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Food Additives & Contaminants: Part B
Surveillance

ISSN: 1939-3210 (Print) 1939-3229 (Online) Journal homepage: https://www.tandfonline.com/loi/tfab20

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To cite this article: Wangia Ruth Nabwire, James Ombaka, Christopher Peyton Dick, Christian Strickland, Lili Tang, Kathy Siyu Xue & Jia-Sheng Wang (2020) Aflatoxin in household maize for human consumption in Kenya, East Africa, Food Additives & Contaminants: Part B, 13:1, 45-51, DOI: 10.1080/19393210.2019.1690053

To link to this article: https://doi.org/10.1080/19393210.2019.1690053

FOOD ADDITIVES & CONTAMINANTS: PART B
2020, VOL. 13, NO. 1, 45–51
https://doi.org/10.1080/19393210.2019.1690053

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Aflatoxin in household maize for human consumption in Kenya, East Africa

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ABSTRACT

The objective of this study is to determine the occurrence and level of aflatoxins (AFs) contamination in freshly harvested maize for human consumption in rural Kenya. Maize kernels and freshly milled maize flour (n = 338) were collected from households in Siaya and Makueni counties. While both counties are representatives of different environmental and climate conditions, Makueni County is the area with reported outbreaks of aflatoxicosis. Samples were analysed for AFB_1, AFB_2, AFG_1, and AFG_2 using Ultra High-Pressure Liquid Chromatography with Fluorescence detection. AFs were detected in 100% of the samples with the range of 2.14–411 µg/kg. The geometric mean of total AFs in all samples from Makueni County is 62.5 µg/kg with 95% CI: 53.7, 71.4 while in Siaya County is 52.8 µg/kg with 95% CI: 44.0, 61.7. This study showed that AFs contamination is prevalent in maize-based foods in the region.

ARTICLE HISTORY
Received 10 June 2019
Accepted 3 November 2019

KEYWORDS
Aflatoxins; AFB_1; total aflatoxins; household maize; rural Kenya

Introduction

Aflatoxins (AFs) are a group of mycotoxins commonly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* under favourable warm and humid climate (Villers 2014). They were discovered and characterised in the 1960s after the reported death of more than 100,000 turkey poults fed on AF-contaminated feed in England (Kensler et al. 2011). Since their discovery, AFs have been associated with acute and chronic toxicity in both animal and human populations (IARC 1993, 2002). While 16 structurally related AFs have been characterised, only four major groups, namely AFB_1, AFB_2, AFG_1, and AFG_2 are extensively studied due to common occurrence in food supplies. AFs contaminate agricultural products including maize, peanuts, sorghum, rice, cassava, spices, and nuts (IARC 1993, 2002). Therefore, AFs contamination poses a significant food safety issue and potential risks to human and animal health (CAST 2003; IARC Working Group Reports 2015). *A. flavus* produces AFB_1 and AFB_2 while *A. parasiticus* mainly produces AFG_1 and AFG_2 and is confirmed to produce all four major AFs (Cole and Cox 1981). AFB_1 is the most potent mycotoxin; the International Agency for Research on Cancer (IARC), classified AFB_1 as Group I human carcinogen due to sufficient evidence from animal and human epidemiology studies that associated AFB_1 exposure to increased risk of developing primary liver cancers (IARC 1993, 2002).

In humans, acute toxicity resulting from exposure to high levels of AFs in the diet was reported in India with a case fatality rate of 10% in humans and 100% in dogs (Tandon et al. 1977). In Kenya, aflatoxicosis was reported in 1981 which was also preceded by deaths of farm animals (Ngindu et al. 1982). More recently, consumption of AF-contaminated grain caused aflatoxicosis in large human populations with a case fatality rate of 40% in Kenya and 50% in Tanzania (Azziz-Baumgartner et al. 2005; Daniel et al. 2011; Kamala et al. 2018). Aflatoxicosis is characterised by vomiting, jaundice, abdominal pain, oedema, convulsions, sudden liver failure and ultimately death (Mwanda et al. 2005). While acute toxicities associated with exposure to high levels of AFs are rare events worldwide, cases occur and are concentrated in high-risk regions such as Makueni County of Kenya. Cumulative exposure to low quantities of AFs through the diet over a period of time is more widespread and is the leading cause of liver cancer in adult populations in the developing world (Kew 2013; Magnussen and Parsi 2013). In children populations, exposure to AFs through weaning foods and the diet is associated with immune suppression, micronutrient deficiency and possible growth impairments (IARC Working Group Reports 2015; Githanga et al. 2019). Populations that rely on maize products as staple food need to be assessed for ultimate health consequences associated with chronic consumption of AF-contaminated maize.

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AFs are closely regulated in most countries with maximum limits ranging from 5 to 20 µg/kg for food destined for human consumption (USFDA 2000; Wu et al. 2013). Kenya, given its troubled past with aflatoxicosis, has a maximum limit of 10 µg/kg in maize and maize products (East African Community 2013). The European Union's standard is the most strict with a maximum limit of 2 µg/kg for AFB_1, and 4 µg/kg for total AFs (European Commission 2006). Despite good agricultural practices combined with rigorous regulation, it is common to find detectable levels of AFs in food commodities. AFs contamination of food supplies presents a continuous challenge throughout the food chain and is a major risk factor for food insecurity in low- and middle-income countries.

In this study Ultra High-Pressure Liquid Chromatography with Fluorescence detection (UHPLC-FLD) is preferred for simultaneous analysis of AFs contamination in maize products, because of high sensitivity and specificity (Wacoo et al. 2014; Alshannaq and Yu 2017), with the purpose to determine occurrence and level of AFs in freshly harvested maize for human consumption collected from rural Kenya.

Materials and methods

Sampling

This study is part of the larger cross-sectional study aimed at establishing AFs exposure levels in children between the ages of 6 and 12 years. The overall objective of the study is to comparatively assess if dietary exposure to AFs contributes to micronutrient deficiency, immune suppression and growth impairment. The research and study protocols were reviewed and approved by the Joint Ethics Committee of the University of Nairobi and Kenyatta National Hospital in Kenya.

Randomised multistage stratified sampling was used in the identification of schools, and selection of study participants from Siaya and Makueni counties. All participants concern and questions were addressed before subject recruitment. Informed consent was explained in local dialect and parents who agreed to study procedures were asked to complete a questionnaire. Parents who provided informed consent were asked to provide 150 g of household maize and/or flour used for daily meal consumption.

A total of 338 samples of maize products (173 milled flour samples and 165 maize grain samples) were collected and prepared for UHPLC-FLD analysis. Milled flour samples were sieved through a 1.0 mm sieve, while the maize grain samples were first ground into a fine texture using a Ninja Professional 1100-W Blender (Euro-Pro Operating LLC, Newton, MA) and then passed through a 1.0 mm sieve. All samples were weighed into sealable plastic storage bags, labelled and stored under refrigeration at 4°C until analysis.

Chemicals and reagents

AFB_1, AFB_2, AFG_1, and AFG_2 standards were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). HPLC grade water was purchased from Avantor Performance Materials (Centre Valley, PA) and methanol was purchased from Honeywell (Morristown, NJ). Sep-pak Classic C18 Cartridges were purchased from Waters Corporation (Milford, MA). An extraction solution was prepared using a ratio of 70:30 methanol: HPLC grade water. Individual AFs standards including AFB_1, AFB_2, AFG_1, and AFG_2 were dissolved in dimethyl sulphoxide (DMSO) and used as stock solutions. A standard curve was created from dilutions of the stock solutions in concentrations of 0.0, 1.0, 2.5, 5.0, 10, 20, and 40 µg/kg.

Sample extraction and clean-up

Due to the heterogeneous nature of AFs contamination, each sample was thoroughly mixed and multiple scoops randomly taken from different parts of the plastic bag. This method has been used in previous studies conducted in rural Kenya (Lewis et al. 2005; Daniel et al. 2011). Up to 5 g of each sample was weighed into a centrifuge tube and 25 ml of 70% MeOH added, then vortexed for 1 min. The suspension was centrifuged for 5 min at 4000 rpm and filtered through Whatman 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 30 µl into a UHPLC vial for analysis.

UHPLC conditions

The Thermo Scientific Dionex UltiMate 3000RS UHPLC system was used for separation of target analytes. 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 mobile phase A consisted of 10% Methanol and 90% HPLC grade water while B contained 100% Methanol. The flow rate was 0.4 ml/min and column temperatures were maintained at 50°C. For each sample, 10 µL was injected to the Acclaim column (Acclaim RSLC 120C18 2.1 × 150 mm, 2.2 µm 120 Å, Thermo Scientific, Waltham, MA). Total run

is 25 min with the gradient at 0.0 min specified for 95A:5B, at 6.0 min 50A:50B, at 10.0 min 5A:95B, and from the 15th minute, the program is specified to run 95A:5B until the end. Control samples were prepared in duplicate every day. In the first step of sample processing, milled maize samples with known AF concentration were spiked with 5.0 µg/kg of AFB_1. In addition, during UHPLC-FLD analysis, two blanks of 25% methanol, two standards containing 2.5 µg/kg of AFB_1 and two standards containing a mixture of AFB_1, AFB_2, AFG_1, and AFG_2 in equal concentrations were used for quality controls.

Method validation

Method validation parameters reported herein (Table 1) are selectivity, linearity, the limit of detection (LOD) and limit of quantification (LOQ). The performance of the method was in accordance with the criteria of Regulation EC No 401/2006 (European Commission 2006). The parameter used for selectivity is retention times with averages of 8.31, 8.72, 9.16, and 9.46 min for AFG_2, AFG_1, AFB_2, and AFB_1, respectively. The calibration curves used to quantify AFs were determined by linearity assumption. Linearity was accomplished by injecting in duplicates standard solutions of AFG_2, AFG_1, AFB_2, and AFB_1 at concentrations of 0.0, 1.0, 2.5, 5.0, 10, 20, and 40 µg/kg and then constructing standard curves. Linearity parameters were based on the regression coefficients (r^2) of the standard curve of each AFs group which were over 0.998. The LOD and LOQ were defined as 3.3 and 10 times the standard deviation, respectively, divided by the slope of the calibration curve for each mycotoxin (Firdous et al. 2014; Janic Hajnal et al. 2017). The parameter used to validate accuracy is the recovery rate of AFs after spiking milled maize flour samples with known AF levels. Relative standard deviation was used to validate precision.

Statistical methods

Statistical analyses were accomplished by the use of SAS software version 9.4 (Cary, North Carolina) and Microsoft Excel 365 Office. Any value below the LOQ was excluded from statistical analysis. The concentrations reported in this study were adjusted for recovery of each AF. Total AFs were calculated by summing AFB_1, AFB_2, AFG_1, and AFG_2. Results were analysed by ANOVA assuming a level of significance at $p < .05$ and 95% confidence Interval. Significant differences in contamination levels were evaluated according to post hoc Tukey's honestly significant differences and two-sample t-test assuming unequal variances.

Results

A detection rate of 100% for total AFs was found in all 338 samples (Table 2). AFB_1 is the most dominant in all samples, contributing up to 97.1% of the total AFs. The next is AFG_1, then AFB_2, and AFG_2 contributed the least percentage to total AFs. Up to 128 (37.9%) samples did not have detectable levels of AFG_1. The geometric mean of AFB_1 in all samples (338) is 57.9 µg/kg with 95% CI: 51.0, 64.7. The median is 59.3 µg/kg and the range of AFB_1 is 1.69–404 µg/kg. The geometric mean of AFG_1 in detectable samples is 0.58 µg/kg with 95% CI: 0.43, 0.73. The median is 0.62 µg/kg and the range of AFG_1 is 0.01–8.38 µg/kg. The geometric mean of AFB_2 in all samples (n = 338) is 0.40 µg/kg with 95% CI: 0.31, 0.48. The median is 0.45 µg/kg and the range of AFB_2 is 0.06–5.83 µg/kg. The maize flour and kernels were least contaminated by AFG_2. The geometric mean of AFG_2 in all samples (n = 338) is 0.17 µg/kg with 95% CI: 0.08, 0.25. The median is 0.23 µg/kg and the range of AFG_2 is 0.01–7.01 µg/kg. When the four groups of AF were combined, the geometric mean of total AFs in all samples (n = 338) is 59.6 µg/kg with 95% CI: 52.8, 66.5. The median is 61.8 µg/kg and the range of AFB_1 is 2.11–411 µg/kg.

The geometric mean of total AFs in all samples from Makueni County is 62.5 µg/kg with 95% CI: 53.7, 71.4 µg/kg (Table 3). The median is 63.0 µg/kg and the range of total AFs in samples from Makueni County is 5.77–411 µg/kg. The geometric mean of total AFs in all samples from Siaya County is 52.8 µg/kg with 95% CI: 44.0, 61.7; the median is 58.1 µg/kg and the range of total AFs in samples from Siaya County is 2.14–252 µg/kg. The geometric mean of AFB_1 in maize kernels from Siaya County is 66.6 µg/kg with 95% CI: 50.9, 82.3, the median is 66.5 µg/kg with a range of 1.69–247 µg/kg. The geometric mean of AFB_1 in maize kernels from Makueni County is 62.5 µg/kg with 95% CI: 51.8, 73.1, the median is 60.6 µg/kg with a range of 14.0–338 µg/kg.

Table 1. Method verification parameters.

Analyte	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)	RSD (%)	Retention time (min)
AFB_1	0.036	0.12	89.4	8.56	9.46
AFB_2	0.001	0.01	94.1	8.73	9.16
AFG_1	0.002	0.02	87.7	6.95	8.72
AFG_2	0.001	0.01	92.3	9.26	8.31

Table 2. AF contamination (µg/kg) in maize products (n = 338).

Aflatoxin	GM (95% CI)	Mean ± SD	Median	Range
AFB_1	57.9 (51.0–64.7)	76.2 ± 63.9	59.3	1.69–403
AFG_1	0.58 (0.44–0.73)	0.99 ± 1.11	0.62	0.01–8.38
AFB_2	0.40 (0.31–0.48)	0.66 ± 0.79	0.45	0.06–5.83
AFG_2	0.17 (0.08–0.25)	0.47 ± 0.79	0.23	0.01–7.01
AFtotal	59.6 (52.8–66.5)	77.9 ± 64.3	61.8	2.14–411

Table 3. Difference in AFs contamination levels (µg/kg) between kernels and flour.

	n	GEOMEAN (95% CI)	Mean ± SD
All samples	338	59.6 (52.8–66.5)	77.9 ± 64.3
Flour	173	60.2 (50.0–70.2)	79.4 ± 67.6
Kernels	165	59.0 (49.7–68.3)	76.3 ± 60.9
Makueni samples	242	62.5 (53.7–71.4)	83.1 ± 70.1
AFB_1 in flour	120	59.0 (44.9–73.1)	85.6 ± 78.2
AFB_1 in kernels	122	62.5 (51.8–73.1)	77.4 ± 59.8
Siaya samples	96	52.8 (43.9–61.7)	64.7 ± 44.2
AFB_1 in flour	53	41.4 (34.5–48.2)	47.4 ± 25.4
AFB_1 in kernels	43	66.6 (50.9–82.3)	82.0 ± 51.9

Maize kernels appear to be more contaminated by AFB_1 compared to maize flour. In Siaya County, the maize kernels' geometric mean is 66.6 µg/kg with 95% CI 50.9, 82.3; median is 66.5 µg/kg with the range of 1.69–247 µg/kg, which is significantly higher than AFB_1 levels in flour samples whose geometric mean is 41.4 with 95% CI 34.5, 48.2. The median is 40.5 µg/kg with the range of 13.9–118 µg/kg ($p < .0001$). While AFB_1 in kernel samples from Makueni has higher contamination levels than flour samples from the same region, these results are not statistically significant ($p > .05$). In flour samples collected from Makueni, the geometric mean of AFB_1 is 59.0 µg/kg with 95% CI: 45.0, 73.0, the median is 65.8 µg/kg with the range of 4.17–403 µg/kg. In Siaya, AFB_1 contamination levels of flour samples have a geometric mean of 41.4 µg/kg with 95% CI: 34.5, 48.2, a median of 40.5 µg/kg with a range of AFB_1 from 13.9 to 118 µg/kg.

Overall, 95.3% of all maize samples exceeded the United States Food and Drug maximum limit of 20 µg/kg for total AFs in household grain for human consumption (Table 4). If considering Kenya Bureau of Standards maximum limit of 10 µg/kg, 97.5% of the maize sample exceeded the limit and are considered unfit for human consumption. Up to 25% (84/338) of the samples had total AFs contamination levels higher 100 µg/kg, which is 10 times over the Kenya Bureau of Standards maximum limit. The current study reports a maximum level of total AFs contamination to be 411 µg/kg, a value over 40 times the maximum limit.

Discussion

Maize and maize products are highly susceptible to AFs contamination. The geometric mean of total AFs in all samples (n = 338) is 59.6 µg/kg with 95% CI: 52.8, 66.5. The geometric mean of total AFs in all samples from Makueni County is 62.5 µg/kg with 95% CI: 53.7, 71.4 while in Siaya County is 52.8 µg/kg with 95% CI: 44.0, 61.7. In rural parts of south and southwestern Ethiopia, total AFs in maize samples were generally above 24 µg/kg with a reported maximum of 513 µg/kg (Getachew et al. 2018). In another study conducted in Ethiopia, AF-contaminated complementary foods intended for consumption by young children aged 5 years and under with a mean range of 0.3–9.9 µg/kg (Ayelign et al. 2018). In Serbia, the highest reported level of AFB_1 in maize samples is 8.8 µg/kg with overall mean contamination levels of 0.53 µg/kg of AFB_1 (Torovic 2018). In Tunisia, mean total AFs reported were 11.08 ± 8.84 µg/kg mainly contributed by AFGs (Jedidi et al. 2017). These studies show that AFs contamination is still prevalent in maize-based foods in low- and middle-income countries and presents a significant challenge to food safety.

The Kenyan population relies on maize as a staple, with an estimated consumption rate of 400 g/person/day (Kilonzo et al. 2014). It has been estimated that Kenyans are exposed to AFs in the range of 4.3–554 ng kg^{-1} bw day^{-1}, whereas, on average, Australians and Americans are exposed to 0.8 ng and 0.26 ng kg^{-1} bw day^{-1}, respectively, from AF-contaminated maize and maize-based products (Wambui et al. 2017). This shows that the Kenyan population is highly susceptible to AF exposure through the diet and are at a greater risk of developing adverse health outcomes in adulthood if early measures to mitigate AF exposure do not take in effect.

Previous studies were mainly conducted in Makueni County due to past aflatoxicosis outbreaks linked to maize grain contamination by AFs (Lewis et al. 2005; Daniel et al. 2011). In a study conducted in Kibwezi of Makueni County, 45% of the households consuming maize kernels were exposed to AFs at levels ranging from 18 to 480 µg/kg (Kilonzo et al. 2014). A 3-year (2005–2007) cross-sectional survey conducted in Makueni and Kitui counties found that the overall geometric mean of AFs was 17.8 µg/kg in household maize samples (Daniel et al. 2011). In a different survey, AFs contamination levels were found in maize samples with the range of 0.98–722 µg/kg (Mahuku et al. 2019). In the current study, the total AFs ranged from 2.14 to 411 µg/kg.

Table 4. Distribution of total AFs in maize products from rural Kenya.

Mais	Number of samples in the range (µg/kg)						
Matrix	<9.9	10.0–19.9	20.0–39.9	40.0–59.9	60.0–79.9	80.0–99.9	>100
Flour	4	8	38	35	24	20	45
Kernels	1	3	19	36	39	28	39
Total	5	11	56	71	63	48	84

In another regional survey conducted in six additional counties of Kenya, AFs contamination was detected in 49% of the maize flour samples with reported ranges of 2.0–710 µg/kg, and higher levels of AFs contamination were found in purchased maize compared to household maize (Mutiga et al. 2015). In Makueni County, however, AF contamination is often higher in home-grown maize compared to maize bought from vendors at the market (Daniel et al. 2011; Kilonzo et al. 2014). During aflatoxicosis outbreak years, AFs contaminations up to 48,000 µg/kg in 2005 and 24,400 µg/kg in 2006 were reported in household maize samples (Azziz-Baumgartner et al. 2005; Lewis et al. 2005; Daniel et al. 2011).

The findings of the current study show very high AF contamination levels in household maize for human consumption. To date, there is no documented evidence of any aflatoxicosis cases in Siaya County, and thus no documented surveillance studies from this region. While none of the surveillance studies previously conducted in Kenya reported AF contamination of maize products from Siaya County, this study reports contamination levels of more than five times the maximum limit of 10.0 µg/kg (East African Community 2013). This study shows that rural households should take careful consideration on how maize products are stored to prevent AFs contamination. The higher contamination levels found in Makueni County can be attributed to severe flooding in the region during the growing season when sampling took place. This could also be an indication of the limited capacity of small-scale holder farmers to adapt to climate change (Famine Early Warning Systems 2018; Muema et al. 2018). Higher AFs contamination has also been reported in Serbia's maize-growing regions and Europe due to erratic weather and potential effects of climate change (Battilani et al. 2016; Janic Hajnal et al. 2017; Kos et al. 2018).

AFs contamination is highly variable by region. In a countrywide serological survey, Yard et al. found that the highest AFs exposure levels were reported in Eastern Province where Makueni County is located whereas in Nyanza Province where Siaya County is located, AFs exposure levels in human populations were below the limit of detection (Yard et al. 2013). In the current study, 19% (18/96) of the samples collected from Siaya County were maize kernels and/or flour mixed with either sorghum, millet and/or cassava which tend to have lower AFs contamination levels compared to maize (Sirma et al. 2015). In Makueni County, however, 100% of the samples were pure maize kernels and/or pure maize flour. Moreover, Siaya's climatic conditions are favourable for the cultivation of other grains compared to Makueni, an indication of opportunities to access diversified diets especially in Siaya (CIAT 2016).

AFs contamination often starts when the crops are still in the field. Mahuku et al. conducted a study to assess the prevalence of AFs contamination in physiologically mature maize from farms in six counties of Kenya (Mahuku et al. 2019). The pre-harvest maize was found to be contaminated by AFs with the highest levels of contamination found in Embu (196.3 ± 1202 µg/kg) and Makueni (39.0 ± 132 µg/kg) counties. The mean AFs contamination levels in Kisii (28.5 ± 72.7 µg/kg) and Homabay counties (24.5 ± 94.9 µg/kg) were more than two times the maximum limit while the lowest levels were found in Machakos (10.5 ± 16.5 µg/kg) and Migori (12.7 ± 24.9 µg/kg) counties (Mahuku et al. 2019). In a different study, pre-harvest maize samples collected from Kakamega and Bungoma counties were contaminated with AFB_1 levels below the maximum limit of 10 µg/kg, with the highest recorded level of 17 µg/kg (Alakonya et al. 2009). The low AFs levels could be attributed to heavy rainfall and limited crop stress in the maize-growing region of Kakamega and Bungoma Counties (CIAT 2016). These studies show that AF contamination is present before the maize is harvested and good post-harvest strategies should be implemented to prevent further accumulation of AFs.

AFs contamination is common in most households in rural Africa and presents a significant risk to food security. In the current study conducted in June and July of 2018, freshly harvested maize were sampled from Makueni and Siaya Counties for analysis. AFB_1, the most potent aflatoxin, contributed up to 97.1% of the total AFs. A high prevalence of the novel S-morphology *A. flavus* has been shown to be dominant in Kenya and is known to produce high concentrations of AFB_1 (Probst et al. 2007; Mutegi et al. 2018). In a different study conducted in Tunisia, 85.7% of the maize samples were contaminated with AFGs, which suggests the involvement of *A. parasiticus* fungi, whose occurrence was confirmed by species-specific polymerase chain reactions (Jedidi et al. 2017).

Several solutions have been recommended and used to mitigate AFs contamination. In Kenya and other sub-Saharan African countries, the International Institute of Tropical Agriculture (IITA) is championing the use of AflaSafe, a form of biological control to mitigate AFs contamination of maize before harvest (Bandyopadhyay et al. 2016). In addition to preharvest prevention of AFs contamination, post-harvest methods such as effective sorting, proper drying, and storage that limits AFs producing fungi to thrive are effective strategies in controlling AFs contamination (IARC Working Group Reports 2015;

Bandyopadhyay et al. 2016). Manual sorting is commonly practised in Kenya, but subsistence farmers tend to sell healthy-looking kernels and store the discoloured kernels for own consumption (Bandyopadhyay et al. 2016). This increases the predisposition of consuming aflatoxin-contaminated maize in households. Moreover, industries that perform automatic sorting use discarded kernels to manufacture animal feed which ultimately gets in the food chain through consumption of animal products such as milk and meat. Effective control of AFs contamination of food supplies is resource intensive and will require expertise that may not be available for small-scale holder farmers in most of the developing world.

Conclusions

In the current study, the presence of AFs in food products in households indicates an elevated risk of exposure to populations in Siaya and Makueni Counties. Due to the widespread nature of AFs contamination in Kenya, it is important to educate small-scale holder farmers on the prevention and mitigation of AFs contamination. This should be done in addition to good agricultural practices combined with frequent surveillance.

Acknowledgments

We thank the county governments and communities in Siaya and Makueni counties for supporting this study. We also thank all participating school administrations and field assistants without whom this work may not be possible.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Fieldwork and data collection was supported through small grants from the University of Georgia namely Interdisciplinary and Innovative Research Grant and the Tipton Golias Travel Award. Laboratory analyses were supported by grant ECG-A-00-07-00001-00 from United States Agency for international Development Feed the Future Program Peanut and Mycotoxin Innovation Laboratory. The funders had no role in the design of the study, data collection, laboratory analyses, manuscript draft, data interpretation and the decision to publish results.

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