Research Article

Determinants of recent aflatoxin exposure among pregnant women in rural Zimbabwe

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Received: 02/12/2016; Revised: 12/04/2017; Accepted: 18/04/2017

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mnfr.201601049.

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Keywords: aflatoxin, dietary diversity, pregnancy, spatial variation, temporal variation

Abbreviations: SHINE, Sanitation, Hygiene Infant Nutrition Efficacy Trial; **AFM1,** Aflatoxin M1; **AF**, aflatoxin; **Afl-alb**, aflatoxin-albumin; **LAZ**, length-for-age Z score; **WAZ**, weight-for-age Z score;

Abstract

Scope: Aflatoxins are toxic secondary metabolites of *Aspergillus* species that contaminate staple foods such as maize and groundnuts. Aflatoxin exposure during pregnancy has been associated with adverse birth outcomes in limited-scale surveys in sub-Saharan Africa. The objective of this study was to describe the determinants of aflatoxin exposure, using urinary aflatoxin M1 (AFM1) biomarkers and data generated by the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial for rural Zimbabwean women in early pregnancy. SHINE is a large, cluster-randomized community-based trial in Zimbabwe designed to investigate the independent and combined effects of nutrition and hygiene interventions on early child growth.

Methods and results: Urine samples collected from 1,580 pregnant women in rural Zimbabwe at median gestational age of 13.9 weeks were measured for AFM1. AFM1 was detected in 30% of samples (median of exposed, 162 pg AFM1/mg creatinine; range 30-6046 pg AFM1/mg). In

multivariable ordinal logistic models, geographical location ($p<0.001$), seasonality ($p<0.001$) and dietary practices ($p=0.011$) were significant predictors of urinary AFM1.

Conclusion: This is the largest aflatoxin biomarker survey conducted in Zimbabwe, and demonstrated frequent exposure in pregnant women with clear temporal and spatial variability in aflatoxin biomarker levels.

Graphical Abstract

Aflatoxin exposure during pregnancy has been associated with poor birth outcomes in limited-scale surveys in sub-Saharan Africa. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial is a large, cluster-randomized community-based trial in Zimbabwe designed to investigate the effects of combined nutrition and hygiene interventions on early child growth, and to explore the potential contribution of aflatoxin exposure. The objectives of this paper are to describe the determinants of aflatoxin exposure during early pregnancy among women enrolled in the SHINE trial.

1 Introduction

Mycotoxins are naturally occurring poisons produced by fungi that frequently colonize crops under stress conditions. Aflatoxins (AFs), one of the major groups of mycotoxins from a public health perspective, are toxic secondary metabolites of *Aspergillus* species that contaminate staples such as maize and groundnuts. It is estimated that approximately 0.5 billion people, predominantly those living in developing countries, are at high risk of chronic exposure to high levels of AFs [1]. AFs have been most widely studied in the etiology of liver cancer and shown to be causative [2, 3]. AFs also inhibit protein synthesis and are cytotoxic, teratogenic and immunotoxic [2]

Epidemiologic evidence from multiple countries has additional demonstrated AF exposure in pregnant women, infant cord blood and young children [4-15] suggesting that exposure during early life is widespread. Several studies have demonstrated that AF exposure was associated with poor growth [4, 15, 16]. Gong *et al.* [4] demonstrated a significant inverse dose-response relationship between serum aflatoxin-albumin (AF-alb) biomarker concentration and both length-for-age (LA) and weight-for-age (WA) Z-scores. among young children in rural Benin and Togo, as. In a subsequent longitudinal study in rural Benin, young children in the highest quartile of AF-alb had significantly reduced growth velocity (1.9 cm less growth from baseline, over eight months), compared to those in the lowest quartile of AF-alb, with a dose-dependent relationship after adjustment for age, sex, height at recruitment, socioeconomic status, village and weaning status [16]. In a Gambian study of 138 mother-infant pairs, the average AF-alb in maternal blood during pregnancy (at two time-points) was significantly associated with infant growth velocity from birth to 52 weeks [15]. Exposure to AF in these infants at age 16 weeks, further and significantly contributed to reduced growth velocity over the subsequent 36 weeks [15].

Although it is estimated that mycotoxins contaminate up to 25% of the world's food supply [17], prevalence and severity of exposure vary by geography, season and calendar year, between households within a given area and even within an individual household's different food stores [18, 19]. The extent of AF accumulation varies spatially and temporally, depending on several factors, including the burden and strain of *Aspergillus*; temperature, water and nutrient availability during critical periods of plant development; nitrogen availability; soil texture and chemical properties; and plant density [20, 21]. Drought stress, especially during the flowering and early grain-filling stages, has been associated with increased *A. flavus* growth and AF accumulation in maize [22-25]. Exposure at the household level further depends on post-harvest activities that include drying and/or threshing, storage practices, pest damage, and dietary consumption patterns [26-30]. Accordingly, developing effective interventions to mitigate AF exposure requires a critical understanding of the modifiable and non-modifiable risk factors for household-level AF exposure.

Aflatoxin exposure during pregnancy has been documented in limited-scale surveys in sub-Saharan Africa and Asia [12, 13, 31-34], but little is known about exposure patterns in southern Africa. The objective of this study was to describe the determinants of aflatoxin exposure, using urinary aflatoxin M1 (AFM1) biomarkers and data generated by the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial for rural Zimbabwean women in early pregnancy.

2 Materials and Methods

2.1 Population and data collection

This study was conducted in Chirumanzu and Shurugwi districts in the Midlands Province of rural Zimbabwe, a subsistence farming region with considerable topographic variation. Chirumanzu and

Shurugwi districts, respectively, are further divided into 25 and 24 administrative wards and have populations of 80,000 and 78,000, respectively [37]. Maize and groundnuts are the major food crops with a single harvest season in which planting generally begins with the first rains in November and crops are harvested in April and May [38]. The districts are largely rural and have a high prevalence of child stunting, with a mean LAZ at 24 months of -1.97 in the Midlands province[39].

The data were collected as part of the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial. This is a cluster-randomized community-based trial investigating the independent and combined effects of a nutrition intervention and a water, sanitation and hygiene intervention on stunting and anemia [40]. Briefly, between November 2012 and April 2015, 5280 pregnant women were enrolled at a median gestational age of 13.9 weeks through prospective pregnancy surveillance. Within two weeks of enrollment, a baseline visit was conducted, during which data on household demographics, dietary practices and child care behaviors were collected, along with a maternal mid-stream urine sample. Enrollment into the trial was relatively balanced geographically and temporally with 150-200 baseline visits completed each month. The present analysis is limited to 1580 (77%) of 2054 women enrolled between April 2013-March 2014 (representing one harvest year) for whom archived urinary samples were available. There were no significant differences in the demographic characteristics of women with and without available urine samples (data not shown).

Trial clusters were grouped into four geographic SHINE survey regions, with each served by one research laboratory: St. Theresa's Mission Hospital in southern Chirumanzu; Mvuma District Hospital in Mvuma town, northeast Chirumanzu; Tongogara Clinic in Shurugwi district; and Shurugwi District Hospital in Shurugwi town, Shurugwi district. Written informed consent was obtained from all participants in their local language. Ethical approvals for the SHINE trial and for this study were obtained from the Medical Research Council of Zimbabwe (IRB#:MRCZ-A-1675)

and from the institutional review board of Johns Hopkins Bloomberg School of Public Health, USA (IRB#:00004205).

2.2 AFM1 and creatinine analysis

Urine samples were transported in cooler boxes from participants' homes to the closest field laboratory and then stored at -80C until August 2014. Urine samples were thawed for measurement of AFM1, a validated exposure biomarker of aflatoxin ingestion in the prior 2-3 days [41]. All AFM1 assays were undertaken at the Zvitambo laboratory in Harare. Briefly, urine samples were centrifuged to remove precipitate and diluted with distilled water. AFM1 was measured in duplicate by direct ELISA (Helica, USA), using the manufacturer's standards and controls; ELISA plates were read using a Dynex MRX microplate reader (Dynex Technologies, USA). Serial dilutions conducted on 30 urine samples indicated that multiple dilutions of 1:10 and greater generated statistically similar concentrations (after adjustment for dilution), while more concentrated samples (e.g. 1:5) gave inconsistent data. All samples were therefore analysed initially at a 1:10 dilution and further diluted as necessary to fall within the range of the ELISA standard curve. Two internal quality controls (a negative and a positive) were included on each plate. The calculated detection limit for this assay was 80 pg AFM1/ml urine based upon a t-test of the lowest detectable optical density that was significantly different from 0 pg AFM1 ($p<0.05$). The intra-plate coefficient of variation (CV) for the assay was <3% and inter-plate CV <15%. Urinary creatinine was measured to adjust for differences in urinary flow between individuals, using a colorimetric test with reported intra-plate and inter-plate CVs <10% (Human Diagnostics, Germany). Both AF and creatinine have been found to be stable during storage [42, 43].

Survey Data Variable Pre-processing

Variables used in analyses were taken from the baseline survey, administered at the time of sample collection. A wealth index was created using the tetrachroric PCA command (STATA) including 18 variables on current access to water and sanitation, durable assets, and household characteristics equivalent to the variables used in the DHS rural wealth index [44, 45]. Of the 4,705 participants with a baseline visit, 4,666 (99% of the respondents) contributed data towards the wealth index construction.

A dietary diversity questionnaire (adapted from [46]) with 15 categories that referenced the previous 24 hours of food consumption was used to create an indicator of the proportion of aflatoxin-prone foods (PAPF) consumed (Supplemental Table 1). Foods that are prone to contamination with AF include maize, nuts, milk and vitamin A-rich fruits (mango and papaya) [47-50]. The number of food groups prone to AF contamination was divided by the total number of food groups consumed to create the PAPF.

Seven questions from the Coping Strategy Index (originally designed to measure food insecurity) [51] were used as indicators of AF consumption risk. This was intended to test the hypothesis that coping strategies influence the consumption of aflatoxin-prone foods (Supplemental Table 2). Risky practices include: borrow food, rely on less expensive foods, harvest immature crops and send household members to beg. Protective practices include: limit portion size, reduce number of meals, and gather unusual types of wild food or hunt. For example, limiting portion size at mealtimes would potentially decrease exposure through reduced consumption of staple food, while harvesting immature crops would potentially increase exposure because immature crops are less likely to be fully dried.

GIS Data Sources and Pre-processing

Global positioning coordinates of each cluster representing geographical areas ranging from 43-9,771 square hectares were linked to remotely-sensed, spatially-explicit rainfall estimates for 2012-2014. These data were downloaded from FEWSNET as raster files containing daily total rainfall at 8 km^2 resolution. Rainfall rasters were re-projected into the longitude/latitude coordinates system for further analysis. Responses were extracted as the average values for each cluster and aggregated as monthly and annual rainfall totals. GIS data were extracted from the raster files using the raster package in R (v. 2.0-12 in R. v. 2.15.1, http://www.r-project.org/). Aggregation of variables was performed with an R script.

Elevation data were extracted for each of the coordinates using an online file conversion in which a Google Earth KML file was converted to a csv file and elevation data were added using NASA's Space Shuttle Radar Topography Mission, which includes 90 m resolution for the world (http://www.gpsvisualizer.com).

2.3 Statistical analysis

Urinary AFM1 levels were not normally distributed. Data were grouped into exposure categories representing non-detectable AFM1 (<80 pg AFM1/ ml urine) and three tertiles of detectable AFM1 (30-115, 116-230 and >230 pg AFM1/mg creatinine), henceforth referred to as "low," "medium" and "high" exposure categories. Maternal AFM1 in individual women was linked to demographic information at the household level collected from each woman. Univariate analyses were conducted to assess variation by maternal and household characteristics, dietary practices and environmental characteristics. Trends across groups were examined using the nptrend command in Stata. Multivariate models were tested using ordinal logistic regression. Model 1 represented demographic

variables including location, time since harvest, maternal education and wealth index. Model 2 added additional dietary practices variables, including the dietary diversity ratio, risky dietary practices scale and protective dietary practices scale. Model 3 added the effect of environmental variables rainfall and elevation. Significant time and geographical trends were assessed further by examining urinary aflatoxin levels by calendar month and mapping exposure prevalence by administrative ward. Stata 12.0 was used to conduct all analyses. Graphics were created with R 2.15 and QGIS 2.6.1.

3 Results

Study Participant and Region Characteristics

Baseline characteristics of enrolled women are shown in Table 1. Mean maternal age was 26.8 y, mean mid-upper arm circumference (MUAC) was 26.4 cm and mean gestational age was 13.9 weeks at the time of urine collection. Overall, 43% had completed secondary school, 36% some secondary and 21% some primary or completed primary and 10% were employed. Households had an average of five members, and 8% had electricity. The median farm size was 2 hectares.

Of the four study regions, approximately 30% of study participants were from Mvuma, 14% from Shurugwi, 30% from St. Theresa's and 25% from Tongogara. The mean elevation in the study area was 1,232 m (range= 980-1500 m) and the mean annual rainfall was 907 mm (range= 775-1,070 mm). Figure 1B and 1C illustrate the variation in rainfall and elevation in the study region.

Aflatoxin Exposure

Overall, 30% (484/1,580) of pregnant women had detectable AFM1 in their urine above the detection limit of 80 pg AFM1/ml urine (median concentration 162.5 pg/mg creatinine, interquartile range (IQR) 100.0-286.5). AFM1 values ranged from 31 to 6,046 pg/mg.

Figure 1A depicts the geographical distribution of AFM1 in the two study districts. AFM1 was almost ubiquitous across the districts, with median prevalence in individual administrative wards of 30% (range 0-75%). There were spatial trends in the severity of exposure, with a higher proportion of women being exposed in the southern portion of the study area (St. Theresa's), which is lower in elevation and rainfall.

There was significant variation in AFM1e across the calendar year (Figure 2). Detectable AFM1 decreased somewhat linearly from the time of maize harvest. The pattern in overall exposure prevalence differed by exposure tertile. The highest exposure prevalence occurred in May (63/130 exposed) and January (77/168 exposed), when almost 50% of women had detectable AFM1 in their urine; by contrast, severity of exposure (tertile 3) peaked in September, when almost 10% of women had AFM1 levels above 230 pg/ mg creatinine.

Associations between AF exposure and characteristics

Several baseline factors differed significantly across AFM1 exposure groups (Table 2). A higher proportion of women had secondary education in the high, medium, and low exposure groups compared to the non-detectable group (p value for trend=0.002). Though women's dietary diversity score was not different across AFM1 exposure groups, the PAPF was higher in the AFM1-exposed groups and lowest in the non-detectable group with a significant trend, (p=0.003). Similarly, having protective dietary practices was negatively associated with AFM1-exposed groups although the tertiles of AFM1-exposed groups did not appear to differ from one another, (p=0.026). AFM1 exposure groups did not significantly differ in head of household gender, access to electricity, wealth quintiles, or farm size.

AFM1was consistently higher in the St. Theresa's area, which is lower in elevation than the other areas. There was a strong trend for AFM1 to be higher in households with lower levels of rainfall ($p<0.0001$) and lower areas of elevation ($p<0.0001$).

In multiple ordinal logistic regression, geographic location, time in months since harvest, maternal education, dietary variables and elevation were all significant predictors of AFM1, whereas wealth and rainfall were not (Table 3). In the multivariate models, the St. Theresa's study area had higher odds of AFM1 in models that did not include elevation (OR=2.13, 95% CI =1.57-2.88, P<0.001).

Each additional month since harvest was associated with a 7% decreased odds of level of AFM1 (OR=0.93, 95%CI= 0.90-0.96, P<0.001). For example, if a woman was in the second tertile of exposure in April, she would have a 7 percent odds of being one tertile lower for each month since harvest, suggesting that AF exposure was greatest in April-June and decreased later in the year. Maternal education was significantly associated with increased odds of AFM1 in all models (for model 2 for those completing secondary school, (OR=1.50, 95%CI=1.08-2.06, P=0.015), and in model 3 (OR=1.45, 95%CI=1.04-2.00, P=0.026). The proportion of AF-prone foods (PAPF) consumed was associated with 16% higher odds of elevated of aflatoxin exposure (OR=1.16 95%CI=1.04-1.29, P=0.008). Mothers practicing more than two protective dietary practices (PPDP) had a 46% lower odds of level of AFM1 (OR=0.54, 95% CI=0.36-0.81, P=0.003), whereas mothers practicing one risky practice (PRDP) had a 37% increased odds of elevated of AFM1 (OR=1.37, 95% CI=1.04-1.80, P=0.026). Rainfall was not associated with AFM1 in full models, but each 100-m increase in elevation was associated with a 17% decrease in odds of aflatoxin exposure (OR=0.83,

95% CI=0.72-0.96, P=0.01). In Model 3, when altitude was added to the model, the relative odds ratio of the St. Theresa's location was attenuated (OR=1.40, 95% CI=0.91-2.15, P=0.13).

4 Discussion

AF exposure was common among pregnant women in rural Zimbabwe. Overall, 30% of women assessed early in pregnancy had detectable aflatoxin in their urine, indicating recent ingestion of the toxin. The results show that season, geographical location and dietary practices may be important determinants of AF exposure. Exposure was unrelated to relative wealth, but surprisingly, AFM1 was related to maternal educational status, with mothers who had completed secondary school at increased odds of aflatoxin exposure. Although AFM1 is a short-term biomarker that reflects toxin intake in the prior two to three days [41], it has been found to correlate with serum AF-Alb, a longer-term indicator of cumulative exposure (over 2-3 months), indicating that short-term exposure may be a proxy for longer-term exposure [52, 53].

This is the first comprehensive study of the prevalence and determinants of population-wide aflatoxin exposure in Zimbabwe since the 1980's. Two surveys conducted almost 30 years ago assessed urinary aflatoxin biomeasures and, in a subset of women, breast-milk samples for aflatoxins AFB1, B2, G1, G2 and M1 [35, 36]. In the first survey (n=1,228), exposure by province ranged from 0.7% in Manicaland to 8% in Mashonaland West. The same research group analyzed a larger sample (n=2,553) and found that exposure ranged from 4.1% in Manicaland to 11.4% in Masvingo. In Manicaland, the average altitude is 2,500 meters above sea level, with a maximum temperature of 17°C, which may be a limiting factor for fungal growth [54], whereas the areas with higher levels of contamination were of medium altitude with moderate rainfall. The mean concentration of AFM1

among detectable samples using thin-layer chromatography was extremely high (4.2 ng/ml) [35, 36]. Since this method was likely not as sensitive as the ELISA method we used, some samples detectable in the present study would have been classified as non-detectable in the earlier survey, suggesting that aflatoxin prevalence may previously have been underestimated. Additionally, 11% (6/54) of breast milk samples in the 1980's studies contained detectable levels of aflatoxins. These two studies found relatively low prevalence of exposure, but reported important geographic variability in exposure by altitude and average rainfall patterns, consistent with findings in our study [35, 36].

More recently, using archived Zimbabwean samples from a nationally representative sample of mothers of children under 5 years collected between October and December 2011 (n=287), we found that 17% of maternal urine samples had detectable AFM1 (mean of exposed: 390 pg/ml, range 150-3,740 pg/ml; no creatinine adjustment), with prevalence of exposure varying by region [55]. This is comparable to the current study of SHINE women, in whom the mean urinary concentration of detectable samples was 270 pg/ml before adjustment for creatinine. Overall, the Zimbabwean levels are much higher than those reported from Brazil (range 0.19- 12.7 pg/ mg creatinine) or Egypt (geometric mean 19.7 pg/mg creatinine)[52], but similar to those reported in two recent studies of adults and children in Ghana, and one study in children from Guinea, both considered a high-risk countries [56-58].

We found significant associations of aflatoxin exposure with a range of demographic and dietary variables. Consumption of foods that are prone to AF contamination (maize, groundnuts, milk and vitamin-A fruits) was associated with significantly increased odds of AF exposure, suggesting that increased reliance on these foods is a risk factor. Maternal education was associated with significantly increased odds of AF exposure. It is possible that maternal education is a proxy other risks, but it is

important to note that higher education was not protective against exposure. Although there were significant associations between maternal education and wealth (chi^2=83.7 p<0.001), no association between wealth and AF exposure was found. There are conflicting evidence for associations between wealth and aflatoxin exposure in prior studies. Four studies investigated the socio-demographic determinants of AF exposure: two (Kenya and Ghana) reported lower AF exposure in higher-income households [59, 60] and two (also Kenya and Ghana) reported no relationship [53, 61]. Although AF exposure did not vary across wealth quintiles, it is possible that the pathways to exposure vary among economic subgroups. In our data, we found that 80% of households in the highest wealth quintile reported eating maize from their own field in the last seven days, whereas farmers in the lowest quintile were more likely to source food from the market or as a gift. The pathway through which wealthier farmers may be exposed could be larger grain stores resulting in longer storage time, which may increase the chance for development of AF in the grain. Poorer farmers rely more heavily on donations and market supplies of grains that may be more contaminated than home-grown stores [62].

Although it is commonly assumed that food-insecure farmers would be more at risk for AF exposure, it is possible that risk-inducing and risk-protective coping practices occur together. In our analyses, practices of borrowing food from friends or relatives, relying on less expensive or less preferred foods, harvesting immature crops, and sending household members to beg were associated with increased odds of AF exposure, whereas limiting portion size at meals, reducing number of meals eaten per day and gathering unusual types or amounts of wild food/hunting were predictive of decreased odds of AF exposure (analysis of individual practices in Supplemental Table 2). While these practices would not be recommended for multiple health reasons, this finding does highlight that the relationship between food insecurity and aflatoxin exposure may be complex.

One strength of our study is the relatively large sample size and analysis of specimens collected over a full calendar year, which allowed us to describe both typical exposure within the population and seasonal variability. We found significant temporal and spatial variation in AF exposure in the study area. Although the temporal trend was not linear, exposure prevalence was highest immediately after maize and groundnut harvest and tended to decrease progressively after harvest. In our study area, groundnuts are commonly consumed immediately after harvest. Groundnuts contaminated in the field may be the source of high AF exposure seen in May. These findings suggest that while post-harvest toxin accumulation is an important contributor to household exposure, the levels of exposure at harvest might indicate the importance of pre-harvest toxin accumulation. Pre- and post-harvest practices could be important determinants of AF exposure at the household level [63]. We did not explore these factors because of the short-term nature of AFM1 compared to potentially long-term recollections of the previous harvest season (up to 11 months prior).

This study also had limitations. We only analyzed one urine sample per woman, and therefore could not examine within-person variation, which is likely significant. The AFM1 ELISA is relatively inexpensive, commercially available, and relatively rapid. The detection limit of the ELISA assay we used was 80 pg/ml, whereas other methods such as HPLC are able to detect AFM1 in <10 pg amounts. The implication is that our non-detectable category is a combination of truly-exposed individuals lower than 80 pg/ml and truly non-exposed individuals. Thus, when comparing these results to other studies, our method likely underestimated the true prevalence of low exposure. The true proportion of pregnant women recently exposed to AF would thus likely be greater than the 30% ; assuming that low levels of exposure are hazardous, aflatoxin contamination of food is a potentially significant public health problem in these two districts of Zimbabwe.

Given the high prevalence of AF exposure, strengthening agricultural extension programs would decrease risky pre- and post-harvest practices. Additionally, current dietary interventions focus on increasing household dietary diversity and household food security, but do not account for the potential relationship between changing dietary practices and risk of AF exposure. In our analyses, we showed that a higher reliance on AF-prone foods such as maize, groundnuts, lentils and milk was associated with AF exposure. Certain foods (e.g. groundnuts) that are emphasized to improve dietary diversity may benefit diets, but only if they do not contain dangerous levels of AF..

In summary, we found that at least 30% of pregnant women living in rural Zimbabwe had recently been exposed to aflatoxin, with variation in prevalence by geography, season and dietary practices. This potent liver carcinogen may also contribute to the pathogenesis of adverse birth outcomes and child stunting, potentially at concentrations found in this study, as they were similar to those of other high-risk settings. Establishment of national AF exposure monitoring programs may be necessary to identify at-risk populations and to develop effective mitigation strategies. This study highlights a need to incorporate consideration of AF-prone foods and conditions favoring accumulation into food security programs and public health policy to adequately address the nutritional food safety and security of rural populations, particularly pregnant women and children.

Author contributions: LES and SR conducted lab analyses, LES conducted statistical analyses, LES, MMNM, AJP, PCT, JHH, RJN, SR, AC, GK and RJS have written the manuscript.

Acknowledgements: We are grateful to our funders who supported this work including: USDA NIFA Pre-doctoral Fellowship (2013-67011-21116) and Cornell Atkinson Center for a Sustainable Future Academic Venture Fund. AJP is funded by the Wellcome Trust (108065/Z/15/Z). We acknowledge the support of the Zimbabwean Ministry of Health and Child Care for making this research possible.

The authors have declared no conflicts of interest.

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Tables

Table 1. Characteristics of Pregnant Zimbabwean Women

Characteristic[a]		
Maternal		
Age (Years)	Mean (SD)	26.8 (8.1)
MUAC (cm)	Mean (SD)	26.4 (3.1)
Education %(n)	Primary	21.0 (315)
	Some Secondary	36.4 (546)
	Completed Secondary	42.6 (639)
Dietary Practices		
Dietary Diversity Score	Mean (SD)	3.7 (1.4)
AF-prone foods -PAPF	Mean (SD)	0.24 (0.10)
Risky Dietary [b] Practices % (n)	none	59.2 (924)
	one	27.0 (422)
	two - three	13.7 (214)
Protective Dietary Practices % (n)	none	67.0 (1048)
	one	15.9 (248)
	two - four	17.1 (268)

Household Demographics		
Employed	%(n)	9.8 (153)
Electricity	% (n)	7.6 (119)
Household Size	Mean (SD)	4.9 (2.1)
Wealth Index[c],	Quintile 1	18.9 (296)
% (n)	2	20.0 (314)
	3	19.6 (307)
	4	20.8 (326)
	5	20.7 (325)
Land (Hectares)	Median IQR	2.0 (1.0, 3.6)
Environmental		
Location % (n)	Mvuma	30.2 (478)
	Shurugwi	14.2 (224)
	St. Theresa's	30.2 (477)
	Tongogara	25.4 (401)
Months Since Harvest	Mean (SD)	5.8 (3.6)
Total Rainfall [d]	Mean (SD)	907 (47)
Altitude (m)	Mean (SD)	1232 (125)

[a] Nos. of observations were 1501 (age); 1564 (MUAC); 1500 (education); 1555 (women's dietary diversity score); 1462 af-prone food ratio); 1560 (risky dietary practices); 1564 (protective dietary practices); 1564 (employed); 1562 (electricity); 1496 (household size); 1568 (wealth index); 1301 (land farmed); 1580 (location and time since harvest (months)); 1572 (total rainfall (mm)); 1570 (altitude (m)).
[b] Details for scores are outlined in Supplemental Table 2. Risky practices include borrow food, rely on less expensive foods, harvest immature crops and send household members to beg. Protective practices include: limit portion size, reduce number of meals, gather unusual types of wild food or hunt.
[c] Asset-based wealth index developed using polychoric PCA with 18 variables representing current access to water and sanitation, durable assets, and household characteristics equivalent to the variables used in the DHS rural wealth index[44, 45]. Asset based index developed based on 4666 SHINE households and quintiles calculate.
[d] Total rainfall (cm) for the 2013-14 harvest season.

Table 2. Determinants of urinary AFM1 in Pregnant Zimbabwean Women

Characteristic[a]		AFM1 ND	AFM1 Tertile 1	AFM1 Tertile 2	AFM1 Tertile 3	P-value (trend)-)[b]
Education Primary		23.0 (238)	13.9 (21)	18.6 (31)	16.4 (25)	**0.002**
% (n) Some Secondary		37.1 (382)	33.8 (51)	37.1 (62)	33.6 (51)	
Completed Secondary		39.8 (410)	52.3 (79)	44.3 (74)	50.0 (76)	
Wealth Index[d], Quintile	1	19.3 (211)	18.8 (29)	18.0 (30)	16.9 (26)	0.94
% (n)	2	20.0 (219)	18.2 (28)	17.4 (29)	24.7 (38)	
	3	19.1 (209)	18.2 (28)	19.8 (33)	24.0 (37)	
	4	21.1 (231)	20.8 (32)	22.8 (38)	16.2 (25)	
	5	20.4 (223)	24.0 (37)	22.2 (37)	18.2 (28)	
Dietary Diversity Score Mean (SD)		3.7 (1.4)	3.6 (1.4)	3.8 (1.2)	3.8 (1.4)	0.60
AF-prone foods-PAPF Mean (SD)		0.24 (0.10)	0.27 (0.10)	0.25 (0.10)	0.26 (0.09)	**0.003**
Risky Dietary [c]	none	59.6 (647)	63.4 (97)	55.0 (93)	56.9 (87)	0.56
Practices % (n)	one	26.0 (282)	26.0 (40)	32.7 (55)	29.4 (45)	
	two - three	14.4 (156)	11.0 (17)	11.9 (20)	13.7 (21)	
Protective Dietary	none	65.2 (709)	74.0 (114)	68.4 (115)	71.4 (110)	**0.026**
Practices % (n)	one	15.8 (172)	13.6 (21)	18.4 (31)	15.8 (24)	
	two - four	19.0 (207)	12.3 (19)	13.1 (22)	13.0 (20)	
Land (Hectares) Median IQR		2.0 (1.0,3.6)	2.0 (1.0, 4.0)	1.6 (1.0, 3.6)	1.6 (1.0, 3.0)	0.11
Location % (n)	Mvuma	33.5 (367)	26.4 (41)	21.0 (36)	21.5 (34)	**0.001**
	Shurugwi	14.5 (159)	18.1 (28)	12.3 (21)	10.1 (16)	

St. Theresa's	25.9 (284)	36.1 (56)	41.4 (71)	41.8 (66)	
Tongogara	26.1 (286)	19.4 (30)	25.1 (43)	26.6 (42)	
Months Since Harvest Mean (SD)	6.1 (3.5)	5.6 (3.5)	4.9 (3.7)	5.1 (3.6)	**<0.0001**
Total Rainfall [e] Mean (SD)	910 (48)	903 (45)	895 (41)	901 (51)	**<0.0001**
Altitude (m) Mean (SD)	1242 (126)	1218 (122)	1216 (121)	1195 (118)	**<0.0001**

[a] Nos. of observations were: 1500 (education); 1555 (women's dietary diversity score); 1462 af-prone food ratio); 1560 (risky dietary practices); 1564 (protective dietary practices); 1568 (wealth index); 1301 (land farmed); 1580 (location and time since harvest (months)); 1572 (total rainfall (mm)); 1570 (altitude (m)). Age, MUAC, employment, electricity household size are not shown because there were no hypotheses that these variables would be associated with urinary AFM1 and there were no significant differences across exposure levels.

[b] Nptrend (Stata) command was used to calculate the p values for trends.

[c] Details for scores are outlined in Supplemental Table 2. Risky practices include borrow food, rely on less expensive foods, harvest immature crops and send household members to beg. Protective practices include: limit portion size, reduce number of meals, gather unusual types of wild food or hunt.

[d] Asset-based wealth index developed using polychoric PCA with 18 variables representing current access to water and sanitation, durable assets, and household characteristics equivalent to the variables used in the DHS rural wealth index[44, 45]. Asset based index developed based on 4666 SHINE households and quintiles calculate.

[e] Total rainfall (cm) for the 2013-14 harvest season.

Table 3. Multivariable Ordinal Logistic Models assessing Predictors of urinary AFM1[2]

		Model 1 N=1472 Pseudo R^2=0.0225	Model 2 N=1445 Pseudo R^2=0.0275	Model 3 N=1435 Pseudo R^2=0.0317
Predictor		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Location (ref Mvuma)	Shurugwi	1.20 (0.83, 1.73)	1.14 (0.77-1.68)	0.65 (0.38,1.11)
	St. Theresa	**2.20** **(1.65-2.94)****	**2.13** **(1.57-2.88)****	1.40 (0.91-2.15)
	Tongogara	1.33 (0.98-1.81)	1.34 (0.97-1.86)	0.82 (0.51-1.31)
Time since harvest (months)		**0.93** **(0.90-0.96)****	**0.93** **(0.90-0.96)****	**0.93** **(0.90-0.96)****
SES (quintile)[a]	2	1.08 (0.76, 1.55)		
	3	1.18 (0.83-1.69)		
	4	0.92 (0.64,1.31)		
	5	1.00 (0.71,1.44)		
Mother's education (ref primary)	Some Secondary	**1.38** **(1.00 1.90)***	1.31 (0.94-1.83)	1.26 (0.90-1.76)
	Completed Secondary	**1.61** **(1.17-2.20)****	**1.50** **(1.08-2.06)***	**1.45** **(1.04, 2.00)***
Proportion of AF prone foods[b]			1.15 (1.03, 1.28)	**1.16** **(1.04, 1.29)***
AF-risky Food Security Behaviors	1		**1.41**	**1.37**

[c]		**(1.07, 1.84)***	**(1.04, 1.80)***
	2-4	1.28 (0.84-1.94)	1.25 (0.83, 1.90)
AF-protective Food security Behaviors [d]	1	0.91 (0.66, 1.25)	0.92 (0.67, 1.27)
	2-3	**0.52 (0.35-0.78)****	**0.54 (0.36, 0.81)****
Altitude (Per 100 m increase)[e]			**0.83 (0.72-0.95)****
Total Rainfall (per 10mm increase)[e]			0.99TH (0.95, 1.02)

*p=<0.05; ** p<0.01

[a] Asset-based wealth index developed using polychoric PCA with 18 variables representing current access to water and sanitation, durable assets, and household characteristics equivalent to the variables used in the DHS rural wealth index[44, 45]. Asset based index developed based on 4666 SHINE households and quintiles calculate.

[b] The odds ratio reflects a 10% change in the proportion of aflatoxin-prone foods. For example, a 10% increase on reliance of aflatoxin-prone foods is associated with an 11% increase in the odds of a mother being in the low exposed category vs. the non-detectable category or being in the high category vs. the medium category.

[c] Potentially risky dietary behaviors include: borrow food from friends or relatives, rely on less expensive or less preferred foods, harvest immature crops, and send household members to beg for food.

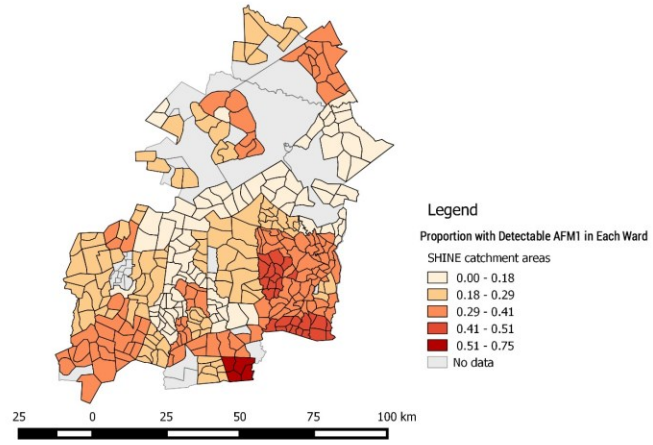
[d] Potentially protective dietary behaviors include: Limit portion size at meals, reduce number of meals eaten per day and gather unusual types or amounts of wild food/hunt.

[e] For rainfall , the odds ratio reflects a 10 millimeter change and for elevation, the odds ratio reflects a 100 meter change.

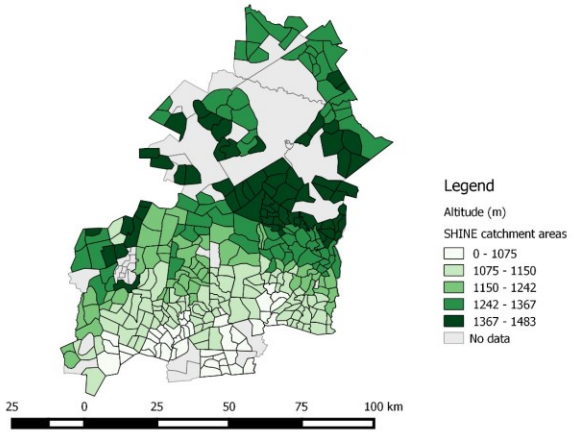
Figure legends

Figure 1. Variation in urinary AFM1 in pregnant women, rainfall and elevation in Chirumanzu and Shurugwi, Zimbabwe. A. Prevalence of pregnant women with detectable urinary AFM1 in each administrative ward. B. Mean elevation in meters in each administrative ward. C. Mean 2013-14 rainfall in millimeters in each ward. "No data" represents areas where there are no study participants. Overall, 30% of women were exposed to aflatoxin and there was significant geographical variation in exposure in the study region with prevalence of exposure highest in the south east portion of the districts. The mean elevation in the study area was 1,232 m (range= 980-1500 m) and the mean annual rainfall was 907 mm (range= 775-1,070 mm).

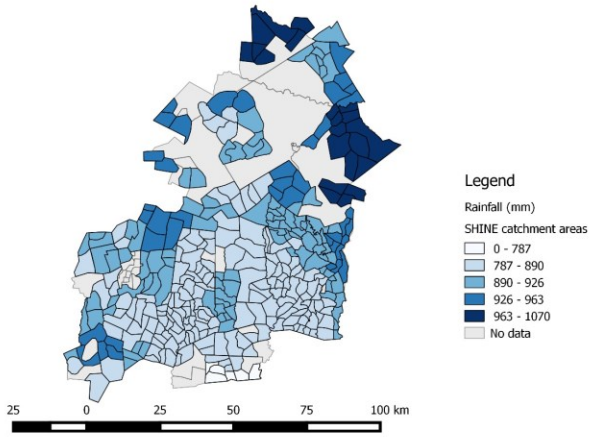
A. **Geographical Distribution of Aflatoxin Exposure in Chirumanzu and Shurugwi**



B. **Altitude in Chirumanzu and Shurugwi (m)**



C. **Rainfall in Chirumanzu and Shurugwi (mm)**



ed.

Figure 2. Temporal variation in levels of urinary AFM1 in 2013-14 in Chirumanzu and Shurugwi, Zimbabwe. The sample size for each month ranges from 96 (September 2013) to 185 (January 2014). Maize harvest occurs in April and groundnut harvest occurs in May. There was significant variation in aflatoxin exposure across the calendar year. Detectable aflatoxin decreased somewhat linearly from the time of maize harvest, but the pattern in overall exposure prevalence differed by exposure tertile. Exposure prevalence peaked in May, when 50% of women had detectable aflatoxin in their urine; by contrast, severity of exposure peaked in September, when almost 10% of women had AFM1 levels above 230 pg/ mg creatinine.