

## Seasonal and geographical differences in aflatoxin exposures in Senegal

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### RESEARCH ARTICLE

#### Abstract

The aim of the study was to determine the geographical and seasonal variations in aflatoxin dietary exposure levels in adults from Senegal. A total of 168 adults (50% male) were recruited from three districts: Nioro du Rip (n=90), located in the Sudan Savannah agro-ecological zone where rainfall is sufficient for groundnut growth; Saint-Louis (n=40) and Mboro (n=38), located in the Sahel zone where groundnuts are produced under irrigated conditions. Diet information and samples were collected at groundnut harvest and post-harvest seasons. Plasma aflatoxin-albumin adducts (AF-alb) and total aflatoxin in household groundnut samples were measured by ELISA and a quantitative thin layer chromatography method, respectively. The blood AF-alb geometric mean was 45.7 pg/mg albumin (range 5.5-588.2 pg/mg). Nioro du Rip had a higher AF-alb level at harvest than Saint-Louis and Mboro (80.0 vs 15.6 and 33.3 pg/mg,  $P<0.001$ ). Similar trends were observed at post-harvest ( $P<0.05$ ). Seasonal trends were not consistent across the districts as Nioro du Rip had a higher AF-alb level at harvest than post-harvest (80.0 vs 58.6 pg/mg,  $P=0.026$ ), whereas Saint-Louis had a higher level at post-harvest than harvest (25.6 vs 15.6 pg/mg,  $P=0.032$ ). It is clear that aflatoxin exposure is prevalent in adults from Senegal and that season and geographical location are strong determinants of aflatoxin exposure.

**Keywords:** aflatoxin exposure, geographical differences, season, groundnut, Senegal

#### 1. Introduction

Aflatoxins are a group of naturally occurring secondary metabolites predominantly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Major food crops frequently contaminated with aflatoxins include: maize, groundnuts, rice and other grains. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most toxic subtype among all aflatoxins and is recognised as a human liver carcinogen by the International Agency for Research on Cancer (IARC, 2002). Fungal growth and toxin production, strongly influenced by high temperatures and humidity, can occur in the field (Torres *et al.*, 2014). Furthermore, aflatoxin contamination levels are likely to increase following harvest and during storage and this is attributable to improper drying and post-harvest storage practices (Pitt *et al.*, 2012; Turner *et al.*, 2005).

Biomarker of exposure reflecting internal doses individually permits reliable measurements of aflatoxin exposure and has been shown to be correlated with dietary intake of aflatoxin (Routledge *et al.*, 2014). Evidence from cross-sectional and longitudinal studies have demonstrated that chronic exposure to aflatoxin measured by the aflatoxin-albumin adduct (AF-alb) biomarker is associated with hepatocellular carcinoma (IARC, 2002), child growth impairment (Gong *et al.*, 2002, 2004), immune modification (Jiang *et al.*, 2005, 2008; Turner *et al.*, 2003) and childhood chronic hepatomegaly (Gong *et al.*, 2012). Aflatoxin is, therefore, listed as one of the mycotoxins of major public health concern (Wild and Gong, 2010).

Aflatoxin exposure is more prevalent in low-income countries, where subsistence farming, food shortage and lack of diet diversity are alongside insufficient

regulatory control and low awareness of aflatoxin risk and management. It is well known that sub-Saharan countries in Africa, owing to their hot and humid tropical climate, have high aflatoxin exposure from *in utero* through to adulthood (Wild and Gong, 2010). Furthermore, these high aflatoxin exposure levels coincide with the high incidence and mortality rates of liver cancer estimated for sub-Saharan African countries (Ferlay *et al.*, 2012).

Senegal is a predominant producer of groundnut (USDA, 2014), a dietary staple crop prone to aflatoxin contamination. There is, however, a paucity of information regarding the level of aflatoxin contamination in groundnuts and the aflatoxin exposure in this population. This current study was, therefore, conducted to determine the level of aflatoxin exposure in adults from Senegal by analysing the level of aflatoxin in blood and groundnut samples. Seasonal and geographical influences on these levels were also investigated. Understanding the extent of these spatio-temporal influences on aflatoxin exposure will aid the development of effective interventions that are necessary for aflatoxin management in Senegal.

## 2. Methodology

### Participants

168 adults (husband and wife pairs) were recruited from three districts of Senegal; Nioro du Rip (n=90), Saint-Louis (n=38) and Mboro (n=40) across three different eco-geographical zones in Senegal to represent geographical variation in aflatoxin contamination levels: Groundnut Basin, Senegal River Valley and Niayes. The Groundnut Basin (Nioro du Rip) is a site of intense rain fed groundnut production and consumption. It is located within the Sudan Savannah agro-ecological zone. Niayes (Mboro) and Senegal River Valley (Saint-Louis) are located within the Sahel agro-ecological zone where groundnuts are produced under irrigated conditions. Participants were recruited and surveyed during the groundnut harvest season and followed up between three and four months later after a period of storage, to determine the impact of storage on aflatoxin contamination levels. At each visit, blood samples were collected from each individual for measurement of the aflatoxin exposure biomarker AF-alb; groundnut samples were collected from each household to measure the level of aflatoxin contamination.

Trained field workers collected socio-demographic (age, gender, ethnicity and education level) and dietary (consumption frequency of groundnut and maize) information via a food frequency questionnaire during the first visit. Written informed consent was obtained from each of the study participants. Ethical approval for this study was obtained from the Ministry of Health in Senegal and the University of Leeds.

### Aflatoxin-albumin adduct analysis

A 5 ml blood sample was taken from each participant at each visit by a qualified nurse. The plasma was separated by centrifugation and stored at -20 °C before shipped on dry ice to the University of Leeds for the assessment of AF-alb level, using a competitive ELISA method. The procedure briefly involved albumin extraction and digestion, followed by purification using Sep-Pak C18 cartridges and then competitive ELISA as described previously (Chapot and Wild, 1991). Three positive and one negative control samples were analysed along with each batch of samples, for the purpose of quality control. All samples were analysed in triplicate and repeated on two separate days. Results were only accepted if the coefficient of variation was less than 25% between the repeats. The assay's limit of detection was 3 pg/mg albumin. For the purpose of data analysis any samples with AF-alb below this limit, were assigned a value of 1.5 pg/mg albumin.

### Total aflatoxins analysis in groundnut samples

Groundnuts at harvest were randomly sampled directly from the field at a time when drying and threshing operations were ongoing or from households where the recently harvested pods were stored. Post-harvest samples were collected from the top, middle and bottom of household containers, respectively, and pooled. After each sample collection events (harvest or post-harvest), the pods were further sun-dried and shelled. Kernels obtained after shelling were mixed thoroughly and sub-divided using a quartering method until approximately a 200 g sample was obtained. The samples (~200 g each) were stored at 4 °C until they were shipped to the International institute of Tropical Agriculture (IITA), Ibadan, Nigeria for aflatoxin analysis.

Aflatoxin analyses were performed using a modification of the AOAC method (AOAC, 1999) described in Atehnkeng *et al.* (2008). A 20 g subsample was ground and blended vigorously with 100 ml of 85% methanol for 3 min. The mixture was further shaken for 30 min and filtered. Following filtration, 25 ml of hexane (40%) and 40 ml of 10% NaCl solution were added to the filtrate, and the mixture was shaken for a minute. The lower phase of the mixture was extracted with 25 ml dichloromethane twice. The pooled extracts were evaporated to dryness in the dark and reconstituted in 1 ml of dichloromethane for the quantitative thin layer chromatography (TLC) assay.

Aflatoxin standards (Sigma-Aldrich, St. Louis, MO, USA) and extracts were separated on TLC plates. AFB<sub>1</sub> and aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), G<sub>1</sub> (AFG<sub>1</sub>) and G<sub>2</sub> (AFG<sub>2</sub>) were quantified using a scanning densitometer, CAMAG TLC Scanner 3 with WinCATS 1.4.2 software (Camag AG, Muttenz, Switzerland). The recovery was 85% and

the assay's limit of detection (LOD) was 1.0 µg/kg. For data analysis, any sample with a level below this limit was assigned a value of 0.5 (½ LOD). Total aflatoxin was calculated by summing AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>.

### Statistical analysis

All analyses were performed using SPSS for Windows version 21.0 (SPSS Inc, Chicago, IL, USA). Results are expressed as geometric mean and 95% confidence intervals or median and interquartile range as variables did not satisfy normality criteria. AF-alb (pg/mg) and total aflatoxin (µg/kg) were positively skewed and therefore log transformed. For continuous variables, differences between groups (gender, age, geographical location and season) were analysed using independent sample t-test, analysis of variance (ANOVA) or the Kruskal-Wallis one-way analysis of variance. Bonferroni correction was used as a post hoc analysis test and polynomial contrasts tested for linear trends in group means. Paired sample t-tests or Wilcoxon signed-rank tests were used to compare the differences of matched variables (repeated measures) within a single group (seasonal differences). For categorical variables (food consumption frequency), Chi-square or Fisher's exact test were used. Correlation (Pearson) analyses was performed to investigate the relationship between husband and wife AF-alb levels also between total aflatoxin in groundnut samples and AF-alb levels. A  $P \leq 0.05$  was deemed statistically significant.

## 3. Results

### Demographic characteristics

A total of 168 adults (50% males) with a mean age of  $39 \pm 12$  years were recruited at the harvest season, and 123 (73%) of them were followed up at the post-harvest season, with the rest missing at blood collection. The majority of participants were of Wolof ethnicity (78%) and educated to at least primary school level (74%). Available blood samples at harvest ( $n=158$ ) and post-harvest ( $n=123$ ) had detectable

AF-alb levels. The geometric mean adduct level of all available blood samples ( $n=281$ ) was 45.7 pg/mg (range 5.5-588.2 pg/mg).

There were no significant differences in AF-alb levels in relation to socio-demographic factors: gender, age, ethnicity or education level. There was a positive correlation between husband and wife pairs' AF-alb levels at harvest ( $r=0.764$ ,  $P<0.001$ ) and at post-harvest ( $r=0.609$ ,  $P<0.001$ ).

### Aflatoxin exposure (AF-alb) by season and geographical location

Overall the AF-alb geometric mean levels were similar between harvest and post-harvest seasons (45.4 vs 46.1 pg/mg,  $P=0.874$ ; respectively). Around 22 and 14% of participants had AF-alb levels above 100 pg/mg at harvest and post-harvest, respectively.

AF-alb levels by location and season are shown in Table 1. At the harvest season participants from Nioro du Rip had a significantly higher geometric mean AF-alb level than both Saint-Louis and Mboro (80.0 vs 15.6 and 33.3 pg/mg,  $P<0.001$ ) and participants from Mboro had a significantly higher AF-alb level than Saint-Louis ( $P<0.001$ ). Similar trends were observed at post-harvest as participants from Nioro du Rip and Mboro had significantly higher AF-alb levels in comparison with participants from Saint-Louis ( $P<0.001$  and  $P=0.034$ ).

In relation to seasonal variations, a consistent trend was not observed across the locations. Nioro du Rip had a higher geometric mean AF-alb level at harvest than post-harvest (80.0 vs 58.6 pg/mg,  $P=0.026$ ), whereas Saint-Louis had a higher level at post-harvest than harvest (25.6 vs 15.6 pg/mg,  $P=0.032$ ). The increase in AF-alb level from harvest to post-harvest in Mboro was not statistically significant ( $P=0.087$ ).

**Table 1. Aflatoxin-albumin adduct levels (AF-alb) in adults by location and season.**

District <sup>1</sup>	Harvest AF-alb (pg/mg albumin) <sup>2</sup>		Post-harvest AF-alb (pg/mg albumin) <sup>2</sup>		P value <sup>3</sup>
	n	Geometric mean (95% CI)	n	Geometric mean (95% CI)	
Nioro du Rip	86	80.0 (67.4, 95.0) <sup>a</sup>	72	58.6 (51.5, 66.7) <sup>a</sup>	0.026
Mboro	37	33.3 (28.6, 38.8) <sup>c</sup>	25	42.6 (30.2, 60.1) <sup>a</sup>	0.087
Saint-Louis	35	15.6 (12.2, 20.0) <sup>b</sup>	26	25.6 (17.6, 37.2) <sup>b</sup>	0.032

<sup>1</sup> Geographical differences analysed using ANOVA with post hoc analysis. <sup>a,b,c</sup> represent significant differences ( $P<0.001$ ) between districts.

<sup>2</sup> AF-alb levels were log transformed for analysis.

<sup>3</sup> Seasonal differences analysed using paired samples t-test.

### Groundnut total aflatoxin contamination

Total aflatoxin was detected in 74% of the groundnut samples at harvest stage and in 34% of the post-harvest samples. Table 2 shows the total aflatoxin levels found in household groundnut samples by location and season. Aflatoxin was detected in 80, 62 and 67% of groundnut samples at harvest from Nioro du Rip, Saint-Louis and Mboro, respectively. During post-harvest, total aflatoxin was detected in 28% of groundnut samples from Nioro du Rip and 4 out of 5 (80%) samples collected from Saint-Louis. Post-harvest groundnut samples were not available to collect from Mboro as most of the groundnuts had been sold following harvest. At harvest, total aflatoxin median level in groundnut did not differ by location ( $P=0.310$ ). In Nioro du Rip the contamination level was lower at the post-harvest season than at the harvest season (median: 0.5 vs 3.4  $\mu\text{g}/\text{kg}$ ,  $P=0.002$ ). In Saint-Louis, contamination level was higher at post-harvest than at harvest; although, the small sample size in this region did not permit statistical comparison on the contamination levels.

### Relationship between blood AF-alb and total aflatoxin in groundnut samples

There was a significant correlation between AF-alb ( $\text{pg}/\text{mg}$ ) and total aflatoxin in groundnut samples ( $r=0.201$ ,  $P=0.018$ ) at harvest time only. When the data was stratified by location, Nioro du Rip was the only location that showed a positive correlation between AF-alb and total aflatoxin in groundnut samples at harvest ( $r=0.286$ ,  $P=0.008$ ).

### Groundnut and maize consumption frequency

Maize and groundnut consumption frequency in the previous week was recorded at the harvest season. Participants were grouped according to how often they

consumed groundnuts (or maize) as low consumption (<4 days per week), or high consumption ( $\geq 4$  days a week). As shown in Table 3, the majority of the participants (70%) were of high groundnut consumption, and their geometric mean AF-alb level was 2.6-fold higher than those of low consumption (62.8 vs 24.0  $\text{pg}/\text{mg}$ ,  $P<0.001$ ). For maize intake, similar results were observed. Participants of high maize consumption had a geometric mean AF-alb level 1.5 fold higher than those of low consumption (61.4 vs 41.1,  $\text{pg}/\text{mg}$  albumin,  $P=0.015$ ). In contrast to groundnut consumption, the majority of participants (64%) were of low maize consumption.

**Table 3. Levels of aflatoxin-albumin adduct (AF-alb) ( $\text{pg}/\text{mg}$  albumin) grouped by groundnut and maize consumption at harvest time.**

Food type and consumption frequency	n	AF-alb ( $\text{pg}/\text{mg}$ ) <sup>1</sup> ; geometric mean (95% CI)	P value <sup>2</sup>
Groundnut			
Low consumption (<4 days/week)	45	24.0 (18.7, 30.8)	
High consumption ( $\geq 4$ days/week)	107	62.8 (56.0, 74.3)	<0.001
Maize			
Low consumption (<4 days/week)	96	41.1 (33.5, 50.3)	
High consumption ( $\geq 4$ days/week)	54	61.4 (48.5, 77.9)	0.015

<sup>1</sup> AF-alb levels were log transformed for analysis.

<sup>2</sup> Differences between consumption frequency groups were assessed using independent samples t-test.

**Table 2. Total aflatoxin ( $\mu\text{g}/\text{kg}$ ) in household groundnut samples by location and season.**

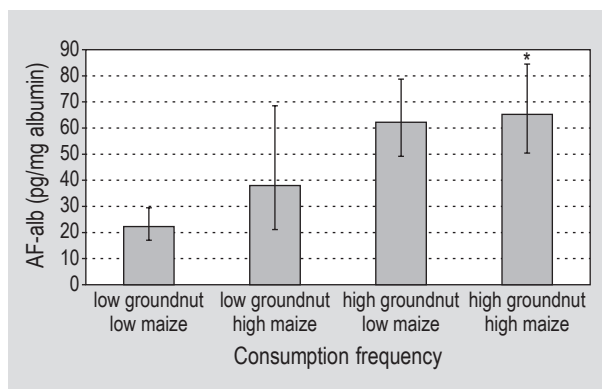
Season	Nioro du Rip	Saint-Louis	Mboro	P value
Harvest				
n(%) detected	36/45 (80)	8/13 (62)	10/15 (67)	
Mean (SD)	20.8 (75.3)	6.5 (10.7)	8.4 (9.4)	
Median (IQ)	3.4 (1.3-11.9) <sup>1</sup>	1.6 (0.5-6.2)	7.2 (0.5-14.4)	0.310 <sup>2</sup>
Range	LOD-504.0	LOD-38.0	LOD-34.5	
Post-harvest				
n(%) detected	10/36 (28)	4/5 (80)	–	
Mean (SD)	51.4 (296.6)	10.9 (6.7)	–	
Median (IQ)	0.5 (0.5-2.4) <sup>1</sup>	12.6 (5.8-16.5)	–	
Range	LOD-1,781.4	LOD-18.2	–	

<sup>1</sup> Seasonal difference analysed using Wilcoxon signed-rank test (Nioro du Rip only) and the difference was significant ( $P=0.002$ ).

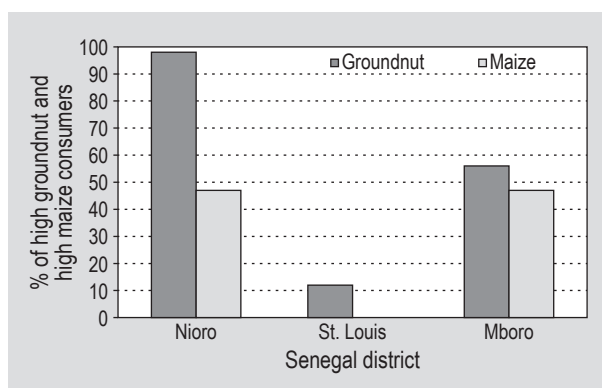
<sup>2</sup> Geographical differences analysed using the Kruskal-Wallis test at harvest time only.

Figure 1 shows the geometric mean (GM) AF-alb levels of participants who were grouped according to how often they reported consuming both groundnuts and maize. Participants who were both low in maize and groundnut consumption had a significantly lower geometric mean AF-alb level compared to participants who were both high in maize and groundnut consumption, and participants who were high in groundnut consumption but low in maize consumption (GM: 22.3 (95% CI: 17.0, 29.5) vs 65.2 (95% CI: 50.4, 84.5) and 62.2 (95% CI: 49.2, 78.7) pg/mg,  $P < 0.001$ ; respectively).

Figure 2 shows the percentage of participants, by geographical location, who consumed groundnuts and maize  $\geq 4$  days per week. More participants from Nioro du Rip reported consuming groundnuts  $\geq 4$  days per week than the other two districts: Saint-Louis and Mboro (98% vs 12-56%,  $P < 0.001$ ). None of the Saint-Louis participants reported consuming maize  $\geq 4$  days per week, while 47% of the participants from both Nioro du Rip and Mboro reported consuming maize this often.



**Figure 1. Geometric mean (95% confidence interval) aflatoxin-albumin adduct (AF-alb) levels of participants grouped according to how often they reported consuming both maize and groundnuts. \*Trend test:  $f=30.2$ ,  $P < 0.001$ .**



**Figure 2. The percentage of participants who reported consuming groundnuts and maize  $\geq 4$  days per week at harvest, by location.**

## 4. Discussion

It is evident from this current study that adults from Senegal are frequently exposed to aflatoxin. All participants had detectable levels of AF-alb with the geometric mean level at 45.7 pg/mg. This mean level is considerably higher than levels previously reported in Senegalese children (Wild *et al.*, 1990). The overall mean adduct level in this current study (45.7 pg/mg) is somewhat similar to the adduct levels (in the range of 30-50 pg/mg) previously reported in adults from Gambia (Castinello *et al.*, 2014; Turner *et al.*, 2007; Wild *et al.*, 1990) but at the same time is notably higher than levels (up to 15 pg/mg) observed in Ugandan adults (Asiki *et al.*, 2014) and adults from lower Kindia region of Guinea (Turner *et al.*, 2005).

In this current study AF-alb was significantly correlated with total aflatoxin in groundnuts at harvest. The AF-alb levels variance by district of Senegal were in agreement with the total aflatoxin levels and the groundnut consumption by district, for example, Saint-Louis had the lowest AF-alb, lowest total aflatoxin contamination and lowest groundnut consumption in the harvest season. These findings suggest that AF-alb biomarker reflects dietary exposure of aflatoxin well and that groundnut consumption and contamination are two major determinants of the exposure levels.

Aflatoxin contamination tends to accumulate after the crops are stored for a period of time (Hell *et al.*, 2000; Pitt *et al.*, 2012). Seasonal trends in AF-alb levels have been reported repeatedly in Sub-Saharan Africa. Studies conducted in Gambia (Castelino *et al.*, 2014; Turner *et al.*, 2000; Wild *et al.*, 2000), Benin (Gong *et al.*, 2004), and Guinea (Turner *et al.*, 2005), for example, have shown that AF-alb levels increased during storage. In this current study a consistent seasonal trend was not observed among the districts. Saint-Louis and Mboro showed higher AF-alb levels at post-harvest than at harvest, while Nioro du Rip, instead, showed a reduction in AF-alb levels from harvest to post-harvest. The higher AF-alb observed during harvest in Nioro du Rip is likely due to increased consumption of aflatoxin contaminated foods. This is supported by the fact that nearly all Nioro du Rip participants reported consuming groundnuts four or more days a week at harvest.

Geographical differences were apparent in AF-alb levels, with Nioro du Rip, located within the Sudan Savannah zone, consistently having the highest level among the three districts. At harvest time the geometric mean AF-alb level in Nioro du Rip was 80.0 pg/mg; 4-fold higher than Saint-Louis and almost 2.5-fold higher than Mboro, and a similar trend was observed at post-harvest. These geographical differences in AF-alb levels are likely due to the different consumption patterns of groundnut and maize and/or the different contamination levels between the districts.

There was a geographical variation in groundnut consumption frequency as the percentage of Nioro du Rip participants that consumed groundnuts four or more days a week was significantly higher than Mboro and Saint-Louis ( $P < 0.001$ ). This higher groundnut consumption frequency reported by Nioro du Rip participants coincided with their higher AF-alb levels compared with the other two districts at harvest.

Previous research implied that aflatoxin contamination of susceptible crops differs across agro-ecological zone in Sub-Saharan Africa (Atehnkeng *et al.*, 2008; Diedhiou *et al.*, 2011; Egal *et al.*, 2005; Hell *et al.*, 2000; Probst *et al.*, 2014). It is thought that the different types of climate and precipitation levels may have an influence on aflatoxin concentration in grains in the field, at harvest and during storage (Cotty and Jaime-Garcia, 2007). In this current study, although no statistical difference was observed for median aflatoxin levels in groundnut samples collected from the three districts, the higher mean level and higher range up to 504  $\mu\text{g}/\text{kg}$  in Nioro Du Rip when compared to other districts suggest it is likely that groundnut contamination varied between the two agro-ecological zones. The Sudan Savanna zone has a hot semi-arid climate that supports rain-fed agriculture, while the Sahel zone has extremely hot temperatures and low precipitation with most crops grown under irrigated conditions. Rain-fed crops are more prone to aflatoxin contamination than crops produced under irrigated conditions (Jones *et al.*, 1981).

A dose response relationship was evident from the food frequency data as participants who consumed groundnuts and maize  $\geq 4$  days per week had the highest geometric mean AF-alb level. The data also indicated that frequent groundnut consumption led to a higher AF-alb level than frequent maize consumption. The majority of people consumed groundnuts more frequently than they consumed maize. Groundnut, typically consumed in the form of snacks, groundnut oil and sauces, is considered a dietary staple and a major cash crop for Senegal. According to the FAO country specific food balance sheets (FAOSTAT, 2014), West African countries such as Senegal tend to have a lower maize supply yet a higher groundnut supply than East African countries (maize: 28.9 vs 56.6 kg/capita/year; groundnuts: 3.4 vs 2.3 kg/capita/year, respectively).

This study did have some limitations. For example, there were a limited number of post-harvest groundnut samples collected from Saint-Louis and no post-harvest groundnut samples were collected from Mboro owing to the groundnuts having been sold after harvest. There was also no measurement of aflatoxin contamination in maize in this current study; although, a recent study found aflatoxin contamination levels ranging between 0 and 852  $\mu\text{g}/\text{kg}$  in maize samples collected in Senegal (Diedhiou *et al.*, 2011). Furthermore, dietary consumption of maize and groundnuts

was only assessed as frequency of consumption. It was, therefore, not possible to completely define the relationship between total aflatoxin intake with AF-alb levels in this population. Nevertheless, Senegal is a major producer and consumer of groundnut and the evidence provided here supports the assumption that groundnut is a major contributor to aflatoxin exposure. Another limitation is that the food consumption frequency data was only collected at harvest time; having groundnut and maize consumption frequency at post-harvest would perhaps explain why Nioro du Rip had such high AF-alb levels at harvest compared to post-harvest.

## 5. Conclusion

Aflatoxin exposure is prevalent in the Senegalese with Nioro du Rip having the highest exposure in the studied population, although seasonal patterns varied among the three districts. The aflatoxin biomarker levels correlated with the frequency of groundnut consumption. Overall, the results from this study reinforce the need for cost-effective interventions to reduce aflatoxin exposure in Senegal, specifically in high-risk areas.

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