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Research Paper

PREVALENCE OF AFLATOXIN CONTAMINATION IN CEREALS FROM NANDI COUNTY, KENYA

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Aflatoxins are secondary fungal metabolites that contaminate cereals, other crops and animal source foods and are a recognized health risk. Kenya has had several outbreaks of aflatoxicosis affecting humans and pets. The aim of this study was to compare aflatoxin levels in cereals, the staple diet of Kenyans, in Nandi where aflatoxicosis has not been reported despite being a maize growing area. Maize, sorghum and millet were sampled from households and also from markets serving various villages in the selected sub-locations (408 samples). The samples were tested for total aflatoxin contamination using cELISA. Households in the study sub-locations depended on homegrown grains than market sourced. Sixty seven point nine percent (72/106), 73.3% (44/60) and 65.7% (67/102) of maize samples collected from Laboret, Kilibwoni and Chepkongony were contaminated with aflatoxins ranging between 0.17-5.3 parts per billion (ppb). Ninety two point nine percent (13/14), 100% (9/9) and 87.5% (14/16) of millet samples from Laboret, Kilibwoni and Chepkongony were positive for aflatoxin at a range of 0.14-6.4 ppb. Fifty percent (9/18), 36.4% (8/22) and 27.3% (6/22) of sorghum samples from Laboret, Kilibwoni and Chepkongony, respectively were contaminated with aflatoxins beyond Kenya Bureau of Standards (KEBS) maximum tolerable limits of 10 ppb. To manage aflatoxin contamination of the cereals in Nandi, the county government needs to step up awareness creation of the dangers posed by chronic aflatoxin exposure to households through cereals and promote good Agricultural practices.

Keywords: Mycotoxin, Aflatoxins, Cereals, Occurrence, East Africa

INTRODUCTION

Aflatoxins are a group of highly poisonous compounds produced in a variety of substrates

by moulds of the species *Aspergillus flavus* and *A. parasiticus*. These moulds grow in foods and feeds under suitable conditions of humidity and

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temperature. They produce aflatoxins as secondary metabolites (meaning, that aflatoxins are not needed for mould metabolism). Ingestion of aflatoxins is the primary source of exposure for humans and animals. There are 4 main aflatoxins commonly encountered in foods/feed namely: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) are metabolites of AFB1 and AFB2 respectively that can occur in milk and milk products from animals/humans consuming feeds/foods contaminated with the B aflatoxins (Applebaum *et al.*, 1982). The foods most at risk for aflatoxin contamination are maize, groundnuts and tree nuts (Bennett and Klich, 2003).

Aflatoxins when ingested cause toxic effects in humans and animals. Depending on the dose, effects are acute or chronic. Several outbreaks of aflatoxicosis have occurred in Uganda, Kenya, and India (Azziz-Baumgartner *et al.*, 2005; Kaaya and Warren, 2005; and Daniel *et al.*, 2011). Kenya has had one major outbreak in 2004 in then Eastern province which caused deaths of 125 persons and 317 reported cases. During that outbreak, market maize was found to be contaminated with aflatoxins at levels ranging 1 to 46,400 ppb (Lewis *et al.*, 2005). The maximum allowable limit in foods and feeds by the Kenya Bureau of Standards is 10ppb and 20ppb respectively (KS EAS 2:2005).

Other than being highly toxic, aflatoxins, are mutagenic, teratogenic and carcinogenic (Massey *et al.*, 1995). Aflatoxin B1 is classified as a class 1 human carcinogen by the International Agency for Research in Cancer (IARC, 2002). Chronic exposure has been linked to the development of liver cancer in humans, which is ranked as the second highest cause of

cancer death worldwide (WHO, 2014). In Kenya, estimates for hepatocellular carcinomas associated with aflatoxin range from 1.05 to 39.9 persons per 100,000 (Liu and Wu, 2010). Stunting in children has also been associated with aflatoxin exposure (Gong *et al.*, 2002).

The aim of the study was to determine the level of contamination of maize, sorghum and millet with aflatoxin from households and markets in Nandi county.

MATERIALS AND METHODS

The sampling was carried out in Nandi county specifically in Nandi north, central and south districts of Kenya between October and December 2010. Nandi county is in the Rift valley province of Kenya, an area that has experienced incidences of esophageal cancer which is associated with fumonisin mycotoxin, a co-contaminant of maize with aflatoxins. This study was done in Laboret, Kilibwoni and Chepkongony sub-locations representing Nandi north, central and south districts respectively. The study sites were purposively selected because of their high maize growing activity and dairy keeping. We acquired ethical approval for the study from the Kenya National Council for Science and Technology.

Household Selection

Stratified random sampling was the method of household selection whereby the sub-locations within the districts were the sampling units. Within each sub-location a list of farmers growing maize and either sorghum or millet formed the sampling frame. Using Rand Between function in MS Excel® random numbers were generated and assigned to each household. The sample size of households was calculated using a proportion of 71% (estimated proportion of an attribute that is

present in the population), level of confidence 95% and desired level of precision 5%. The following formula was applied (Dohoo *et al.*, 2009):

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where

n = Sample size

Z = Z statistic for a level of confidence

P = Estimated proportion of an attribute that is present in the population

d = precision

The number of farmers sampled in each sub-location was proportionally allocated. A total of 261 farmers were sampled and interviewed.

Collection of Samples

From each household, about 500 g each of maize, millet and sorghum were collected in paper bags and kept at room temperature to be analyzed within three months of collection. Households which did not have home grown grains at the time of collection indicated their source of maize, millet and sorghum where we purchased from the specified market.

Analysis of Cereal Grains for Total Aflatoxins

Sample Preparation

Determination and quantification of total aflatoxin content in the samples was done using competitive ELISA (Ridascreen® test Kit). Sample preparation and analysis was carried out as per the manufacturer's protocol. Maize, sorghum and millet samples were ground using a grinder (Grindomix® GM200 knife mill, Retsch GmbH, Germany) at 8000 rpm for 40 seconds to obtain flour. Two grams of the representative

sample was weighed and aflatoxin extracted by adding, 25 ml of methanol/distilled water (70/30; v/v), mixed thoroughly for 10 min at room temperature (RT) using a shaker. The extract was then filtered through Whatman filter paper No. 4 and the filtrate diluted 1:6 in phosphate buffered saline containing 500 µl/l Tween-20 (PBS-Tween).

Competitive ELISA Procedure

Sufficient numbers of antibody coated microtiter wells were inserted into a micro-well holder for all standards and samples to be run in duplicates. Into each well, 50 µl of standard solutions or extracted sample, 50 µl/well of enzyme conjugate and diluted antibody solution were dispensed in duplicates. This was mixed using a shaker for 30 seconds and the plates incubated at room temperature in the dark for 30 minutes to facilitate interaction between the toxins and the antibody. The plate was thereafter washed three times with PBS-Tween, using Wellwash®, (Labsystems, Helsinki, Finland) allowing three minutes between each wash. Substrate solution was then added at 100 µl/well and the plates incubated for 15 minutes in the dark for color to develop. The reaction was stopped by adding 100 µl/well of 1 mol/L H₂SO₄ and the absorbance read at 450 nm in an ELISA plate reader (Labsystems Multiskan® PLUS, Labsystems, Helsinki, Finland). The final amount of aflatoxin present was determined using a software program supplied by the cELISA kits manufacturer (r-biopharm).

Data Analysis

Statistical analysis was performed using SPSS software version 19. To compare proportions of aflatoxin contamination amongst sub-locations we used Chi square test. Significance was reported at 95% ($p < 0.05$) confidence interval.

RESULTS

Household Characteristics

There was a significant difference in the percentages of people with different education levels across the sub-locations. The proportion of persons from Kilibwoni with primary education was greater than those from Chepkongony which in turn did not significantly vary from the proportion in Laboret. A significantly higher percent (1.4%)

of people have university education from Kilibwoni compared to the other sites.

Knowledge on Risks Associated with Consumption of Mouldy Grains

The mentioned health risks associated with consumption of mouldy grains are presented in (Table 1). Cancer as a health risk was identified by a small percentage of the respondents with none mentioning it from Kilibwoni.

Table 1: Household Socio-Demographics of the Study Sub-Locations

Characteristic	Laboret (%)	Kilibwoni (%)	Chepkongony (%)	Test Statistic (p-value)
Gender	(n=594)	(n=372)	(n=563)	0.104
Male	48.3 ^a	55.1 ^b	52.4 ^{a,b}	
Female	51.7 ^a	44.9 ^b	47.6 ^{a,b}	
Age structure	(n= 591)	(n=371)	(n=557)	0.586
<1 years	5.9 ^a	3.8 ^a	5.0 ^a	
2 – 5	21.2 ^a	20.5 ^a	18.1 ^a	
6 – 18	32.3 ^a	35.3 ^a	37.2 ^a	
19 – 35	25.4 ^a	25.9 ^a	27.6 ^a	
36 – 60	13.9 ^a	13.5 ^a	11.0 ^a	
61+	1.4 ^a	1.1 ^a	1.1 ^a	
Dependency (years)	(n=591)	(n=371)	(n=557)	0.638
Dependents (0-14;65+)	52.8 ^a	52.0 ^a	54.9 ^a	
Productive population (15-64)	47.2 ^a	48.0 ^a	45.1 ^a	
Education	(n=579)	(n=359)	(n=557)	0.000
Primary	54.6 ^{a,b}	60.4 ^b	53.7 ^a	
Secondary	18.3 ^a	11.7 ^b	17.1 ^a	
Post-secondary	1.6 ^a	2.5 ^{a,b}	5.0 ^b	
University	.0 ^a	1.4 ^b	0.2 ^a	
Below school admission age	24.4 ^a	22.3 ^a	22.6 ^a	
None	1.2 ^a	1.7 ^a	1.4 ^a	
Mean livestock no. per household				0.272
Cattle	3.8	3.8	3.0	
Sheep	2	0.9	1.5	
Goats	0.1	0.3	0.2	

Note: Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the 0.05 level.

Table 2: Risks Associated with Consumption of Spoilt Grains as Given by Respondents

Risk	Laboret (n=10)%	Kilibwoni (n=63)%	Chepkongony (n=97)%
Stomach upset	24.8 ^a	46.0 ^b	33.0 ^{a,b}
Aflatoxicosis	12.9 ^{a,b}	22.2 ^b	5.2 ^a
Cancer	6.9 ^a	0.0 ^b	1.0 ^b
Death	2.0 ^a	19.0 ^b	2.1 ^a

Note: Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the 0.05 level.

Table 3: Households Practices About Aflatoxins in Study Sub-Locations

Practice	Laboret (%)	Kilibwoni (%)	Chepkongony (%)
Store grains in a crib (n=15)	26.7 ^a	73.3 ^b	0 ^c
Store grains in a granary with iron sheet (n=80)	22.5 ^a	18.8 ^a	58.8 ^b
Store grains in thatched granary (n=18)	38.9 ^a	33.3 ^a	27.8 ^a
Store grains in a bag (n=74)	47.3 ^a	23.0 ^b	29.7 ^b
Shell grains manually by pounding (n=72)	5.6 ^a	26.4 ^b	68.1 ^c
Shell grains by machine (n=104)	75 ^a	25 ^b	0 ^c
Dry on ground on cob (n=23)	0 ^a	73.9 ^b	26.1 ^c
Dry on ground no canvas (n=14)	35.1 ^a	21.4 ^a	42.9 ^a
Dry on ground with canvas (n=182)	50 ^a	17.6 ^a	32.4 ^c

Note: Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the 0.05 level.

Table 4: Percentage Households Disposing their Spoilt Grains Using Various Options

Disposal Option	Laboret (n=101)	Kilibwoni (n=63)	Chepkongony (n=97)
Animal feed	67.3 ^a	81.0 ^{a,b}	84.5 ^b
Busaa	23.8 ^a	22.2 ^a	29.9 ^a
Give away	1 ^a	0 ^a	0 ^a
Throw away	4 ^a	4.8 ^a	10.3 ^a
Leave it in the shamba	13.9 ^a	9.5 ^a	28.9 ^b

Note: Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the 0.05 level.

Table 5: Aflatoxin in Maize in the Various Sub-Locations

Sub-Location	Percentage Positive	Range (ppb)	Mean	Test Statistic
Laboret (n=106)	67.9	0.18-3.6	1.05	0.144
Kilibwoni (n=60)	73.3	0.19-5.3	1.10	
Chepkongony (n=102)	65.7	0.17-3.2	0.83	

Table 6: Percentage Positive, Range and Mean of Total Aflatoxins in Market Maize from Study Sites

Sub-Location	Percentage Positive	Range (ppb)	Mean
Laboret (n=12)	66.7	1.2-1.8	1.608
Kilibwoni (n=13)	69.2	0.7-2.1	0.841
Chepkongony (n=14)	71.4	0.6-2.8	1.153

Table 7: Summary of Total Aflatoxin in Sorghum in the Study Sub-Locations

Sub-Location	Range (ppb)	Mean	Percent Below 10 ppb	Percent Exceeding 10 ppb
Laboret (n=18)	1.9-210.1	48.36	36.4	27.3
Kilibwoni (n=22)	0.15-170.8	20.62	27.3	45.5
Chepkongony (n=22)	0.21-74	8.91	36.4	27.3

Table 8: Levels of Total Aflatoxin in Millet the Study Sub-Locations

Sub-Location	Percentage Positive	Range	Mean
Laboret (n=14)	92.9	0.14-2.9	1.47
Kilibwoni (n=9)	100	0.42-2	1.07
Chepkongony (n=16)	87.5	0.45-6.4	1.47

Household Practices Related to Aflatoxins

Significant difference was found in the storing, shelling and drying practices for grains. A greater percentage (58.8%) from Chepkongony preferred to store grains in granary with iron sheets whereas none stored in a crib. The percentage storing in thatched granary could not be differentiated across the sub-locations. Farming in Laboret was highly mechanized with 75% shelling their maize using machines (Table 3). On options for disposing spoilt grains, majority used it as animal feed or for making local brew called busaa (Table 4).

Sources of Household Maize, Sorghum and Millets

Main source of household consumed maize at the time of sampling was from own farm, this was consumed as stiff porridge known locally as ugali by a majority (98% Laboret, 97.2% Kilibwoni,

97.4% Chepkongony). On average maize flour consumption was 1.64 kg per household per day across the study. That translates to approximately 300 g of ugali per person per day taking into account the average household size of six.

All millet samples were homegrown. Households had mostly sorghum grains sourced from the local markets (69.4% Laboret, 72.6% Kilibwoni and 49.7% Chepkongony).

Total aflatoxins (B1, B2, G1, G2)

Levels of Total Aflatoxin in Maize

A total of three hundred and seven maize samples were analyzed in total. Of these, 39 samples were market sourced. More than 60% of total household samples across the study tested positive for aflatoxins with a range of 0.17-5.3 ppb. All samples were within the acceptable limits of 10 ppb by KEBS (Tables 5 and 6).

Levels of Total Aflatoxin in Sorghum and Millet

Levels of aflatoxin in sorghum analyzed ranged from 0.15 to 210.1 ppb. Kilibwoni had the highest percent (45.5%; 10/18) of samples exceeding maximum tolerance limit of 10 ppb set by KEBS (Table 7). Thirty nine millet samples were analyzed in total with levels ranging from 0.14 to 6.4 ppb. None of the samples exceeded the 10 ppb limit (Table 8).

DISCUSSION

Exposure to dietary aflatoxins is a concern for public health due to its known effects. Aflatoxin's cancer causing potential is due to its ability to produce altered forms of DNA adducts. In humans, epidemiological studies have shown that where there is high incidences of hepatocellular carcinoma there is an association between cancer incidence and the aflatoxin content of the diet (Wu *et al.*, 2011). Ingestion of low to moderate levels of aflatoxins have been associated with stunting in children below five years (Gong *et al.*, 2004), teratogenic effects associated with congenital malformations, and depression of immune responses (Williams *et al.*, 2004).

The low contamination levels of maize with aflatoxins from Nandi concur with results of prevalence studies done in the area (Muthomi *et al.*, 2012). In the study by Muthomi *et al.* (2012) they did not detect any aflatoxin in maize but were able to isolate *Aspergillus* species that are potential toxin producing. The fact that all maize samples tested within KEBS limits is preventive of acute aflatoxicosis incidences. However, the residents are still at risk of effects of chronic exposure to aflatoxins.

In this study, the contamination of sorghum and millet samples was significantly different.

Sorghum had highest contamination levels of aflatoxins across the study areas compared to maize and millet (maximum level of 210.1 ppb). Kilibwoni had the highest percent (45.5%) of sorghum samples exceeding KEBS regulatory limits of 10 ppb aflatoxin. The overall mean of 26.0 ppb of aflatoxins in sorghum is much higher than 15.2 ppb found by Kitya *et al.* (2010) in sorghum samples collected from southern Uganda. The high aflatoxin contamination in sorghum could have been due to contamination of market sorghum as majority of the households bought their sorghum from the local markets (69.4% Laboret, 72.6% Kilibwoni and 49.7% Chepkongony). This disagrees with the results reported by Lewis *et al.* (2005) that the number of home grown maize contaminated with aflatoxin was more than market purchased maize.

On knowledge of risks and practices related to aflatoxins, a higher percentage of respondents (24.8% Laboret, 46.0% Kilibwoni and 33.0% Chepkongony) mentioned stomach upset as a primary risk of consuming spoilt grain compared to the other risks. This is generally true for any food substance if consumed when spoilt. Only 6.9% and 1% of all the respondents in Laboret and Chepkongony respectively identified cancer as a risk arising from consumption of mouldy grains. None from Kilibwoni mentioned it. This indicates lack of awareness on mycotoxin exposure and effects arising from consumption of mouldy grains. Wakhisi *et al.* (2005) found high incidences of esophageal cancer in North Rift valley which is associated with fumonisin exposure.

Storage options significantly differed across the sub-locations. A greater percentage (54.7%) in Laboret stored maize in bags whereas those in Kilibwoni and Chepkongony preferred to store

the maize in cribs and granaries. The granaries and cribs are raised above ground increasing air circulation thus promoting drying of the grains which minimizes fungal growth (Wu and Khlangwiset, 2010).

Attempts should be made to control aflatoxin contamination of home grown maize, millet and sorghum as it will lead to lower contamination levels and reduce the level of household exposure. This is critical as the food products made from maize, sorghum and millets are used by the vulnerable groups (under five years and over 60 years old). Aflatoxin contamination of grains in Nandi poses a risk of chronic exposure to the residents. There is a need for awareness creation on aflatoxins in the country and promotion of proper practices of grain production and storage to prevent contamination with aflatoxins in order to reduce exposure. 🌱

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