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PREVALENCE OF AFLATOXINS IN DIETARY STAPLES IN THE BORDER COUNTY OF BUSIA, WESTERN KENYA

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ABSTRACT

Aflatoxins, secondary metabolites of some *Aspergillus* fungi, are of public health importance. They are major contaminants of cereals and tubers. Data on prevalence of aflatoxin contamination of sorghum, millet and cassava in Busia County are limited. The extent of aflatoxin contamination in dietary staples in Busia County were assessed and potential sources associated with the contamination evaluated. A tool designed to collect sociodemographic profile, food sources and storage locations and vessels and food consumption habits of respondents was loaded onto an Open Data Kit and used in 3 sub-counties. Quantitative data were analyzed using SAS version 15 software. Maize, millet, sorghum, cassava and groundnut samples were collected from 469 households. Competitive Enzyme-linked Immunosorbent Assay method was used to determine total aflatoxin levels in food samples. Sixty-eight percent of the maize samples were sourced from the market. Approximately 75% of maize samples were stored in polypropylene sacks. Samples of all five foods had detectable levels of aflatoxin. Overall, maize had the highest level of contamination (mean 100 ppb; SD 252.9; range 1-1584 ppb) with about a third of maize samples above the East African Community regulatory limits (10 ppb). The levels of aflatoxin ranged from 0.3 to 740 ppb in sorghum, 0.5 to 15 ppb in cassava, from 0.5 to 12 ppb in millet and from 0.1 to 2.8 ppb in groundnuts. The odds of contamination above 10 ppb for market-sourced maize was 1.2 times higher than home-grown maize (OR 1.185, CI 0.554, 2.534). Sorghum stored in buckets had a 12.81 likelihood of having higher than allowable limits of aflatoxin (OR 12.82, CI 2.566, 63.992) than when stored in polypropylene sacks. Aflatoxin is prevalent in the dietary staples consumed in households within Busia County. Residents are at risk of chronic exposure to aflatoxin. Enhanced market surveillance within the county is recommended.

Key words: Aflatoxin, Busia County, Household survey, Kenya, Dietary Staples

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INTRODUCTION

Aflatoxins are harmful fungal metabolites produced mostly by *Aspergillus flavus* and *A. parasiticus* [1]. These molds are prevalent in the tropics, around the equator and are major contaminants of cereal-grains and roots at several stages of food production [2]. They are ubiquitous in the environment but high temperatures and humidity, unseasonal rains during harvest, and improper harvesting and storage practices combine to create conditions suitable for fungal growth resulting in aflatoxin contamination pre- and post-harvest [3].

Globally and nationally, foodborne diseases like aflatoxicosis are important causes of morbidity and mortality as has been reported in Kenya and lately in Tanzania [4, 5]. Aflatoxins compromise the safety of food and exacerbates food insecurity. Of the major agricultural products, maize and groundnuts are more susceptible to aflatoxin colonization as demonstrated by findings from some studies previously conducted [6, 7, 8]. Maize is the main staple in Kenya with an average daily consumption rate of 258 g/person [9]. Aflatoxin is also prevalent in sorghum, millet and groundnuts in parts of the country [10, 11, 12]. Reported estimated daily groundnut consumption rate is 1.1 g/person [13].

Kenya has previously experienced a number of aflatoxicosis outbreaks in Makueni and Kitui in Eastern Province, with high case fatality rates of 39%, attributed to ingestion of contaminated maize. Aflatoxin levels of food samples from case households were as high as 4000 ppb [14, 15, 13]. Additionally, findings from a previous Center for Disease Control and Prevention Aflatoxin sero-survey also showed 100% exposure in sampled population from the Busia region, an indicator of aflatoxin contamination of the dietary staples [16]. One study has reported an aflatoxin prevalence of 7.5% in groundnuts in Busia [10]. Findings from research from other regions both regionally and in country have revealed that poor post-harvest grain management practices can lead to contamination of grains as contamination is exacerbated by high humidity, lack of aeration in storage areas and prolonged storage time with negative impacts on food safety and trade [17, 13]. Data on the prevalence of aflatoxins in staple foods are essential to understand their impact on health and to map out effective mitigation strategies [18]. Region specific knowledge enables the identification of susceptible edible crops that are responsible for exposure to toxins in specific populations. While the likelihood of contamination of many food commodities with aflatoxin remains high, research efforts addressing the aflatoxin problems in Kenya have focused mainly on maize (the staple food) [19]. The aim of this study was therefore to determine the prevalence of aflatoxin contamination in selected food staples of three sub-counties in Busia County and to establish some potential sources of the contamination.

METHODS

Study site and population

The study was conducted in Busia County, one of the four counties in the Western region of Kenya. Busia County lies approximately 431 km, northwest of Nairobi, Kenya's capital city. The County has two border crossing points into Uganda (Busia and Malaba

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towns) (Fig 1). Busia County is a major trading location that accounts for substantive trade between the two East African countries of Kenya and Uganda. The primary economic activities in Busia County are cash crop and subsistence farming, fish farming, trade in farm produce and artisanship. Figure 1 below provides an illustration of the study area.

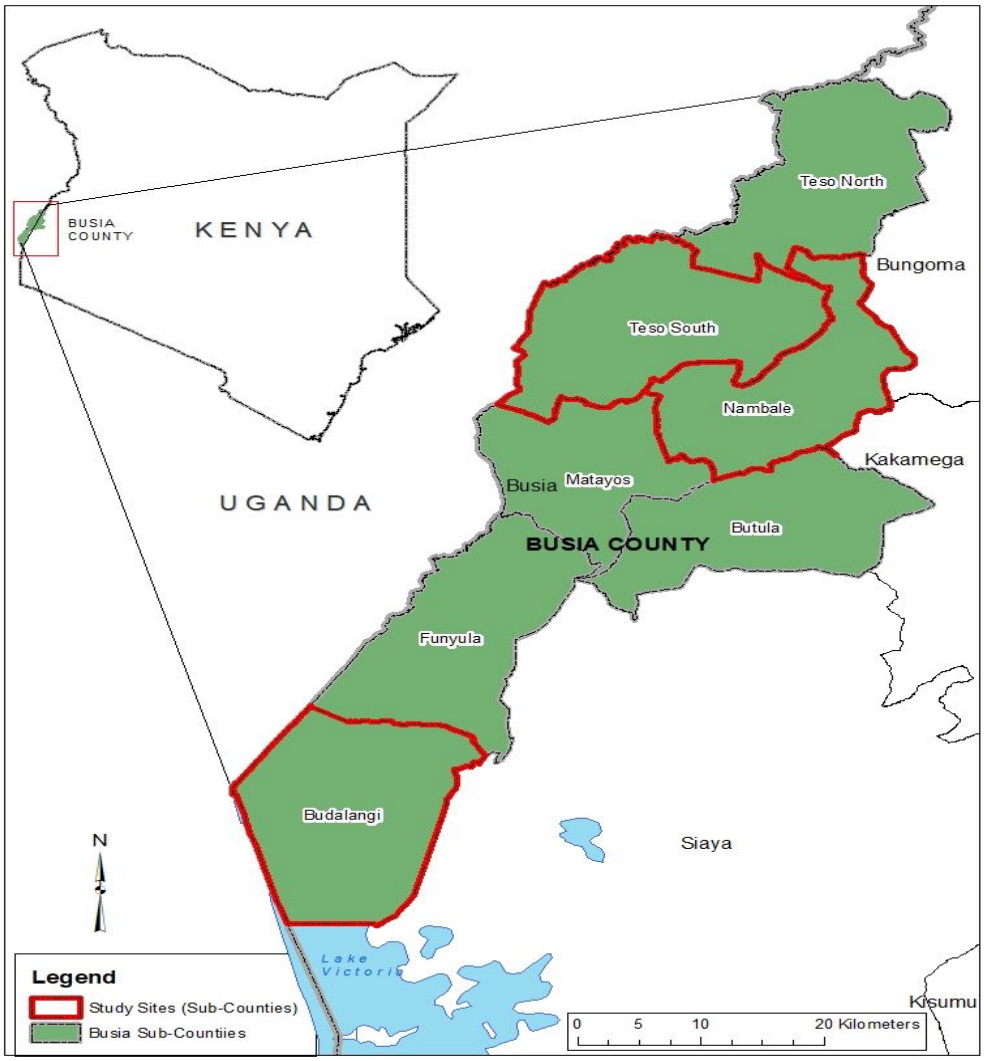

Figure 1: Aflatoxin study sampling areas within Busia County

Study design and site selection

A cross-sectional household survey was conducted during a one-month period (June 2018) in 3 sub-counties within Busia County. The sampling method was guided using a 3-stage cluster sampling design using Chromy's sequential sampling in SAS, version 15 (Statistical Analysis Software Institute, Cary, NC, USA) [20] . First, 3 sub-counties were randomly selected from a total of 7 sub-counties. The smallest sub-areas for which population data was available was the sub-location [21]. Second, 4 locations namely Bukhayo East, Bunyala Central, Amukura and Ochude and 4 of 70 sub-locations namely,

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Okiludu, Amukura, Buyofu and Magombe East were randomly selected. Locations, sub-locations, villages and households were distributed based on probability proportional to the size (PPS).

The sample size was determined based on reported aflatoxin prevalence of 7.5% in groundnuts in Busia and 17.5% and 44.9% in groundnuts and maize respectively in Uganda [22, 10]. Since maize had a 2.5-fold higher prevalence of contamination compared to groundnuts, a 19% prevalence was assumed. A 19% frequency of outcome factor was hypothesized, with 95% confidence limits and a design effect of 2.

Sample size (n) = [DEFF*Np(1-p)]/ [$(d^2/Z^2_{1-\alpha/2})$*(N-1)+p*(1-p)] [23]

Where,
N= Total population (893,681)
P = Probability of exposure/contamination in the population (19%)
DEFF = Design effect for cluster survey (2)
d = Absolute precision on either side of the proportion (95%)
$Z_{(1-\alpha/2)}$ = 1.96
The calculated sample size of 472 was obtained.

Household selection and enrolment
Households were identified using stratified systematic sampling. The total number of households was divided by the sample size to get a sampling interval of 6. Sampling in each sub-location started at the households closest to the central landmark, which was identified with guidance from the village elder, and community health volunteers. Four teams sampled households simultaneously moving towards the four cardinal directions. Vacant households at the time of visit or potential respondents who declined to participate were replaced with the next closest household.

Inclusion criteria included households with a respondent (≥18 years) who had information on food sources and food preparation practices present at the time of the survey and households with respondents whose weekly diet comprised of ≥4 meals prone to aflatoxin contamination (maize, groundnuts, sorghum, millet or cassava).

Data and food sample collection
At the household, geographical coordinates were captured using Open Data Kits (ODK) after obtaining informed written consent. Data was collected using a pre-tested structured questionnaire installed on the ODK. The questionnaires were organized in the following 4 sections (1) socio- demographic profile; (2) food source and storage practices; (3) food consumption; (4) food preparation methods availability. From each household, at least one representative food sample weighing 250 – 500 g was collected. This was done by taking grab samples from top, middle and bottom of food storage bag or container then mixing the total to get a homogeneous sample of the whole. In order to control moisture content, paper bags were used for sample transportation.

When assessing food source and storage factors associated with aflatoxin contamination, only (i) samples collected from households whose respondents provided information on

food source and storage practices and (ii) samples that had a proportion with aflatoxin levels above 10 ppb, were considered. Food samples were categorized as aflatoxin contaminated and not fit for human consumption if they had above 10 parts per billion (ppb) the East African regulatory limit for aflatoxins in grains and having detectable aflatoxin if they had >0.1 ppb (the lowest detectable level and negative if less than 0.1 ppb). Frequencies of various types of food samples above this limit by food sources, sub location and storage vessels were calculated

Determination of levels of Aflatoxin

Total aflatoxins in the food samples were analyzed with enzyme-linked immune-sorbent assay using a commercial competitive ELISA (Helica Biosystems' Total aflatoxin kits LOT No. AF102815, CAT No. 941AFL01M-96) according to the manufacturer's instructions.

Data was exported from ODK to Access and analyzed using Statistical Analysis System (SAS) version 9.4. Study household data were analysed descriptively. Bivariate analysis was performed to determine the association between a food sample being contaminated with aflatoxin (above 10 ppb) as the outcome of interest and demographic characteristics and exposure factors such as source of food and vessels used for food storage. Data was weighted to account for the survey sampling design. SAS procedures accounted for multi-stage stratified sampling designs producing reliable standard errors and confidence intervals. Odds ratios and 95% confidence intervals (CIs) were computed with a P-value of < 0.05 considered. Getis- Ord Gi* tool within ArcGIS software was used for hot-spot analysis ([24, 25] and thematic maps were used to present the data.

Ethical approval was obtained from the Kenyatta National Hospital-University of Nairobi Ethical Review Committee (Ref: KNH-ERC/A/114).

RESULTS AND DISCUSSION

Description of Study population and demographics

Of the 469 households that met the inclusion criteria from 3 sub-counties in Busia County, 23% were in Budalang'i, 29% in Nambale and 48% in Teso South. The socio-demographic characteristics of respondents (n=469) are presented in Table 1. The median age of respondents was 43 years (range, 20-93 years), and majority (99%) were females (n=457). Among the respondents, majority, (61%) had completed pre-primary school education, about a third (27%) had completed primary level education while only 1.5% had completed college education. Lowest levels of literacy were observed in Budalang'i sub-county while the highest were in Nambale sub-county. The distribution of the household members was relatively similar across the 3 sub-counties.

Description of samples and associated levels of Aflatoxin

Food samples collected (n=493) comprised of maize (230), cassava (99), millet (43), sorghum (41), groundnut (32), and composite samples (a blend of one or more of the foods (48). Aflatoxin was detected in all maize, sorghum, millet, cassava and groundnuts samples. These findings indicate pervasive contamination of the county's dietary staples during the time of this study. This extensive contamination indicates that residents of

Busia County are chronically exposed to aflatoxin through their dietary staple. This could also be a possible explanation of the reported 100% exposure to aflatoxin in a sero-survey among humans from this region of Kenya in 2007 [16]. This population is at risk of negative health effects like hepatocellular carcinoma and stunting associated with aflatoxin exposure [26]. To the best of the authors' knowledge, this is the first study to document the incidence of aflatoxin contamination in maize, sorghum, cassava and millet in this sub-county.

Contamination was relative across food types. The levels of contamination ranged from 1.0 to 1584 ppb in maize, 0.3 to 740 ppb in sorghum, 0.5 to 15 ppb in cassava, 0.5 to 12 ppb in millet and 0.1 to 2.8 in groundnuts. Contamination was highest in maize followed by sorghum, cassava, millet and least in groundnut samples. The proportion of maize contaminated with aflatoxin >10 ppb from Budalang'i, Nambale and Teso South was 3%, 22% and 37% respectively. Median aflatoxin levels in maize from Nambale was 231.7ppb (n=59), Budalang'i 3.5ppb (n=63) and Teso-South 228.5ppb (n=108) (Table 2).

Of the 41 sorghum samples collected, only 4 (10%) had levels of aflatoxin >10ppb. All 4 samples were from Budalang'i. Notably, only one millet (n=43) and one cassava (n=99) sample had aflatoxin contamination above 10ppb, while none of the groundnut samples (n=32) had aflatoxin contamination above 10ppb in all the three sub-counties.

All the 48 composite/blended samples had detectable levels of aflatoxin. However, only 3 blends: Maize and cassava blend (67%, n=3); cassava and sorghum blend (11%, n=13); and maize and sorghum blend (33%, n= 3) had contamination levels above 10 ppb. All the blended samples with levels of >10 ppb were from Nambale and Teso-South sub-counties (Table 3).

Relative severity of aflatoxin contamination of cereals observed is consistent with a study in West Africa that established that maize was more prone to aflatoxin colonization than groundnuts, sorghum and millet [7]. Additionally, the detection levels in maize samples in this study are much higher than those reported in Bungoma (45%), Homa Bay (66%) and Rachuonyo (93%) [27]. While aflatoxin quantity in food may not translate to exact exposure levels, based on the findings in this study, exposure through maize posed the greatest public health risk given the daily frequency consumption rate of 258g/person and high aflatoxin levels when compared to exposure through groundnuts, millet, sorghum and cassava [9, 13]. Surprisingly, while all the groundnut samples had detectable levels of aflatoxin, none were above the Kenya Bureau of Standards (KEBS) (10ppb) and all were within the European Union (EU) regulatory limit of 4 µg/kg. This differs from findings by Mutegi *et al.* [24] where 37.3% of groundnuts from Busia were contaminated though 87% were within the EU limits [28]. It is possible that the post-harvest management practices of groundnuts have improved in these study sites.

In this study, contamination varied widely among the maize samples (range 1.0 - 1584ppb). These levels are comparable to some levels reported in Eastern Kenya, in some of the regions where outbreaks of aflatoxicosis were reported [29, 15]. Lewis *et al.* [15] found 55% of maize from markets in Kitui, Machakos and Thika, with aflatoxin

levels greater than the then regulatory limits of 20ppb. In addition, the aflatoxin levels ranged from 1ppb to 46,400ppb. The aflatoxin levels in maize reported in this study are much higher when compared to another study conducted in 3 sub-counties in Nandi county which reported aflatoxin levels in maize (range 0.17-5.3ppb) [30]. While 100% of maize samples in this study had detectable levels of aflatoxin, only 67.9% of the samples in Nandi were positive for aflatoxin. Here, the highest level of aflatoxin in millet was 12 ppb against 6.4 ppb while sorghum was 15-fold higher at 740 ppb compared to 48.36 ppb in the Sirma study [12]. This would suggest that maize is the main source of exposure followed by sorghum in Busia but also that residents of Busia are more exposed to higher levels of aflatoxin compared to residents in Laboret, Kilibwoni and Chepkongony sub-locations of Nandi.

Factors associated with aflatoxin contamination

Food Source and storage

It is noteworthy that while this study was a household survey, almost two-thirds (68%) of the maize samples had been sourced from the local markets. Majority (68%, n=138) of the maize samples were bought from the local markets, whereas 29% (n=59) were home- grown and 3% (n= 6) had been gifted by relatives. Market sourced maize had a more likelihood (OR1.185) of having levels of aflatoxin above regulatory limits (10ppb) compared to homegrown maize, however, this association was not statistically significant. These findings are similar to those reported by Mutiga *et al.* [23] who observed significantly less contamination among home grown maize when compared to purchased maize from Nyanza region [27]. Contamination of maize in the market could be a result from poor post-harvest handling practices at source or exposure to humid conditions in storage or storage in poorly aerated containers and spaces at the stores in the market or prolonged storage periods. This would suggest that most contaminated maize could have been more of a grain management issue at the market. Indeed, high prevalence of aflatoxin in market samples have been reported in Burundi and Eastern Kenya [15, 31]. These findings differ with those reported during the 2004 aflatoxicosis outbreak investigations which found homegrown maize as the primary risk factor for developing aflatoxicosis [4]. These findings would also suggest that if the causes of contamination are not urgently addressed, there is a high likelihood of contamination to be exacerbated there by resulting in rejection of the market produce by potential buyers or lots being sold at lower prices. Reduction in prices of maize will also affect the market sellers, financially. A divergent observation was noted for sorghum. Further shown is a non-significant association between the source of maize or sorghum and the level of aflatoxin (*P* value = 0.076 for maize, and 0.6821 for sorghum). Almost three quarters of the maize was stored in a polypropylene sack (n =137). Of these, 35.7% had aflatoxin levels more than 10ppb, while maize stored in a bucket had 25.7% levels above the allowable limit. The odds of getting a high level of aflatoxin in maize stored in polypropylene sacks was 1.6 times higher than the odds of high level of aflatoxin in maize stored in buckets; however, this association was not significant (Table 4).

There were clusters with high aflatoxin contamination values ($p<0.05$) in 2 of the 3 sub-counties; Teso- South (Okiludu and Amukura sub-locations) and Nambale (Buyofu sub-location). Most of these clusters were in Okiludu sub-location, in villages closer to the border with Otimong' sub-location of Teso South (Figure 2).

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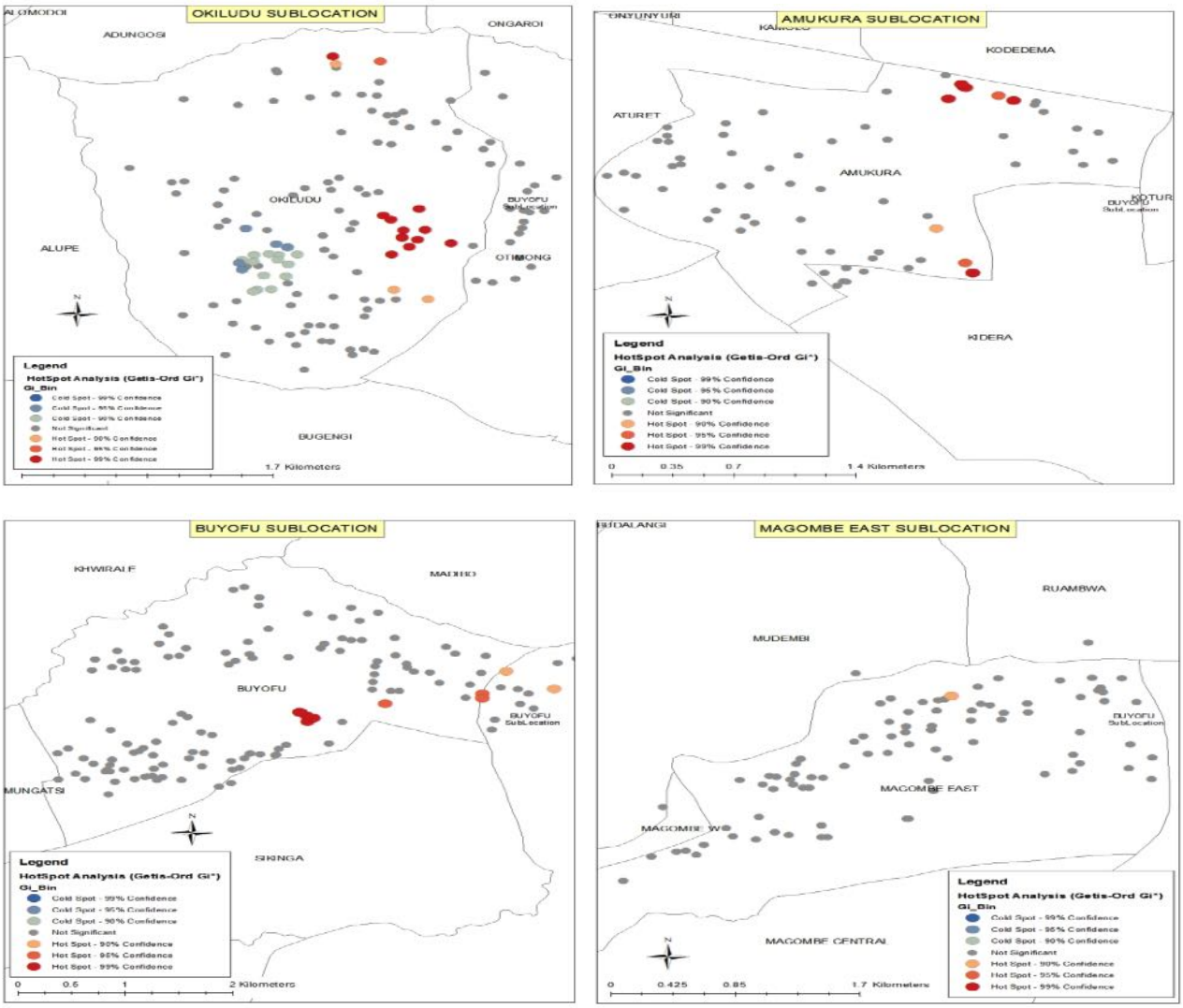

Figure 2: Aflatoxin hotspots within Busia County in June 2018

There were several outliers in Teso-South and some in Nambale sub-county with disproportionately high levels of aflatoxin in food samples. The outliers could be indicative of possible hot spots (99% CI). This study provides, for the first time, a general outlook of the state of aflatoxin contamination of the main staples in Busia County. These high levels of aflatoxins when consumed by humans are likely to result in acute exposure. Negative health effects because of chronic exposure to aflatoxin have been reported [26]. This data only gives a snap-shot of possible hotspots in one month during the same season. It gives an added analytical value to the findings from the laboratory tests. This spatial pattern analysis assists in consistent identification of priority areas of both agricultural and health management interventions.

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This study had one limitation. Data on market storage conditions were not collected because data was collected at household level thus levels of aflatoxin contamination in market sourced grains could not be correlated with storage conditions at the store.

CONCLUSION

The present study was designed to investigate the prevalence of aflatoxin in staple grains and tubers in Busia County. It is now evident that aflatoxin contamination of staples in the county is prevalent but the severity of contamination varies across the various food types. The results have shown that maize was most contaminated with over quarters of the samples having levels above Kenya Bureau of Standards regulatory limit. Most grains, maize, sorghum, millet and groundnuts are sold through informal marketing systems countrywide thus are not monitored for aflatoxin by the local regulatory authorities in Busia County. While aflatoxin is an unavoidable nuisance, maximum tolerable levels need enforcement. This study provides, for the first time, a general outlook of the state of aflatoxin contamination of the main staples in Busia County. Aflatoxin contamination hotspots were identified. This data only gives a snapshot of the hotspots in one month during the same season. It gives an added analytical value to the findings from the laboratory tests. This spatial pattern analysis assists in consistent identification of priority areas of both agricultural and health management interventions.

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Declaration of competing financial interest

The authors declare they have no actual or potential competing financial interest.

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Table 1: Demographic characteristics of study participants by sub-county, Busia county, 2018

		Budalang'in (%)	Nambalen (%)	Teso-South n (%)	Total N (%)
	Total no. of respondents	108	136	225	469
Sex	Female	101(93.5)	134(98.5)	222(98.7)	457(97.4)
	Male	7(6.5)	2(1.5)	3(1.3)	12(2.6)
Age	Median (IQR)	50.5 (29)	40 (22.5)	42 (23)	43 (26)
	Range	[21, 90]	[20, 85]	[20, 93]	[20, 93]
Ethnicity	Bantu	105(97.2)	114(83.8)	36(16)	255(54.4)
	Nilotes	3(2.8)	4(2.9)	36(16)	43(9.2)
	Nilo-Hamites	0(0)	18(13.2)	153(68)	171(36.5)
Education Level*	Pre-primary	73(67.6)	74(54.4)	140(62.2)	287(61.2)
	Primary	21(19.4)	44(32.4)	62(27.6)	127(27.1)
	Secondary	4(3.7)	13(9.6)	19(8.4)	36(7.7)
	College and above	1(0.9)	4(2.9)	2(0.9)	7(1.5)
	Refused	9(8.3)	1(0.7)	2(0.9)	12(2.6)
Household Members	Median	5	6	6	6
	Range	[1, 10]	[2, 13]	[1, 13]	[1, 13]

** Level of education completed by the respondent*

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Table 2: Distribution of Aflatoxin levels in food samples from study households by food type and sub county, Busia 2018

Site	Food type	% >10ppb	Median (ppb)	Range (ppb)
Budalang'i	Cassava (n= 7)	0	0.5	0.5 – 7.5
	Groundnuts (n=0)	-	-	-
	Maize (n= 63)	2.9	3.5	1.0 – 1584
	Millet (n= 10)	1	1.5	0.5 – 12
	Sorghum (n= 23)	1.7	2.0	0.5 – 740
Nambale	Cassava (n= 47)	2.1	2.6	1.0 – 15
	Groundnuts (n= 25)	0	0.8	0.1 – 2.8
	Maize (n= 59)	22	231.7	3.0 – 1456
	Millet (n= 7)	0	0.6	1.0 – 2.0
	Sorghum (n= 4)	0	1.3	2 – 3.5
Teso-South	Cassava (n= 45)	0	0.6	1.0 – 3.5
	Groundnuts (n= 7)	0	0.7	0.5 – 2.1
	Maize (n= 108)	37	228.5	3.5 – 1432
	Millet (n= 26)	0	0.6	1.5 – 2.5
	Sorghum (n= 14)	0	1.2	2.0 – 5.5

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Table 3: Distribution of Aflatoxin levels in composite/blended food samples, Busia 2018

Sub-county	Composite/blended sample	% samples >10ppb	Median	Range
Budalang'i	cassava and sorghum flour (n=2)	0	2.0	2.0 – 2.0 [a]
	maize and sorghum flour (n=7)	0	3.5	1.0 – 8.0
	maize and cassava flour (n=0)	-	--	--
	maize, sorghum and cassava flour (n=2)	0	5.5	2.0 – 9.0
	sorghum and millet flour (n=0)	-	--	--
	cassava and millet flour (n=1)	0	1.5	1.5 – 1.5
	maize and millet flour (n=1)	0	1.0	1.0 – 1.0
	maize, millet and cassava mixed flour (n=2)	0	1.5	1.5 - 1.5
Nambale	cassava and sorghum flour (n=4)	0	0.5	1.8 – 2.5
	maize and sorghum flour (n=0)	0	--	-
	maize and cassava flour (n=3)	67	317.3	330 – 644.0
	maize, sorghum and cassava flour (n=2)	100	1030.3	743 – 1472.0
	sorghum and millet flour (n=0)	-	--	-
	cassava and millet flour (n=0)	-	--	-
	maize and millet flour (n=1)	0	3.0	3.0 – 3.0
	maize, millet and cassava mixed flour (n=0)	-	--	
Teso-South	cassava and sorghum flour (n=13)	11	60.5	3.5 – 195.0
	maize and sorghum flour (n=3)	33	98.6	1.5 – 172.0
	maize and cassava flour (n=2)	0	0.4	1.8 – 2.0
	maize, sorghum and cassava flour (n=0)	-	--	-
	sorghum and millet flour (n=4)	0	0.9	1.8 – 2.5
	cassava and millet flour (n=1)	0	0.5	0.5 - 0.5
	maize and millet flour (n=0)	-	--	-
	maize, millet and cassava mixed flour (n=0)	-	--	-

[a]Lower limit of detection is 0.1ppb

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Table 4: Distribution of Aflatoxin levels in by source and storage, Busia 2018

Food type	Source	≤10ppb n(%)	>10ppb n(%)	OR (95% CI)	*P* - Value
Maize	Home-grown (n=59)	45 (72.65)	14(27.35)	Ref	.0760
	Market (n=138)	99 (69.15)	39 (30.85)	1.185(0.554, 2.534)	
	Other* (n=6)*	1(5.95)	5(94.05)	41.977 (1.708, >999)	
Sorghum	Home-grown (n=14)	13 (94.83)	1(5.17)	1.129(0.550, 2.316)	.6821
	Market (n=13)	12 (94.2)	1(5.80)		
	Storage				
Maize	Polypropylene sack (n=137)	96 (64.27)	41(35.73)	1.611(0.642, 4.042)	
	In bucket (n=55)	39 (74.35)	16(25.65)	Ref	.2398
Sorghum	In Polypropylene sack (n=25)	24(97.06)	1(2.94)	Ref	.0096
	In Bucket (n=3)	2 (72.03)	1 (27.97)	12.815(2.566 63.992)	

*These were described as either having been received from a relative or neighbour

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