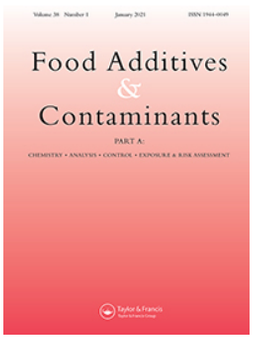

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Human aflatoxin exposure in Uganda: Estimates from a subset of the 2011 Uganda AIDS indicator survey (UAIS)

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ABSTRACT

Aflatoxins are carcinogenic mycotoxins that contaminate a variety of crops worldwide. Acute exposure can cause liver failure, and chronic exposure can lead to stunting in children and liver cancer in adults. We estimated aflatoxin exposure across Uganda by measuring a serum biomarker of aflatoxin exposure in a subsample from the 2011 Uganda AIDS Indicator Survey, a nationally representative survey of HIV prevalence, and examined its association with geographic, demographic, and socioeconomic variables. We analysed a subsample of 985 serum specimens selected among HIV-negative participants from 10 survey-defined geographic regions for serum aflatoxin B1-lysine (AFB1-lys) by use of isotope dilution LC-MS/MS and calculated results normalised to serum albumin. We used statistical techniques for censored data to estimate geometric means (GMs), standard deviations, and percentiles. We detected serum AFB1-lys in 71.7% of specimens (LOD = 0.5 pg/mg albumin). Unadjusted GM AFB1-lys (pg/mg albumin) was 1.33 (95% CI: 1.21–1.47). Serum AFB1-lys was higher in males (GM: 1.57; 95% CI: 1.38–1.80) vs. females (GM: 1.12; 95% CI: 0.97–1.30) (P = .0019), and higher in persons residing in urban settings (GM: 2.83; 95% CI: 2.37–3.37) vs. rural (GM: 1.10; 95% CI: 0.99–1.23) (P < .0001). When we used a multivariable censored regression model to assess confounding and interactions among variables we found that survey region, gender, age, occupation, distance to marketplace, and number of meals per day were statistically significant predictors of aflatoxin exposure. While not nationally representative, our findings provide an improved understanding of the widespread burden of aflatoxin exposure throughout Uganda and identify key geographic, demographic, and socioeconomic factors that may modulate aflatoxin exposure risk.

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aflatoxin; mycotoxin; human exposure; aflatoxin-lysine biomarker; Uganda

Introduction

Aflatoxins are polyketide mycotoxins produced by certain strains of *Aspergillus flavus* and *A. parasiticus*. Contamination of food crops with aflatoxins is a worldwide concern, particularly in oil-rich crops such as maize, grains, and groundnuts (Williams et al. 2004; Wild and Gong 2010). Growth of *Aspergillus* in and on agricultural crops is common; however, aflatoxin accumulation occurs mainly in poor field and/or storage conditions, such as drought, high humidity, insect damage, insufficient drying or dry storage. Aflatoxin exposure is of concern in developing countries where food insecurity can lead to these poor field and/or storage conditions, and regulatory enforcement can be inconsistent (Williams et al. 2004; Wild and Gong 2010). Acute exposures (aflatoxicosis) can lead to gastrointestinal distress, jaundice, liver failure, and death (Williams et al. 2004), whereas chronic exposures have been associated with deleterious effects on foetal and child growth (Turner et al. 2007; Khlangwiset et al. 2011), immunodeficiency (Jiang et al. 2005, 2008), and linked to hepatocellular carcinoma (Ross et al. 1992; Liu and Wu 2010).

Aflatoxin exposure and its associated health burden can be difficult to estimate accurately (Liu and Wu 2010). Aflatoxin contamination of important food crops is widespread in Uganda (Lukwago et al. 2019). Research on human aflatoxin exposure in Uganda, however, is sporadic with most studies to date having focused on food surveys and potential routes of exposure (Lopez

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and Crawford 1967; Alpert and Hutt 1971; Sebunya and Yourtee 1990; Kaaya et al. 2001) rather than assessing exposure through biomarkers (Kang et al. 2015). A link has been established between aflatoxin exposure and hepatocarcinoma in Uganda (Alpert et al. 1968; Alpert and Hutt 1971), but no large-scale population exposure studies using biomarker measurements have been performed. Serum aflatoxin biomarkers have been detected in neighbouring countries such as Kenya (Yard et al. 2013).

Knowing that foodstuffs in Uganda are susceptible to aflatoxin contamination and that neighbouring countries have exposures and outbreaks of aflatoxicosis, a better understanding of the distribution of aflatoxin exposure in Uganda is needed in order to develop effective preventative measures. The objective of our study was to estimate aflatoxin exposure in Uganda, and to identify demographic, socioeconomic and geographic variables associated with aflatoxin exposure by measuring a biomarker of aflatoxin exposure in a serum subsample of the 2011 Uganda AIDS Indicator Survey (2011 UAIS).

Materials and methods

Study population

The 2011 Uganda AIDS Indicator Survey (2011 UAIS) (Ministry of Health Uganda 2012) was a nationally representative, population-based survey of HIV prevalence in which 21,741 blood specimens (12,153 from women and 9,588 from men) were collected from approximately 11,750 households. Details of the survey methods of the 2011 UAIS have been published (Ministry of Health Uganda, 2012). We selected a stratified random sample of the HIV-negative specimens from subjects aged 15–59 years as our study subsample. The strata were defined by geography (10 regions defined in the 2011 UAIS) and gender. From each of the 20 region × gender strata, we selected a simple random sample of 50 HIV-negative specimens to yield an initial study sample of 1000 specimens. Of these specimens, we found that nine specimens were either duplicates or could not be linked back to the survey data, and six specimens could not be analysed for sample quality reasons. We thus reported results from 985 serum specimens.

Survey questionnaire data

We linked the serum specimens to household- and individual-level 2011 UAIS questionnaire data that included demographic, socioeconomic, and various other variables including health status and food security data. Demographic variables consisted of sex, residence type, age, marital status, religion, and ethnic group. Socioeconomic variables were wealth quintiles, education, employment status, and occupation. Food security variables were distance to nearest marketplace, meals consumed per day, frequency satisfying the food needs of the household, source of drinking water, and record of illness in 3 of the preceding 12 months.

Laboratory measurements

The adduct that aflatoxin forms with serum albumin is a robust biomarker of aflatoxin exposure, having a half-life in the body of approximately 20 days that permits observation of potential exposures over a longer period of time than other biomarkers (Gan et al. 1988). In our study, we used serum aflatoxin B1-lysine (AFB1-lys), hydrolysed from aflatoxin B1 bound to serum albumin, as a biomarker of aflatoxin exposure. We measured serum AFB1-lys by use of high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (McCoy et al. 2005), and serum albumin by use of a colorimetric assay performed on a Roche Cobas c501 clinical analyser (Doumas et al. 1971). For the LC-MS/MS analysis, serum was first amended with an isotopically labelled ($^2H_9$) AFB1-lys internal standard (IS) and subjected to proteinase digestion. The resulting AFB1-lys and IS were then extracted by use of mixed-mode anion exchange reversed-phase solid phase extraction. Eluates were reconstituted, chromatographically separated using a C_{18} column, and detected by use of positive electrospray ionisation LC-MS/MS. The LC-MS/MS calibration range for serum AFB1-lys was 0.025–10 ng/mL, and the limit of detection (LOD) was 0.03 ng/mL. The LOD for serum albumin was 0.2 g/dL. We calculated serum AFB1-lys results normalised to serum albumin in pg/mg albumin and assumed an approximate LOD of 0.5 pg/mg albumin for our normalised results.

Statistical analyses

We used SAS 9.4 to perform all statistical analyses. Our albumin-normalised AFB1-lys data appeared approximately log-normally distributed with approximately 30% of the values left censored at 0.5 pg/mg albumin (the LOD estimate for albumin normalised results) (Figure 1). Consequently, we used censored regression techniques based on maximum likelihood estimation (MLE) methods to estimate descriptive statistics that included geometric means (GMs), selected percentiles, and their respective 95% confidence intervals. We used PROC LIFEREG with a log-normal distribution to estimate GMs stratified by study variable categories, and a Type III Wald Chi-Square test statistic for bivariate significance testing across categories for each variable. Both the Wald chi-square test and 95% confidence intervals (CIs) for the GM were calculated using the estimate of the asymptotic covariance matrix. Percentiles were derived from the estimated log-normal distribution. One consequence of using a log-normal model was an equivalence between the MLE GM and median (50^{th} percentile). We limited our analyses to single-variable stratification for simplicity and to avoid strata with few observations.

We developed a multivariable censored regression model using MLE to identify statistically important factors associated with aflatoxin exposure after adjustment for confounding and interactions among variables. In the initial model, we included the 2011 UAIS design variables (region, urbanicity and gender) and all other demographic and socioeconomic variables that were statistically significant ($P < .05$) in our bivariate analyses. For the multivariable model, we recoded some categorical variables for parsimony and treated age as a continuous variable including both a linear and quadratic effect. Retaining the original 2011 UAIS design variables (region, urbanicity and gender), we then used a stepwise backwards elimination procedure to generate a reduced model, sequentially removing variables that failed to meet the 0.05 significance level. After each elimination step, we evaluated confounding by determining if any changes in variable parameter estimates exceeded 30%. Lastly, we used a step-up procedure to evaluate pairwise interactions.

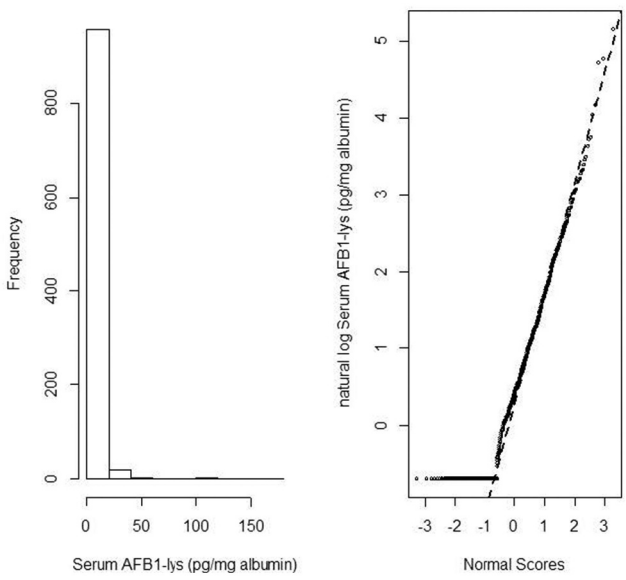

Figure 1. Frequency of serum AFB1-lys biomarker concentrations in study participants.

Comparing our results with other measurement techniques

Serum AFB1-lys measurements obtained by use of different measurement techniques are not directly comparable (McCoy et al. 2008). When discussing our study findings in the context of other studies we expressed results from other studies as LC-MS/MS equivalents based on the following conversion equations: radioimmunoassay (RIA; $\text{AFB1-lys}_{LC\text{-}MS/MS} = \text{AFB1-lys}_{RIA} \div 32$); enzyme-linked immunosorbent assay (ELISA; $\text{AFB1-lys}_{LC\text{-}MS/MS} = \text{AFB1-lys}_{ELISA} \div 3.3$); LC-fluorescence ($\text{AFB1-lys}_{LC\text{-}MS/MS} = \text{AFB1-lys}_{LC\text{-}fluorescence} \div 0.71$) (Wang et al. 1996, McCoy et al. 2008).

Results

Overall exposure and geographic variables

We detected serum AFB1-lys in 71.7% of our subset samples (n = 985, LOD 0.5, Table 1). The overall GM AFB1-lys concentration (measured as pg/mg albumin) for the subset was 1.33 (95% CI 1.21–1.47). We found that geographic region and residence type (urban vs. rural) were associated with aflatoxin exposure in the unadjusted data. Unadjusted GM serum AFB1-lys concentrations were nearly three times

higher for urban households (GM 2.83; 95% CI 2.37–3.37) versus rural (GM: 1.10; 95% CI 0.99–1.23) (Type III Wald Chi-Square $P < .0001$) (Table 1). Noting that the district of Kampala was entirely urban, whereas the urbanicity of the remaining districts ranged from 3% to 19%, we also looked at serum AFB1-lys concentrations among these two urban settings. The GM AFB1-lys concentration for Kampala was 3.35 (95% CI 2.67–4.20) while the GM AFB1-lys concentration across all non-Kampala urban households (data not shown) was 2.36 (95% CI 1.80–084, n = 95, Type III Wald Chi-Square $P = .58$). Geographically, the highest unadjusted GM serum concentrations of AFB1-lys were found in Kampala and its immediate surroundings (Central 1 region; GM: 2.28; 95% CI 1.77–2.94). Residents of the East Central, Mid Northern, and North East regions had the next highest GM biomarker concentrations, while the lowest was observed in the Mid-western region (GM 0.46; 95% CI 0.28–0.73) (Figure 2, Table 1).

Demographic variables

Except for age, all demographic variables we studied were significantly associated with unadjusted estimates of aflatoxin exposure (Table 1). Unadjusted serum AFB1-lys (pg/mg albumin) was higher in males (GM 1.57; 95% CI 1.38–1.78) versus females (GM 1.12; 95% CI 0.97–1.30) (Type III Wald Chi-square $P = .0019$). Respondents who self-

Table 1. Geometric mean (GM) and selected percentiles of serum AFB1-lys concentrations (pg/mg albumin) by demographic characteristics, 2011 UAIS subsample aged 15–59 y, 2011.

Characteristic	n	GM (95% CI)	2.5th percentile	97.5th percentile	%>LOD	Range
Overall	985	1.33 (1.21–1.47)	0.08 (0.06–0.09)	23.4 (19.7–27.7)	72	<LOD–174
Sex ($P = .0019$*)						
Men	493	1.57 (1.38–1.78)	0.06 (0.04–0.08)	22.8 (17.7–29.6)	77	<LOD–174
Women	492	1.12 (0.97–1.30)	0.10 (0.08–0.13)	23.6 (18.9–29.5)	67	<LOD–118
Residence ($P < .0001$)						
Urban	192	2.83 (2.37–3.37)	0.254 (0.18–0.35)	31.4 (23.1–42.8)	89	0.500–174
Rural	793	1.10 (0.99–1.23)	0.062 (0.05–0.08)	19.7 (16.1–23.8)	68	0.500–118
Age (y) ($P = .065$)						
15 to <20	201	1.34 (1.10–1.64)	0.09 (0.06–0.14)	19.4 (13.7–27.6)	74	<LOD–56.6
≥20 to <26.5	193	1.30 (1.06–1.60)	0.09 (0.06–0.14)	18.7 (13.1–26.8)	72	<LOD–37.6
≥26.5 to <33	213	1.61 (1.33–1.96)	0.10 (0.07–0.15)	25.3 (17.9–35.7)	77	<LOD–174
≥33 to <43	190	1.43 (1.13–1.81)	0.07 (0.04–0.11)	31.2 (20.6–47.4)	72	<LOD–112
43 to 59	188	1.00 (0.77–1.28)	0.04 (0.02–0.08)	23.1 (14.9–35.9)	63	<LOD–118
Region ($P < .0001$)						
Central 1	98	2.28 (1.77–2.94)	0.19 (0.12–0.31)	26.8 (17.1–42.0)	85	<LOD–27.2
Central 2	99	1.02 (0.79–1.33)	0.09 (0.05–0.16)	11.9 (7.49–19.0)	69	<LOD–31.4
Kampala	97	3.35 (2.67–4.20)	0.36 (0.24–0.55)	30.8 (20.8–45.6)	94	<LOD–41.8
East Central	98	1.73 (1.36–2.21)	0.16 (0.10–0.27)	18.2 (11.8–28.0)	81	<LOD–32.7
Mid Eastern	98	1.01 (0.71–1.44)	0.04 (0.02–0.10)	23.7 (12.8–44.0)	62	<LOD–27.8
North East	98	1.41 (0.99–1.99)	0.06 (0.03–0.11)	36.2 (19.7–66.8)	71	<LOD–174
West Nile	100	0.82 (0.56–1.22)	0.03 (0.01–0.06)	26.6 (13.6–52.1)	59	<LOD–64.2
Mid Northern	97	1.58 (1.24–2.00)	0.16 (0.10–0.25)	15.7 (10.3–24.0)	80	<LOD–56.6
South Western	101	1.09 (0.83–1.42)	0.09 (0.05–0.15)	13.5 (8.47–21.7)	70	<LOD–24.4
Mid Western	99	0.46 (0.28–0.73)	0.01 (0.004–0.04)	17.8 (8.47–37.4)	47	<LOD–112
Marital Status ($P = .0157$)						
Never in union	239	1.55 (1.31–1.84)	0.12 (0.09–0.17)	20.2 (15.0–27.4)	78	<LOD–56.6
Married	517	1.15 (1.01–1.32)	0.07 (0.05–0.09)	20.5 (16.1–26.0)	69	<LOD–118
Living with partner	104	1.73 (1.28–2.34)	0.09 (0.05–0.17)	32.8 (19.2–56.1)	74	<LOD–26.4
Other	125	1.43 (1.05–1.94)	0.06 (0.03–0.11)	35.7 (20.9–61.1)	71	<LOD–174
Religion ($P = .0450$)						
Catholic	420	1.26 (1.07–1.48)	0.06 (0.04–0.08)	27.8 (20.9–36.9)	69	<LOD–118
Anglican/Protestant	322	1.27 (1.08–1.50)	0.08 (0.06–0.11)	19.9 (15.0–26.5)	72	<LOD–174
Muslim	132	1.90 (1.52–2.39)	0.15 (0.10–0.24)	23.8 (16.0–35.5)	82	<LOD–37.6
Other	111	1.21 (0.92–1.58)	0.08 (0.05–0.15)	17.3 (10.8–27.9)	70	<LOD–41.8
Ethnic Group ($P < .0001$)						
Baganda	178	1.96 (1.63–2.37)	0.17 (0.12–0.24)	23.1 (16.5–32.2)	83	<LOD–41.8
Banyankore	87	0.93 (0.67–1.29)	0.06 (0.03–0.12)	15.1 (8.56–26.6)	66	<LOD–24.4
Iteso	83	0.78 (0.55–1.11)	0.05 (0.02–0.10)	13.3 (7.29–24.2)	60	<LOD–118
Basoga	85	2.39 (1.92–2.98)	0.32 (0.21–0.48)	17.8 (12.1–26.3)	89	<LOD–32.7
Alur/Jopadhola	60	0.28 (0.13–0.60)	0.01 (0.00–0.04)	15.2 (5.14–45.1)	37	<LOD–21.3
Bagisu/Sabiny	58	1.47 (1.03–2.11)	0.11 (0.05–0.23)	20.3 (10.7–38.5)	74	<LOD–17.9
Langi	63	1.26 (0.92–1.71)	0.12 (0.06–0.23)	13.0 (7.49–22.5)	73	<LOD–15.0
Lugbara/madi	69	1.10 (0.70–1.72)	0.04 (0.01–0.09)	34.4 (15.6–75.5)	65	<LOD–64.2
Other	302	1.45 (1.21–1.74)	0.07 (0.05–0.10)	29.8 (21.6–41.2)	73	<LOD–174

Notes: AFB1-lys, aflatoxin B1-lysine CI, confidence interval; GM, geometric mean
*P = Type III Wald Chi-Square

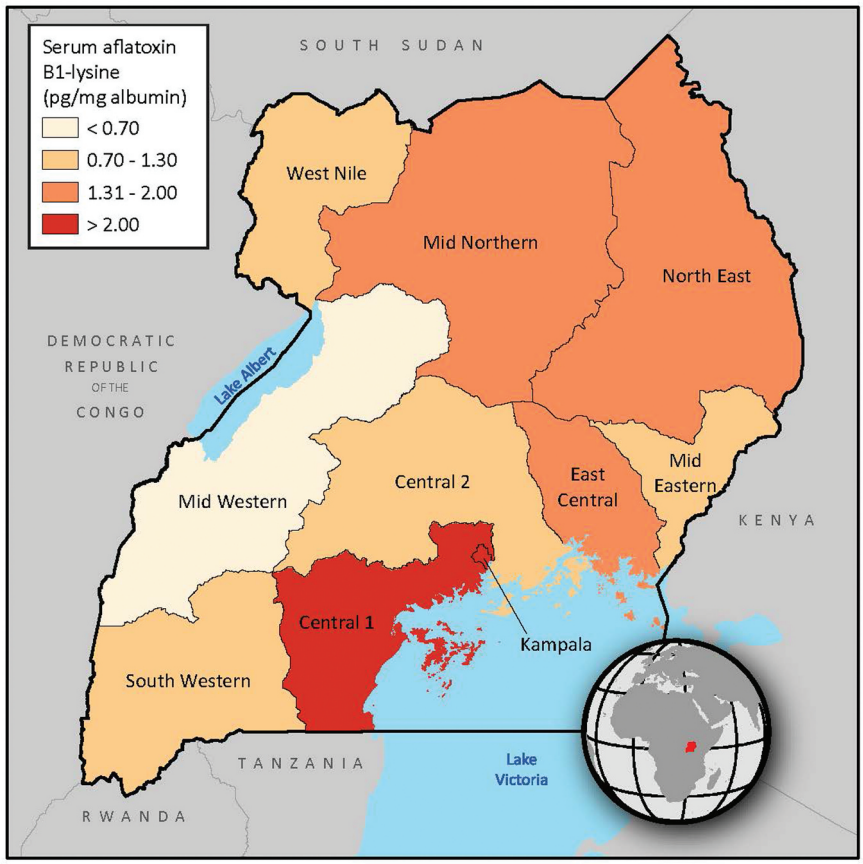

Figure 2. Geometric mean serum AFB1-lys (pg/mg albumin) by survey-defined region, 2011 UAIS subsample aged 15–59, 2011.

identified as Basoga or Baganda ethnicity had almost twice as high GM AFB1-lys concentrations relative to the individuals who self-identified with other ethnicities (Type III Wald Chi-Square $P < .0001$). The relationship between AFB1-lys concentration and age appeared to be non-linear, with the highest concentration (pg/mg albumin) observed among those aged 26.5–33 y (GM 1.61; 95% CI 1.33–1.96) and those aged 43–59 y had the lowest (GM 1.00; 95% CI 0.77–1.28).

Socioeconomic variables

We found that wealth, education, and type of occupation were significantly associated with unadjusted estimates of aflatoxin exposure (Table 2). Serum AFB1-lys (pg/mg albumin) concentrations of individuals in the highest wealth quintile (GM 2.51; 95% CI 2.16–2.93) were at least twice that of individuals in all other wealth quintiles (Type III Wald Chi-Square $P < .0001$). Serum AFB1-lys was also highest in those possessing higher education (GM 2.16; 95% CI 1.51–3.10) and lowest in individuals with only a primary level of education (GM 1.07; 95% CI 0.93–1.23) (Type III Wald Chi-Square $P < .0001$). No significant associations were observed with employment status in the past year; however, persons in agricultural occupations had serum AFB1-lys concentrations that were significantly lower (GM 0.94; 95% CI 0.81–1.08) than those in other occupations (GM 1.94; 95% CI 1.66–2.26) (Type III Wald Chi-Square $P < .0001$).

Health and food security variables

We observed significant associations between health and food security variables and unadjusted estimates of aflatoxin exposure (Table 3). Unadjusted serum AFB1-lys concentrations (pg/mg albumin) were highest in individuals closest (<1 km) to a marketplace (GM 1.96; 95% CI 1.66–2.31) and lowest for those furthest (>5 km) away (GM 0.87;

Table 2. Geometric mean (GM) and selected percentiles of serum AFB1-lys concentrations (pg/mg albumin) by socioeconomic characteristics, 2011 UAIS subsample aged 15–59 y, 2011.

Characteristic	n	GM (95% CI)	2.5th percentile	97.5th percentile	%>LOD	Range
Overall	985	1.33 (1.21–1.47)	0.08 (0.06–0.09)	23.4 (19.7–27.7)	72	<LOD–174
Wealth quintiles ($P < .0001$*)						
Poorest	208	1.21 (0.95–1.52)	0.05 (0.03–0.09)	27.7 (18.4–41.7)	68	<LOD–118
Poorer	176	0.84 (0.65–1.08)	0.04 (0.02–0.08)	16.8 (10.9–26.0)	61	<LOD–56.6
Middle	192	1.13 (0.90–1.41)	0.06 (0.04–0.10)	20.6 (13.8–30.5)	68	<LOD–174
Richer	174	1.11 (0.88–1.40)	0.07 (0.04–0.11)	18.5 (12.4–27.7)	68	<LOD–23.8
Richest	235	2.51 (2.16–2.93)	0.25 (0.19–0.33)	25.2 (19.3–32.8)	89	<LOD–41.8
Education ($P < .0001$)						
No education	137	1.43 (1.11–1.86)	0.08 (0.05–0.14)	25.3 (16.0–40.0)	72	<LOD–41.2
Primary	570	1.07 (0.93–1.23)	0.05 (0.04–0.07)	22.3 (17.5–28.4)	66	<LOD–174
Secondary	220	1.88 (1.59–2.21)	0.17 (0.12–0.23)	21.0 (15.7–28.2)	84	<LOD–37.6
Higher	58	2.16 (1.51–3.10)	0.15 (0.07–0.29)	32.0 (17.0–60.2)	85	<LOD–56.6
Employment ($P = .1953$)						
Currently working	734	1.31 (1.17–1.46)	0.08 (0.06–0.10)	22.9 (18.8–27.9)	71	<LOD–174
Have a job, but on leave	15	1.39 (0.56–3.44)	0.05 (0.01–0.39)	36.3 (7.26–182)	67	<LOD–11.3
Employed in the past year	30	0.81 (0.45–1.46)	0.05 (0.01–0.18)	14.1 (5.15–38.7)	60	<LOD–16.3
Unemployed in the past year	206	1.52 (1.24–1.87)	0.09 (0.06–0.14)	25.3 (17.6–36.4)	75	<LOD–41.2
Occupation ($P < .0001$)						
Not working	206	1.52 (1.24–1.87)	0.09 (0.06–0.14)	25.3 (17.6–36.4)	75	<LOD–41.2
Agricultural	438	0.94 (0.81–1.08)	0.06 (0.04–0.08)	15.3 (11.8–19.7)	64	<LOD–112
Other	341	1.94 (1.66–2.26)	0.12 (0.09–0.17)	31.0 (23.6–40.8)	80	<LOD–174

AFB1-lys, aflatoxin B1-lysine; CI, confidence interval; GM, geometric mean
*P = Type III Wald Chi-Square

95% CI: 0.69–1.10) (Type III Wald Chi-Square $P < .0001$). Exposure trends relating to food security characteristics show that AFB1-lys concentration is elevated in those groups reporting having the most (≥4 meals per day, GM 3.87; 95% CI 2.51–5.97) and fewest meals per day (1 meal per day, GM 2.04; 95% CI 1.57–2.64). This was also true for those with the most and least trouble meeting the food needs of the household, as compared to the groups in the middle (2 meals per day GM 1.06; 95% CI 0.93–1.21; 3 meals per day GM 1.54; 95% CI 1.30–1.82) for each for these characteristics (Type III Wald Chi-Square $P < .0001$).

Multivariable model

In our multivariable model, the differences we observed in unadjusted AFB1-lys concentrations (pg/mg albumin) associated with education, religion, wealth, marital status and water supply were no longer statistically significant (Table 4). When we removed these variables from our full multivariable model to obtain a final reduced model, the remaining independent covariates we found to be significantly associated with AFB1-lys concentrations were region ($P < .0001$), gender ($P < .0001$), age (linear $P = .0201$; quadratic $P = .0128$), number of meals per day ($P < .0001$), distance to the market ($P = .045$), and occupation ($P = .0002$). The type of residence (urban vs. rural) was no longer statistically significant at the 0.05 significance level after controlling for the other factors in the reduced model ($P = .0614$). Our reduced model estimated that GM AFB1-lys concentrations were 1.46 times higher among males vs. females ($P < .0001$), 1.47 times higher among all other occupational trades compared to agricultural trades ($P = .0002$), and 1.44–1.46 times higher for those residing <1 km and 1–2 km versus >5 km from a marketplace ($P = .012$ and 0.0092, respectively) (Table 4). All the survey regions, except West Nile ($P = .1348$), had significantly higher GM AFB1-lys concentrations compared to the Mid-western Region. The effect of age was non-linear (inverse U-shape), and GM AFB1-lys was highest in individuals aged 33.5 y after controlling for the other variables in the model. The only statistically significant interaction we identified existed between survey-defined geographic region and number of meals (results not shown, $P < .0001$). For this reason, inferences about the effect of the number of meals should be approached with caution as the effect varied by region.

Discussion

We have presented serum AFB1-lys concentrations from a stratified simple random sample of HIV-negative persons between ages 15–59 who

Table 3. Geometric mean (GM) and selected percentiles of serum AFB1-lys concentrations (pg/mg albumin) by food security characteristics and health, 2011 UAIS subsample aged 15–59 y, 2011.

Characteristic	n	GM (95% CI)	2.5th percentile	97.5th percentile	%>LOD	Range
Overall	985	1.33 (1.21–1.47)	0.08 (0.06–0.09)	23.4 (19.7–27.7)	72	<LOD–174
Distance to nearest market place ($P < .0001$*)						
<1 km	266	1.96 (1.66–2.31)	0.14 (0.10–0.19)	27.5 (20.5–36.9)	81	<LOD–174
1–2 km	258	1.49 (1.23–1.80)	0.08 (0.06–0.12)	26.9 (19.2–37.5)	74	<LOD–112
3–5 km	249	1.07 (0.89–1.29)	0.07 (0.05–0.10)	16.7 (12.0–23.2)	68	<LOD–118
>5 km	199	0.87 (0.69–1.10)	0.04 (0.03–0.07)	17.5 (11.6–26.3)	62	<LOD–56.6
Meals per day ($P < .0001$)						
1 meal	130	2.04 (1.57–2.64)	0.12 (0.07–0.19)	36.0 (22.7–57.0)	79	<LOD–174
2 meals	544	1.06 (0.93–1.21)	0.06 (0.05–0.08)	18.3 (14.5–23.1)	67	<LOD–112
3 meals	287	1.54 (1.30–1.82)	0.10 (0.07–0.14)	24.6 (18.2–33.2)	76	<LOD–64.2
≥4 meals	24	3.87 (2.51–5.97)	0.46 (0.22–0.97)	32.4 (15.4–67.9)	100	0.81–41.8
Frequency satisfying the food needs of the household ($P = .0350$)						
Always	68	1.63 (1.09–2.44)	0.07 (0.03–0.16)	38.7 (19.1–78.6)	75	<LOD–174
Often	119	1.58 (1.19–2.08)	0.09 (0.05–0.15)	28.9 (17.7–47.3)	76	<LOD–118
Sometimes	361	1.26 (1.09–1.45)	0.09 (0.07–0.13)	17.2 (13.3–22.2)	72	<LOD–31.4
Seldom	156	1.04 (0.80–1.33)	0.06 (0.03–0.10)	18.7 (12.0–29.1)	65	<LOD–27.2
Never	281	1.48 (1.23–1.79)	0.07 (0.05–0.11)	29.4 (21.1–41.0)	73	<LOD–112
Source of drinking water ($P < .0001$)						
Protected spring	171	1.11 (0.88–1.41)	0.07 (0.04–0.11)	19.0 (12.6–28.7)	68	<LOD–64.2
Protected well	357	1.33 (1.13–1.56)	0.08 (0.05–0.11)	23.3 (17.6–30.9)	71	<LOD–174
Public tap/standpipe	142	2.45 (1.97–3.05)	0.19 (0.13–0.29)	31.7 (21.6–46.5)	86	<LOD–56.6
River/dam/lake/pond/stream	80	0.67 (0.45–0.98)	0.03 (0.01–0.08)	13.3 (6.91–25.4)	56	<LOD–31.4
Unprotected spring	62	0.96 (0.65–1.40)	0.06 (0.03–0.14)	15.0 (7.69–29.2)	65	<LOD–17.0
Unprotected well	102	1.30 (0.96–1.77)	0.07 (0.04–0.13)	24.4 (14.2–42.1)	71	<LOD–24.4
Other	71	1.76 (1.29–2.41)	0.14 (0.07–0.25)	22.9 (13.1–39.9)	79	<LOD–27.2
Sick for at least 3 months during the past 12 months ($P = .7104$)						
Yes	43	1.48 (0.96–2.28)	0.10 (0.04–0.24)	22.4 (10.4–48.4)	74	<LOD–21.4
No	835	1.32 (1.19–1.47)	0.07 (0.06–0.09)	24.2 (20.0–29.1)	71	<LOD–174

AFB1-lys, aflatoxin B1-lysine CI, confidence interval; GM, geometric mean
*P = Type III Wald Chi-Square

were selected to be part of the UAIS. Our data showed widespread evidence of aflatoxin exposure, finding detectable serum AFB1-lys concentrations (≥0.5 pg/mg albumin) in >70% of our subsample. After covariate adjustment, we found that survey-defined geographic region, sex, age, number of meals consumed per day, distance to marketplace, and occupation were significant predictors of serum AFB1-lys concentration. Although not a nationally representative sample, our work is the first study to provide a biomarker-based estimate of exposure to aflatoxin in a subsample of the Ugandan population sampled across various regions of the country. We believe our study provides contextual data that will assist in interpreting serum AFB1-lys measurements observed in other studies and may ultimately help guide intervention efforts to reduce aflatoxin exposure in Uganda.

While direct comparisons of results from other studies are not straightforward due to methodological differences, the overall unadjusted estimate of GM serum AFB1-lys concentration for our Uganda subsample (1.33 pg/mg albumin; 95% CI 1.21–1.47 pg/mg albumin) is comparable to data shown by others in the region. Yard et al. (2013) performed an analogous study of aflatoxin exposure in Kenya in which serum AFB1-lys concentrations were measured in a similarly stratified (sex and survey-defined region) random sample of HIV-negative serum samples from the 2007 Kenya AIDS Indicator Survey (2007 KAIS). Using the exact same LC-MS/MS methodology as our study, Yard et al. found a slightly higher detection frequency (78% ≥0.5 pg/mg albumin) and median concentration (1.78 pg/mg albumin, 95% CI 1.46–2.12 pg/mg albumin) in their 2007 KAIS subsample.

Within Uganda, several studies have looked at serum AFB1-lys concentrations in population cohorts residing in the 2011 UAIS Central 1 region. Kang et al. (2015) looked at serum AFB1-lys concentrations in two population cohorts from the Central 1 region. In one cohort (General Population Cohort Study), which consisted of individuals aged ≥13 y from contiguous rural villages concentrated in a single sub-county of Kalungu

Table 4. Unadjusted[a] and adjusted[b] (full- and reduced-multivariate model) ratios of geometric mean (GM) serum AFB1-lys concentrations (pg/mg albumin) relative to a reference group[c] by selected variables, 2011 UAIS subsample aged 15–59 y, 2011.

		Unadjusted		Full model		Reduced model	
Characteristic	*n*	GM ratio (95% CI)	*P*	GM ratio (95% CI)	*P*	GM ratio (95% CI)	*P*
Region							
Central 1/Kampala	98/97	4.48 (3.11–6.46)	<0.0001	2.90 (1.98–4.26)	<0.0001	2.95 (2.03–4.28)	<0.0001
Central 2	99	1.61 (1.06–2.46)	0.0265	1.53 (1.02–2.30)	0.0422	1.57 (1.04–2.35)	0.0301
East Central	98	2.82 (1.86–4.27)	<0.0001	2.32 (1.54–3.50)	<0.0001	2.39 (1.59–3.59)	<0.0001
Mid-Eastern	98	1.85 (1.22–2.82)	0.0041	2.1 (1.39–3.16)	0.0004	2.12 (1.41–3.20)	0.0003
Mid-Northern	97	2.56 (1.69–3.88)	<0.0001	2.96 (1.95–4.49)	<0.0001	2.94 (1.94–4.45)	<0.0001
North East	98	2.47 (1.63–3.76)	<0.0001	2.16 (1.42–3.31)	0.0004	2.24 (1.47–3.42)	0.0002
South Western	101	1.81 (1.20–2.75)	0.0051	1.96 (1.30–2.96)	0.0012	1.93 (1.29–2.89)	0.0015
West Nile	100	1.59 (1.04–2.41)	0.031	1.35 (0.90–2.03)	0.1504	1.36 (0.91–2.05)	0.1348
Mid-Western	99	1		1		1	
Sex							
Male	493	1.35 (1.63–1.12)	0.0019	1.46 (1.74–1.22)	<0.0001	1.46 (1.73–1.22)	<0.0001
Female	492	1		1		1	
Residence							
Urban	192	2.49 (1.99–3.12)	<0.0001	1.24 (0.89–1.73)	0.2029	1.31 (0.99–1.73)	0.0614
Rural	793	1		1		1	
Marital status							
Never in union	239	1.18 (0.95–1.47)	0.1337	0.94 (0.71–1.24)	0.6514		
Married/living with partner/other	746	1		1			
Religion							
Catholic/Anglican/Protestant	742	0.84 (0.68–1.05)	0.1273	0.93 (0.75–1.14)	0.4755		
Muslim/other	243	1		1			
Wealth quintiles							
Richest	235	2.24 (1.81–2.77)	<0.0001	1.13 (0.83–1.54)	0.4348		
Poorest/poorer/middle/richer	750	1		1			
Education							
Secondary/higher	278	1.18 (0.95–1.47)	0.1337	0.94 (0.71–1.24)	0.6514		
Primary/none	707	1		1			
Occupation							
Agricultural	438	0.53 (0.44–0.64)	<0.0001	0.69 (0.57–0.85)	0.0004	0.68 (0.56–0.83)	0.0002
Other/not working	547	1		1		1	
Distance to nearest marketplace							
<1 km	266	2.15 (1.63–2.82)	<0.0001	1.40 (1.04–1.89)	0.0265	1.46 (1.09–1.95)	0.012
1–2 km	258	1.67 (1.26–2.20)	0.0003	1.41 (1.07–1.85)	0.0149	1.44 (1.09–1.89)	0.0092
3–5 km	249	1.18 (0.89–1.56)	0.2441	1.23 (0.94–1.60)	0.1307	1.24 (0.95–1.61)	0.1142
>5 km	199	1		1		1	
Meals per day							
1 meal	130	0.53 (0.28–0.99)	0.0473	0.78 (0.43–1.42)	0.4158	0.78 (0.43–1.41)	0.4039
2 meals	544	0.28 (0.15–0.49)	<0.0001	0.43 (0.25–0.76)	0.0034	0.44 (0.25–0.76)	0.0036
3 meals	287	0.4 (0.22–0.72)	0.0023	0.56 (0.32–0.97)	0.0396	0.57 (0.32–0.99)	0.0449
≥4 meals	24	1		1			
Source of drinking water							
Other	71	0.71 (0.47–1.08)	0.1102	0.83 (0.56–1.24)	0.3677		
Protected well/protected spring	528	0.52 (0.40–0.68)	<0.0001	1.06 (0.78–1.45)	0.7095		
Unprotected well/unprotected spring/river/dam/lake/pond/stream	244	0.41 (0.30–0.55)	<0.0001	0.87 (0.62–1.23)	0.4331		
Public tap/standpipe	142	1		1			

[a]Full model includes all covariates that were statistically significant in bivariate analyses ($P \leq 0.05$), n = 972
[b]Reduced model based on stepwise backwards regression. Significance level to stay in model α = 0.05, n = 972
[c]The reference group in each comparison is the category denoted as "1"
AFB1-lys, aflatoxin B1-lysine; CI, confidence interval; GM, geometric mean

district, a similar median serum concentration of 2.23 pg/mg albumin was observed for samples collected across 8 time points from 1989 to 2010. In the other cohort (Rakai Community Cohort Study), which consisted of adults aged 15–49 y from 50 villages across the Rakai district, a lower median serum concentration of 1.67 pg/mg albumin was observed for samples collected from 2000 to 2003. Asiki et al. (2014) also looked at serum AFB1-lys concentrations in Kalungu district (General Population Cohort Study) but found a higher GM concentration of 3.48 pg/mg albumin in their subset of adults aged 18–89 y. These concentration differences may be attributable to a variety of factors, including time of sampling and methodological differences. Elements of our own study design, such as potential oversampling of rural, lower-populated districts and only including HIV-

negative specimens, may also explain cases where our observed AFB1-lys concentrations appear to be lower.

We found gender to be a significant predictor of aflatoxin exposure in our subsample, with GM AFB1-lys concentrations estimated to be 1.46 times higher in males versus females in our final reduced multivariable model (95% CI: 1.22–1.73; $P < .0001$). By comparison, many other studies failed to observe statistically significant gender-based differences in AFB1-lys concentrations, examples of which include subpopulations of Uganda (Asiki et al. 2014; Kang et al. 2015), Kenya (Yard et al. 2013), Ghana (Jolly et al. 2006), and the UK (Turner et al. 1998). A 2013 study of pregnant women and recent mothers (<2 y) from the former Eastern Province of Kenya found a median AFB1-lys concentration of 10.5 pg/mg albumin (Leroy et al. 2015), which was considerably higher than the estimate for females in our 2011 UAIS subset. When interpreting gender-based differences in serum biomarker concentrations, the possibility of gender-based differences in serum albumin concentrations should also be considered. We saw a small but statistically significant difference in the mean serum albumin concentrations of males versus females (~3% higher in males) in our dataset (data not shown), consistent with observations in other populations (Weaving et al. 2016).

Aflatoxin exposure appeared to show an inverse U-shaped association with age in our subsample. When we included age as a continuous variable in our multivariable analyses, including both a linear and quadratic effect, both these effects were statistically significant, suggesting the association of serum AFB1-lys concentrations with age may be non-linear. Our model suggests that AFB1-lys concentrations are highest in individuals aged 33.5 y. Studies looking at aflatoxin exposure in two cohorts residing in the 2011 UAIS Central 1 showed unadjusted GM AFB1-lys concentrations appeared to be highest in individuals aged 20–39 y (Kang et al. 2015) and higher in adults vs. children (Asiki et al. 2014). Contrary to our observations, studies in Kenya (Yard et al. 2013) and Ghana (Jolly et al. 2006) showed the highest biomarker concentrations in older individuals.

The highest concentrations of AFB1-lys were found in samples from residents of Kampala and its immediate surroundings, with East Central, Mid Northern, and North East regions having the next highest biomarker concentrations. The lowest concentration was observed in the Mid-western region. The reasons for the differences in geographic exposure are likely complex and multifactorial. The diets of individuals in different geographic regions may vary and contribute to differences in aflatoxin exposure. This phenomenon has been observed previously in West Africa where differences in biomarker concentration were related to crops consumed (Egal et al. 2005). Diet and microclimate have also been implicated in regional differences in AFB1-lys adduct concentrations in sub-Saharan African countries (Xu et al. 2018). There are many climate variables that influence aflatoxin production and contamination of crops, and the exact mechanisms at play here are unknown (Cotty and Jaime-Garcia 2007). It has been shown that rates of hepatocarcinoma and crop contamination follow a similar geographic trend across Uganda as observed in biomarker concentrations (Alpert and Hutt 1971). In particular, the former Teso and Karamoja districts (2011 UAIS North East region), which had high incidence of aflatoxin contamination, had higher AFB1-lys concentrations in our subsample. This trend was also true for Buganda Province (Kampala and the 2011 UAIS Central 1 and Central 2 regions) in which 28.9% of food samples were contaminated with aflatoxin.

Rural populations have been shown to have higher concentrations of adducts than urban (Wild et al. 2000), which is the opposite of what we observed in this study. However, a trend of higher serum AFB1-lys concentrations in urban versus rural residents was also observed in Kenya (Yard et al. 2013). This may be because storage and transport of food crops can lead to an increase in aflatoxin contamination, as seen in peanut products, which trend towards higher contamination in retail markets as opposed to those same products when purchased at wholesalers (Kaaya et al. 2006). Muzoora et al. (2017) also showed a similar trend towards urban products being more contaminated than rural products, with more urban Ugandan peanut samples (67.1%) testing positive than rural. We also found higher concentrations of biomarker in individuals with non-agricultural occupations, which was consistent with our observed urban versus rural

difference no longer being as large or statistically significant after controlling for occupation. We observed high unadjusted biomarker concentrations in both Kampala and non-Kampala urban households, and while the concentrations appeared slightly higher in Kampala households this difference was not statistically significant. Further studies with the goal of examining regional differences as it relates to population density and foods consumed, as well as the roles of food storage and transport, are necessary to clarify what role urbanicity and/or market distance plays with aflatoxin exposure.

While our study has provided valuable insight into aflatoxin exposure across Uganda and its association geographic, demographic, and socioeconomic variables, we acknowledge that there are limitations to our study. Most importantly, we point out that our data are from a subsample of the 2011 UAIS, a study originally designed to assess HIV-exposure, not aflatoxin exposure. We were unable to use the original study design weights in our analyses, and so our results are not representative population-based estimates. In the absence of using sampling weights, our analysis of approximately the same number of serum samples from each of the survey-defined regions may have resulted in an oversampling of the rural population. This, along with our decision to only analyse HIV-negative samples may have also influenced the concentrations observed. To avoid presenting results based on small numbers of observations we only performed single variable stratified analyses. In addition, we predominantly show bivariate associations within the data, and thus confounded by other variables may be present. No food sampling data were available in the 2011 UAIS to correlate with exposure, nor were there food diaries from survey participants. Due to the temporal distribution of sampling in the 2011 UAIS, there is no clear "snapshot" of time during which exposure occurred, which presents an additional challenge in associating dietary exposure, as seasonal variation in toxin concentration of crops and subsequent exposure is known to occur (Castelino et al. 2014). Our use of the serum AFB1-lys adduct biomarker does provide a longer window of exposure compared to other markers, but not having time of sampling to correlate to seasonal variation and/or food contamination is a limitation. Additionally, there is no established link between clinical outcome and biomarker concentration, and no associated health-outcome data were available for those individuals surveyed, thus we cannot assess any association of health with biomarker concentration.

Continuing to investigate aflatoxin exposure, in both the presence and absence of aflatoxicosis outbreaks, is essential to improving our understanding of the many factors associated with aflatoxin exposure, and a better understanding of these factors is key to developing strategies to reduce exposure and improve health outcomes. This study, and many previous, show a high prevalence of aflatoxin biomarkers in populations in and surrounding Uganda. There is a strong need for education and surveillance measures to be incorporated to reduce population exposure to aflatoxins.

Disclaimer

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