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# Determinants of aflatoxin exposures in Kenyan School-aged children

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**ABSTRACT**

Aflatoxins are naturally occurring food toxins known to contaminate cereals with a carry-over effect in milk and meat products from farm animals raised on contaminated feed. In children, continuous consumption of aflatoxin-contaminated food is linked to immune suppression, vaccine interference and growth faltering while in adult populations, carcinogenesis in the liver has been established. We evaluate the main determinants of aflatoxin exposures among children recruited from primary schools in Makueni and Siaya Counties. A five-part questionnaire was administered to collect information from randomly selected participants. Aflatoxin$B_1$-lysine adducts in children's sera and total aflatoxins in food samples were analyzed by High-Performance Liquid Chromatography with Fluorescence detection. Using Chi-squared tests and Kruskal–Wallis tests, children from low-income households had the highest aflatoxin exposure, p-value = 0.0029. Smaller family size, greater food diversity, and good farming practices were associated with low aflatoxin exposures $p < 0.001$. Individual households living under severe levels of poverty were evidently exposed to higher levels of aflatoxins.

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Aflatoxins; children; determinants; socio-economic factors

## Introduction

Aflatoxins are a group of naturally occurring food contaminants produced by the soil-borne *aspergillus spp* fungi mainly *Aspergillus flavus* and *Aspergillus parasiticus* (IARC 2002; CAST 2003). Aflatoxins contaminate a variety of food crops including cereals, legumes, oilseeds, nuts, spices, coffee and tea (Bandyopadhyay et al. 2007; CAST 2003). Even though aflatoxin producing *Aspergillus* species occur worldwide, a higher prevalence in Sub-Saharan Africa and southeast Asia is evident due to hot humid climates and suboptimal control strategies (IARC 2002; Okoth 2016). Aflatoxin contamination presents a persistent challenge to food safety. Contamination can occur at every stage of the food supply chain including drying, during transport, in poor storage conditions, and in the market (Kang'ethe et al. 2017; Dembedza et al. 2019). Overall, more than 25% of the world's food supply is estimated to be contaminated by aflatoxins (Williams et al. 2004; Smith et al. 2015). Worldwide, estimated 4.5 billion people are susceptible to aflatoxin exposures through dietary sources or in occupational settings such as grain handling. Furthermore, dietary exposure or inhalation presents significant risks to human health.

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Since their discovery in the 1960s, aflatoxins have been established to be carcinogenic, mutagenic, immunotoxic, genotoxic and a probable contributor to growth faltering in children in low and middle-income countries (IARC 2002, 2015). In a recent study done among Kenyan children, Githang'a et al. (2019) reported immune-modulation and interference with hepatitis B vaccine efficiency resulting from dietary exposures in early life. Specifically, despite routine immunization, children with higher aflatoxin-metabolites in both sera and urine samples did not develop sufficient antibodies against Hepatitis B viral infections (Githang'a et al. 2019). Aflatoxins by nature are invisible to the naked eye with no distinctive smell. Dietary exposure to aflatoxin-contaminated food within a short period of time results in aflatoxicosis, a medical condition characterized by jaundice, bile duct proliferation, edema and in severe cases, sudden liver failure and deaths (Williams et al. 2004; Azziz-Baumgartner et al. 2005). The case fatality rate of aflatoxicosis in Kenya and Tanzania was reported to range from 39% to 50% (Williams et al. 2004; Daniel et al. 2011). Historically, Kenya's Makueni County is an established hotspot for aflatoxin exposures, with previous fatal aflatoxicosis outbreaks and widespread contamination of grain products (Bandyopadhyay et al. 2007; Azziz-Baumgartner et al. 2005). On the contrary, Siaya County has neither reported cases of aflatoxicosis nor publicly available surveillance data on contamination levels of food products. This project sought to explore determinants of widespread aflatoxin contaminants in food and human exposures, and to contribute early surveillance data on aflatoxin contamination levels in food and possible human exposure in Siaya County.

## Materials and methods

This study forms part of a larger cross-sectional study designed to establish the extent of aflatoxin exposure in the children population and the associated health outcomes. A detailed description of the study setting, rationale, and significance is provided in the study protocol (Wangia et al. 2019). The Kenya Joint Ethics committee of the University of Nairobi and Kenyatta National Hospital approved the study protocol and procedures. Randomized multistage stratified sampling was used in the identification of schools, and selection of study participants; mainly children aged six to 12 years and their mothers. School administrations were requested to convene parent meetings at a convenient time and location. The meetings were open to the public and were presided by the chairperson of the parents and teachers' association of each respective schools. Full disclosure of study details was given to all participants before completing informed consent forms. Mothers who provided informed consent were asked to complete a five-part questionnaire.

The study setting was rural farming communities that produce grain in small quantities for household use, and thus none had commercial storage facilities from survey results. Sampling was done when food supplies were low with households anticipating a new harvest in a month, and thus, most of the mothers couldn't provide 1 kg of recommended food samples. Instead, mothers were instructed to scoop flour/grain from at least five different parts of the kitchen container and send it to study administrators who thoroughly mixed the samples for laboratory analysis. A total of 338 samples was collected, milled, passed through a 1.0 mm sieve, labeled and stored under refrigeration at 4°C awaiting analysis. During sample extraction and cleanup, samples of 5 g were placed into a centrifuge tube, 25 ml of 70% MeOH added, then vortexed for 1 minute. The suspension was then centrifuged for 5 minutes at 4000 rpm and filtered through Whatman's No.1 Filter paper. A portion of 2.0 ml filtrate was transferred into a polypropylene tube and diluted with 3.6 ml HPLC water. Cleanup was done through a Sep-Pak cartridge and syringe barrel in the fume hood. Samples were eluted by 1.0 ml MeOH and dried using Labconco Centrivap concentrator (Kansas City, MO). The samples were reconstituted with 25% MeOH, centrifuged and filtered before transferring vials of 30 µl into Thermo Scientific Dionex UltiMate 3000RS UHPLC for analysis. Mobile phase A consisted of 10% Methanol and 90% HPLC grade water and B contained 100% Methanol. The flow rate was 0.4 ml/min and column temperatures were maintained at 50°C. Excitation and emission wavelengths for fluorescence detection were set at

360 nm and 435 nm, respectively, and 362 nm and 450 nm for UV detection. The performance of the method was in accordance with the criteria of Regulation EC No 401/2006 (European Commission 2006). Detailed data on grain analysis and aflatoxin contamination levels have been previously published (Nabwire et al. 2019).

In addition to parental consent, children's assent was required and about 6–8 ml of blood was obtained by certified phlebotomists and separated into sera for aflatoxin biomonitoring. The serum samples were analyzed for $AFB_1$-lysine adducts, a validated biomarker for aflatoxin exposure in human populations, using High-Performance Liquid Chromatography with Fluorescence Detection (Wang et al. 2001; Kang et al. 2015). Briefly, thawed serum samples were placed in a 56°C water bath for 30 minutes to deactivate any pathogens if present. Albumin and total protein content were then quantified using a spectrophotometer. An aliquot of 150 µl of serum was digested with Pronase in the ratio 1:4 for 3 hours in a water bath maintained at 37 °C. The digested samples were then loaded onto an Oasis Max cartridge from Waters Co. (Milford, Ma, USA), purified over a vacuum chamber manifold, sequentially washed and then eluted with 2% formic acid in methanol. The eluents were evaporated to dryness and reconstituted for HPLC injection in a 1200 liquid chromatography system (Agilent Technologies, Wilmington, DE, USA). Chromatographic separation was achieved using Zorbax Eclipse XDB-C18 column (5 µm particle size, 250 × 4.6 mm). The mobile phase consisted of 20 mM ammonium phosphate monobasic (pH 7.2) and 100% methanol to achieve separation within 25 minutes at a flow rate of 1.0 ml/min. Final $AFB_1$-lysine adducts concentration in albumin was obtained by manual integration and calculated using calibration curves then adjusted for albumin content.

Characteristics of the study population were summarized with proportions for categorical variables and with median and interquartile ranges (IQR) for continuous variables. The distribution of $AFB_1$-lysine adduct by different variables was summarized as continuous (geometric mean and median) and categorical variables (proportion). Kruskal-Wallis test was used to determine significant associations between categories if any. Chi-squared tests were applied to accept or reject independence between $AFB_1$-lysine and different variables. In the tests for independence, the global hypothesis test revealed that the groups were significantly different from each other. SAS v9.4 (Cary, NC), R version 3.6.1 (Vienna, Austria) and Microsoft Excel 365 Office were used for statistical analyses. The level of significance was set at p-value ≤0.05.

## Results

Up to 811 mothers were interviewed with respondents varying in age. The median age of mothers enrolled in the study was 36 years Interquartile Range (30, 43). An overall 79.8% of the mothers weremarried and only 28.4% had more than 8 years of formal education. The average number of children per woman is four children, Interquartile Range (3–5) while the overall median family size is made up of six people, Interquartile Range (5–7) with bigger family sizes of up to seven in Siaya County. The characteristics of children and mothers in the study population are shown in Table 1.

The study population was mainly made up of subsistence farmers, who grow food for household use. Among these, 72% use pesticides and/or fertilizer during farming. After harvest, 93.0% of farming households in Siaya store produce in maize sacks and only 39.4% in Makueni use maize sacks with a majority preferring hermetic bags for storage. Total aflatoxin in all food samples quantified by High-Performance Liquid Chromatography had a geometric mean (GM) of 59.6 µg/kg with (95% CI: 52.8, 66.5) µg/kg. Food Samples collected from Makueni County had higher total aflatoxin contamination levels, GM = 62.5 µg/kg with (95% CI: 53.7, 71.4) µg/kg while Siaya' contamination levels were slightly lower in comparison, GM = 52.8 µg/kg with (95% CI: 44.0, 61.7) µg/kg. Detailed data have been previously reported (Nabwire et al. 2019).

The food frequency questionnaire collected estimates of 24-hour dietary recall, weekly, monthly and yearly estimates of food groups consumed per household. The food consumption patterns were categorized into 10 groups, namely, 1) cereal and grain products, 2.) starchy roots and tubers, 3.)protein

**Table 1.** Characteristics of children and mothers in the study population.

| Characteristics | | N (%) | Makueni n = 327(%) | Siaya n = 484(%) | p-value |
|---|---|---|---|---|---|
| Age of child | Median (IQR) | 9 (8,11) | 9 (7, 11) | 10 (8, 11) | 0.663 |
| Mothers' Age | Median (IQR) | 36 (30,43) | 36 (31,42) | 37 (30, 45) | 0.279 |
| Sex of the child | Male | 372 (45.9) | 163 (49.8) | 209 (43.2) | 0.072 |
| No. meal/day | Median (IQR) | 3 (2,3) | 2.90 (0.48) | 2.43 (0.56) | <0.001 |
| Family Size | Median (IQR) | 6.0 (5.0, 7.0) | 5.34 (1.77) | 6.96 (2.70) | <0.001 |
| Marital Status | Married | 657 (82.3) | 285 (87.2) | 372 (76.9) | <0.001 |
| | Not Married† | 141 (17.7) | 29 (8.9) | 112 (23.1) | |
| ††Formal Education | Pre/Primary | 565 (70.7) | 183 (56.0) | 382 (78.9) | <0.001 |
| | Secondary/Post | 234 (29.3) | 134 (41.0) | 100 (20.7) | |
| Household Income+ | Low <$50 | 422 (54.9) | 80 (24.5) | 342 (70.7) | <0.001 |
| | Middle$50-100 | 202 (26.3) | 110 (33.6) | 92 (19.0) | <0.001 |
| | High >$100 | 145 (18.8) | 104 (31.8) | 41 (8.5) | |
| Living Conditions | Good | 93 (11.9) | 52 (15.9) | 41 (8.5) | <0.001 |
| | Fair | 547 (69.9) | 224 (68.5) | 323 (66.7) | |
| | Poor | 142 (18.2) | 22 (6.7) | 120 (24.8) | |
| Mothers Occupation | Farmer | 513 (63.2) | 219 (67.0) | 294 (60.7) | 0.084 |
| | Other | 298 (36.7) | 108 (33.0) | 190 (39.3) | |
| Spouse Occupation | Farmer | 359 (44.3) | 171 (52.3) | 188 (38.8) | <0.001 |
| | Other | 451 (55.7) | 156 (47.7) | 295 (61.0) | |
| Grow maize | Yes | 719 (90.9) | 279 (85.3) | 440 (90.9) | <0.001 |
| | No | 72 (9.1) | 28 (8.6) | 44 (9.1) | |
| Pesticide or fertilizer use | Yes | 567 (72.0) | 216 (66.1) | 351 (72.5) | <0.001 |
| | No | 220 (28.0) | 86 (26.3) | 133 (27.5) | |
| Aflatoxin knowledge | Yes | 474 (59.8) | 277 (84.7) | 197 (40.7) | <0.001 |
| | No | 319 (40.2) | 32 (9.8) | 287 (59.3) | |
| Identify aflatoxins | Yes | 433(47.8) | 220 (67.3) | 213 (44.0) | <0.001 |
| | No | 474 (52.3) | 85 (26.0) | 271 (56.0) | |

Reported values are IQR – Inter Quartile Range (IQR) and MeanSD and proportions; †not married includes single and widowed mothers, ††Formal education is based on the Kenya's 8 years of primary, 4 years of secondary and 4 years of post-secondary education, thus pre/primary refers to all mothers who got formal education for 8 years or less and secondary/post refers to all mothers who received more than 8 years of formal education. □living conditions refer to the respondents general perception of their daily life , ‡Other occupations included teachers, nurses, police officers, other civil servants and petty traders+Household Income amounts in Kenya Shillings(KES), 100KES = 1USD – cutoffs based on frequency distribution of incomes in populations sampled where mothers who reported monthly income of less than KES 5,000=~50USD were categorized as low, >5,000 ≤ 10,000=~>50USD≤100USD were categorized as middle income and monthly income levels of more than KES 10,000=~100USD were categorized as high-income earners

sources, 4.)Legumes, 5.)Vegetables, 6.)Fruits, 7.)Oils, and Fats, 8.)Sugar/Sugar cane products, 9.)Dairy Products, and 10.) Beverages. Food frequency data shows that the study population had a dietary diversity score of less than 4, characterized by limited diversification in food choice across both counties.

From Table 2, children in households with more than three meals a day consistently had higher aflatoxin exposure in both counties, an indication of overreliance on maize as a staple. Moreover, biomonitoring data show that aflatoxin $B_1$-lysine adducts in sera were higher among children in Makueni, median levels of 13.19pg/mg of albumin and lower in Siaya, median levels 9.01pg/mg of albumin, p-value = 0.0001. Table 2 also shows an analysis of aflatoxin metabolites in children's sera when categorized by different variables. Even though the children's exposure levels are neither dependent on their mother's marital status (p-value = 0.48) nor the level of formal education (p-value = 0.09), we get valuable information on characteristics of the household. Across households growing maize in both counties, aflatoxin exposure is reduced, evidently by lower $AFB_1$-lysine metabolites in children's sera (p-value = 0.21). Nonetheless, there are no significant differences whether households choose to use pesticides and fertilizers or not in their farming practices (p-value = 0.49). As expected, children in a household where mothers were aware of the aflatoxin contamination problem had consistently lower exposures (p-value = 0.0005) and were more likely to be knowledgeable of mitigation strategies (p-value 0.09).

**Table 2.** Aflatoxin $B_1$- Lysine adducts in children's sera by different variables.

| Mother characteristics | n | Median (95%CI) pg/mg albumin | Geomean (95%CI)pg/mg albumin | Mean pg/mg albumin | $X^2$ | p-value |
|---|---|---|---|---|---|---|
| Marital status | | | | | | |
| Married | 602 | 10.19 (9.06, 11.31) | 10.26(9.51, 11.06) | 21.11 | 0.50 | 0.48 |
| Not married | 131 | 12.27 (10.44,14.10) | 10.74(9.27, 12.44) | 19.58 | | |
| Formal Education | | | | | | |
| Pre/primary | 512 | 10.08(8.98,11.18) | 10.06(9.27,10.93) | 19.46 | 6.41 | 0.09 |
| Secondary/post | 222 | 11.67(9.82,13.51) | 10.33(9.02, 11.83) | 22.44 | | |
| Household Income | | | | | | |
| Low | 383 | 9.90 (8.59, 11.20) | 9.65 (8.84,10.54) | 18.23 | 4.56 | 0.10 |
| Middle | 189 | 9.75 (8.41,11.10) | 9.59 (8.50,10.82) | 17.35 | | |
| High | 133 | 13.32 (9.50,17.13) | 12.14 (10.0,14.74) | 29.11 | | |
| Grow Maize | | | | | | |
| Yes | 662 | 10.28 (9.20,11.36) | 10.24 (9.53,11.01) | 21.19 | 1.55 | 0.21 |
| No | 64 | 11.93 (10.29,13.56) | 12.27 (9.98,15.09) | 21.65 | | |
| Pesticide/Fertilizer Use | | | | | | |
| Yes | 527 | 10.39 (9.11,11.66) | 10.21 (9.42,11.06) | 21.21 | 0.47 | 0.49 |
| No | 195 | 10.44 (8.90,11.98) | 11.11 (9.76,12.64) | 21.59 | | |
| Aflatoxin knowledge | | | | | | |
| Yes | 448 | 11.90 (10.64,13.17) | 11.74(10.72,12.85) | 25.46 | 11.97 | 0.0005 |
| No | 280 | 9.33 (8.02,10.64) | 8.48 (7.71, 9.33) | 13.14 | | |
| Identify aflatoxins | | | | | | |
| Yes | 409 | 11.50 (10.18, 12.82) | 11.3(10.25,12.46) | 25.96 | 2.96 | 0.09 |
| No | 315 | 9.62 (8.38, 10.86) | 9.23 (8.44, 10.09) | 14.02 | | |

* all median, geometric mean and arithmetic mean values are log serum aflatoxin lysine adducts

Exposure patterns from Table 2 are corroborated by data in Table 3 which details the variation of total aflatoxin levels in household food products by different variables. Smaller family sizes had less contaminated foods and thus lower aflatoxin levels in food samples (p-value 0.181). Even though higher contamination levels in food products were evident among women who were not married, there are no significant differences in contamination levels across categories of marital status and education levels. Households that purchase grain have the highest total aflatoxin levels, GM 69.23 µg/kg (95% CI 60.30,79.49) µg/kg, p-value = 0.0923 compared to households that practice subsistence farming. Furthermore, households that grew maize had lower aflatoxin contamination level GM 59.20 µg/kg (95% CI 55.28,63.41) µg/kg, p-value = 0.0792. Farmers who stored maize in traditional maize sacks had higher total aflatoxin contamination in grain, GM 60.51 µg/kg (95% CI 55.39 66.09) µg/kg, p-value = 0.114 compared to households who preferred modern storage methods including the use of hermetic storage bags. Additionally, aflatoxin knowledge was associated with lower total aflatoxin contamination in grain products, p-value = 0.3547. While there were no significant differences in contamination levels between middle- and high-income households low-income households had the highest total aflatoxins in grain GM, 73.21 µg/kg (95% CI 64.92, 82.55) µg/kg, p-value = 0.0029. This indicates that children from low-income households were more likely to be exposed to the highest aflatoxin contamination levels in grain.

## Discussion

Maize is the dietary staple in Kenya characterized by breakfasts of porridge gruel made from maize flour, lunch meals of mixed maize and beans while dinner is mainly stiff-like porridge meal locally known as 'ugali' served with vegetables and some form of protein (Daniel et al. 2011; Githanga et al 2019; Nabwire et al. 2019). Food insecure households characterized by a dietary diversity score of less than 4 consistently showed higher aflatoxin exposure levels in both the current and past studies (Kang'ethe et al. 2017; Githang'a et al. 2019). Family size is used as a proxy for normal financial resources available per household member (Jolly et al. 2006; Obuseh et al. 2010; Shuaib et al. 2012).

**Table 3.** Total Aflatoxin levels in household food products by different variables.

| Variables | Geometric Mean µg/kg (95% CI) | Median µg/kg (95% CI) | P-value |
|---|---|---|---|
| Family Size | | | 0.1810 |
| <6people | 58.36 (53.43, 63.74) | 57.88(49.89, 65.87) | |
| ≥6people | 62.38 (56.88,68.40) | 65.41(59.49, 71.33) | |
| Number of meals per day | | | 0.3274 |
| <3 | 62.72(55.35,71.08) | 68.85(62.15,75.55) | |
| ≥ 3 | 60.08(54.56,65.61) | 59.26(54.99,63.86) | |
| Marital status | | | 0.6621 |
| Married | 60.08 (56.11, 64.34) | 63.07(57.80,68.34) | |
| Not married | 65.67 (54.75, 78.77) | 67.10(54.99,79.21) | |
| Education | | | 0.1652 |
| Pre/Primary | 56.61(52.31,61.27) | 60.23 (53.65,66.81) | |
| Secondary/Post | 67.16(60.56,74.48) | 66.08 (58.23,73.94) | |
| Household Income | | | 0.0029 |
| Low | 73.21 (64.92,82.55) | 72.74 (61.72,83.76) | |
| Middle | 54.67 (49.13,60.82) | 61.26(53.46,69.05) | |
| High | 54.42 (47.17,61.67) | 53.79(49.04,59.00) | |
| Food Source | | | 0.0923 |
| Purchase | 69.23(60.30,79.49) | 70.74(60.47,81.01) | |
| Farm | 59.45(53.06,66.61) | 56.66(48.59,64.73) | |
| Both | 57.17(52.05,62.80) | 63.07(56.70,69.44) | |
| Grow Maize | | | 0.0792 |
| Yes | 59.20(55.28,63.41) | 61.43(55.85,67.02) | |
| No | 79.17(64.2197.62) | 67.61(53.5381.69) | |
| Pesticide/fertilizer use | | | 0.3065 |
| Yes | 59.00(54.4763.90) | 61.92(54.2369.61) | |
| No | 66.16(58.9674.22) | 66.32(57.5175.13) | |
| Maize Storage | | | 0.1114 |
| Maize sacks | 60.51(55.3966.09) | 67.99(62.0373.95) | |
| Other | 56.47(50.5263.13) | 50.44(43.6357.25) | |
| Aflatoxin Knowledge | | | 0.3547 |
| Yes | 56.39(49.6963.99) | 63.07(55.3770.77) | |
| No | 61.9957.5566.78 | 62.17(55.2769.07) | |

Bigger families in Makueni county were more susceptible to higher aflatoxin contamination in food products and ultimate dietary exposure (p-value = 0.0295) compared to Siaya. Households with smaller families of five or fewer members had lower aflatoxin exposures on average.

Among rural households practicing subsistence farming, lower aflatoxin contamination in grain in addition to lower exposures in children's sera (p-value = 0.0792) was evident. Among farming households, the volume of production is small and sufficient only for an individual household. Thus, the need for commercial storage facilities required for long-term storage was non-existent. Traditionally, harvested grains including maize were stored in outdoor-elevated granaries with adequate aeration to limit the proliferation of fungi and aflatoxin contamination (Azziz-Baumgartner et al. 2005; Strosnider et al. 2006). However, limited technological advancements have contributed to non-conventional methods of storing food products which further promotes aflatoxin contamination.

In the current study, 93.0% of the households in Siaya and 39.4% in Makueni use maize sacks for storage. Maize storage in sacks was associated with high aflatoxin exposure levels with worse effects observed in Makueni County, p-value = 0.0257. Up to 46.8% of the farmers recruited from Makueni county prefer multi-layered hermetic storage bags designed to reduce post-harvest losses caused by pests-damage and contamination by aflatoxins (Dembedza et al. 2019). A study in Ghana reported that 25% of the rural households that store harvested maize experienced significantly higher levels of aflatoxin contamination (Jolly et al. 2015).

Furthermore, the use of pesticides and/or fertilizer was consistently associated with lower aflatoxin contamination levels in both grain and children's sera. Good farming practices including the use of fertilizers and pesticides have been associated with lower aflatoxin contamination of grain

products in other Kenyan regions (Njeru et al. 2019). General knowledge of aflatoxins and overall ability to identify moldy food products was associated with lower household aflatoxin levels. In addition to subsistence farming, other participants were teachers, nurses, police officers, civil servants, petty traders and/or unemployed.

Lastly, socio-economic conditions play a major role in exposure levels. While more than half (51.3%) of survey respondents reported low monthly incomes of less than KES 5000 (~USD 50), all things considered, most participants perceive their daily life to range from fair to good. A previous study among women in Kenya's Meru County reported that women with higher household expenditure, better food security, land ownership, and higher household incomes experienced lower aflatoxin contamination at the household level (Leroy et al. 2015). Additionally, epidemiological studies in Ghana by Shuaib et al. established that individuals with higher incomes and education levels were more likely to be aware of health risks linked to consumption of aflatoxins contaminated food products, thereby, more likely prioritize food safety (Obuseh et al. 2010; Shuaib et al. 2012). In Guatemala, households that purchased maize from the market had higher aflatoxin levels in maize supplies than households that produced the maize themselves (Voth-Gaeddert et al. 2020). Taken together, these studies show that having sufficient financial resources improves an individual's ability to afford higher quality foods because safe foods are likely to cost more. It is possible that participants in our study may not have the excessive disposable income to purchase additional food products and instead rely on locally produced food.

This study was limited in the following ways. First, it was not possible to collect a minimum of 1 kg maize samples for laboratory analysis. Mothers who provided informed consent produced grain in small quantities for household use, and thus none had a commercial storage facility from survey results. Furthermore, sampling was done when food supplies were low, with households anticipating a new harvest in a month. Mothers were instructed to scoop flour/grain from different parts of their kitchen container and submit to study administrators who thoroughly mixed the provided samples before laboratory analysis. To offset this limitation and corroborate personal exposures, bio-monitoring for aflatoxin $B_1$-lysine adducts in children was done to evaluate personal exposures. Second, results from this study cannot be used to determine cause and effect, nor can they be used to analyzing behavior change due to ethical limitations on contacting study participants in the future.

## Conclusion

In Kenya and most parts of the developing world, food safety can be challenging to attain due to widespread contamination of food supplies by aflatoxins. In our study populations where corn is a staple for many households, participants recruited from Makueni County were more likely to have higher aflatoxin exposure levels compared to participants who reside in Siaya County. Makueni County experience extreme plant stress from drought, and high ambient humidity which promotes colonization of food items with aflatoxin-producing *Aspergillus* fungi.

The main determinants of lower aflatoxin contamination and human exposure in this study were established to be good farming practices, food security, and improved socio-economics. Households with sufficient produce for the season, and those who utilized pesticides, fertilizers and other available good farming practices experienced lower aflatoxin contamination in food supplies and ultimately lower levels of aflatoxin $B_1$- lysine adducts in children's sera. Additionally, households storing foods in hermetic bags to prevent pests' infestations and proliferation of fungi; and those generally aware of aflatoxins experienced lower exposures. Intervention strategies to mitigate aflatoxin exposures will be effective when resources are invested in rural household communities that rely on subsistence farming for dietary staples. Additionally, encouraging food diversity by promoting food groups less susceptible to aflatoxin contamination is poised to mitigate negative health outcomes associated with aflatoxin exposures.

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## Disclosure of interest

The authors report no conflict of interest.

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## References

Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, et al. 2005. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ Health Perspect. 113(12):1779–1783.

Bandyopadhyay R, Kumar M, Leslie JF. 2007. Relative severity of aflatoxin contamination of cereal crops in West Africa. Food Addit Contam. 24(10):1109–1114. doi:10.1080/02652030701553251.

CAST. 2003. Mycotoxins: risks in plant, animal, and human systems. Ames, IA: Council for Agricultural Science and Technology, 2002. (Task force report: no 139.

Daniel JH, Lewis LW, Redwood YA, Kieszak S, Breiman RF, Flanders WD, Bell C, Mwihia J, Ogana G, Likimani S, et al. 2011. Comprehensive assessment of maize aflatoxin levels in Eastern Kenya, 2005-2007. Environmental Health Perspectives. 119(12):1794–1799. doi:10.1289/ehp.1003044.

Dembedza MP, Chidewe C, Benhura MA, Mvumi B, Manema LR, Nyanga LK. 2019. Effectiveness of hermetic maize grain storage technology in limiting aflatoxin exposure in women and children from smallholder farming areas. World Mycotoxin J. 12(3):233–244.

European Commission. 2006. COMMISSION REGULATION (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

Githang'a D, Wangia RN, Mureithi MW, Wandiga SO, Mutegi C, Ogutu B, Agweyu A, Wang JS, Anzala O. 2019. The effects of aflatoxin exposure on Hepatitis B-vaccine induced immunity in Kenyan children. Current Problems in Pediatric and Adolescent Health Care. 49(5):117–130. doi:10.1016/j.cppeds.2019.04.005.

IARC. 2002. IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Lyon (France): IARC Press; p. p. 185.

IARC. 2015. Mycotoxin control in low and middle income countries. IARC Working Group Report No9. Lyon (France): International Agency for Research on Cancer.

Jolly P, Jiang Y, Ellis W, Awuah R, Nnedu O, Phillips T, Wang JS, Afriyie-Gyawu E, Tang L, Person S, et al. 2006. Determinants of aflatoxin levels in Ghanaians: sociodemographic factors, knowledge of aflatoxin and food handling and consumption practices. International Journal of Hygiene and Environmental Health. 209 (4):345–358. doi:10.1016/j.ijheh.2006.02.002.

Jolly PE, Akinyemiju TF, Jha M, Aban I, Gonzalez-Falero A, Joseph D. 2015. Temporal variation and association of aflatoxin B(1) albumin-adduct levels with socio-economic and food consumption factors in HIV positive adults. Toxins (Basel). 7(12):5129–5140. doi:10.3390/toxins7124868.

Kang MS, Nkurunziza P, Muwanika R, Qian G, Tang L, Song X, Xue K, Nkwata A, Ssempebwa J, Lutalo T, et al. 2015. Longitudinal evaluation of aflatoxin exposure in two cohorts in south-western Uganda. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 32(8):1322–1330. doi:10.1080/19440049.2015.1048749.

Kang'ethe EK, Korhonen H, Marimba KA, Nduhiu G, Mungatu JK, Okoth SA, Joutsjoki V, Wamae LW, Shalo P. 2017. Management and mitigation of health risks associated with the occurrence of mycotoxins along the maize value chain in two counties in Kenya. Food Qualit Saf. 1(4):268–274. doi:10.1093/fqsafe/fyx025.

Leroy JL, Wang J-S, Jones K. 2015. Serum aflatoxin B1-lysine adduct level in adult women from Eastern Province in Kenya depends on household socio-economic status: A cross sectional study (Article). Soc Sci Med. 146:104–110. doi:10.1016/j.socscimed.2015.10.039.

Nabwire WR, Ombaka J, Dick CP, Strickland C, Tang L, Xue KS, Wang J-S. 2019. Aflatoxin in household maize for human consumption in Kenya, East Africa. Food Additiv Contam: Part B. 1–7. 13(1):45–51.

Njeru NK, Midega CAO, Muthomi JW, Wagacha JM, Khan ZR. 2019. Influence of socio-economic and agronomic factors on aflatoxin and fumonisin contamination of maize in western Kenya. Food Sci Nutr. 7(7):2291–2301. eng. doi:10.1002/fsn3.1070.

Obuseh FA, Jolly PE, Jiang Y, Shuaib FM, Waterbor J, Ellis WO, Piyathilake CJ, Desmond RA, Afriyie-Gyawu E, Phillips TD. 2010. Aflatoxin B1 albumin adducts in plasma and aflatoxin M1 in urine are associated with plasma concentrations of vitamins A and E. International Journal for Vitamin and Nutrition Research. 80(6):355–368. doi:10.1024/0300-9831/a000021.

Okoth S 2016. Improving the evidence base on aflatoxin contamination. Series: Agriculture and Nutrition/CTA Working Paper 16/13. Wageningen: Technical Center for Agricultural and Rural Cooperation.

Shuaib FM, Jolly PE, Ehiri JE, Ellis WO, Yatich NJ, Funkhouser E, Person SD, Williams JH, Qian G, Wang JS. 2012. Socio-demographic determinants of aflatoxin B1-lysine adduct levels among pregnant women in Kumasi, Ghana. Ghana Med J. 46(4):179–188.

Smith LE, Prendergast AJ, Turner PC, Mbuya MN, Mutasa K, Kembo G, Stoltzfus RJ. 2015. The potential role of mycotoxins as a contributor to stunting in the SHINE trial. Clinical Infectious Diseases. 61(Suppl 7):S733–737. doi:10.1093/cid/civ849.

Strosnider H, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R, Brune M-N, DeCock K, Dilley A, Groopman J, Hell K, et al. 2006. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. Environmental Health Perspectives. 114(12):1898–1903. doi:10.1289/ehp.9302.

Voth-Gaeddert LE, Stoker M, Torres O, Oerther DB. 2020. The influence of local market and household factors on aflatoxin presence in maize and symptoms of its exposure to children in Guatemala. International Journal of Environmental Health Research. 30(3):312–326. doi:10.1080/09603123.2019.1594721.

Wang JS, Abubaker S, He X, Sun G, Strickland PT, Groopman JD. 2001. Development of aflatoxin B(1)-lysine adduct monoclonal antibody for human exposure studies. Applied and Environmental Microbiology. 67(6):2712–2717. doi:10.1128/AEM.67.6.2712-2717.2001.

Wangia GDP, Wang JS, Anzala OA. 2019. Aflatoxin exposure in children age 6–12 years: a study protocol of a randomized comparative cross-sectional study in Kenya, East Africa. Pilot Feas Stud. 5(1):141. doi:10.1186/s40814-019-0510-x.

Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. The American Journal of Clinical Nutrition. 80(5):1106–1122. doi:10.1093/ajcn/80.5.1106.