res. 2000;36(2):187–201.

25. Chen G, Gong YY, Kimanya ME, Shrima CP, Routledge MN. Comparison of urinary aflatoxin M1 and aflatoxin albumin adducts as biomarkers for assessing aflatoxin exposure in Tanzanian children. Biomarkers. 2017;23(2):131–6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28114823.

26. Ouko E. Sources and levels of human exposure to aflatoxins in Makueni county, KENYA. 2014. Available from: https://www.semanticscholar.org/paper/Sources-and-levels-of-human-exposure-to-aflatoxins-Ouko/6c278e2427f5ad4190649336ebe4d6062778de49

27. Lei Y, Fang L, Akash MSH, Rehman K, Liu Z, Shi W, et al. Estimation of urinary concentration of aflatoxin M1 in Chinese pregnant women. J Food Sci. 2013;78(11):T1835-8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24102482.

28. Obuseh FA, Jolly PE, Jiang Y, Shuaib FMB, Waterbor J, Ellis WO, et al. Aflatoxin B1 albumin adducts in plasma and aflatoxin M1 in urine are associated with plasma concentrations of vitamins A and E. J Natl Res Coun Thal Sci Soc. 2007;39(2):128. Available from: https://books.google.co.th/books?id=U_kIoBQGmC.

29. Kumi J, Dotse E, Asare GA, Ankhsh N-A. Urinary Aflatoxin M1 Exposure in Ghanaian Children Weaned on Locally. African J Sci Res. 2015;4(6):28–32. Available from: https://www.researchgate.net/publication/289345310_ Urinary_aflatoxine_M1_exposure_in_Ghanaian_Children_Weaned_on_locally_prepared_nutritional_food

30. Ministry of Agriculture: Addis Ababa. AGRO-ECOLOGICAL ZONES OF ETHIOPIA. 2018. Available from: http://hdl.handle.net/123456789/2517

31. Summer PE, Lee D. Reducing Aflatoxin in corn during harvest and storage. UGA Coop Ext Bull 1231. 2017;6.

32. Chen C, Mitchell NJ, Gratz J, Houpt ER, Gong Y, Egner PA, et al. Aflatoxin exposure in Kenya, 2007: a cross-sectional study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2013;30(7):1322–31.

33. Shuaib FM, Jolly PE, Ehiri JE, Ellis WO, Yatich NJ, Funkhouser E, et al. Socio-demographic determinants of aflatoxin B1-lysine adduct levels among pregnant women in Kumasi, Ghana Ghana Med J. 2012;46(4):179–88.

34. Leroy JL, Wang JS, Jones K. Serum aflatoxin B1 -lysin adduct level in adult women from Eastern Province in Kenya depends on household socio-economic status: a cross sectional study. Soc Sci Med. 2015;146:104–10. https://doi.org/10.1016/j.socscimed.2015.10.039.

35. Gong YY, Egal S, Hounsa A, Turner PC, Hall AJ, Cardwell KF, et al. Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: The critical role of weaning. Int J Epidemiol. 2003;32(4):556–62. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12919209.

36. Jolly PE, Akinyemiju TF, Jha M, Aban I, Gonzalez-Falero A, Joseph D. Temporal variation and association of aflatoxin B1 albumin-adduct levels with socio-economic and food consumption factors in HIV positive adults. Toxins (Basel). 2015;7(12):5129–40.

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