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ABSTRACT
Aflatoxins are a major class of fungal toxins that have food safety importance due to their economic and health impacts. This pilot aflatoxin exposure biomonitoring study on 84 individuals was conducted in a rural (Ilumafon) and a semi-urban community (Ilishan Remo) of Ogun state, Nigeria, to compare aflatoxin exposures among the two population cohorts. First morning urine samples were obtained from the participants, and the urinary aflatoxin M$_1$ (AFM$_1$) levels were measured by a quantitative Helica Biosystems Inc. ELISA kit assay. About 99% (83 out of 84) of the urine samples had detectable AFM$_1$ levels in the range of 0.06 to 0.51 ng mL$^{-1}$ (median: 0.27 ng mL$^{-1}$). The mean urinary AFM$_1$ levels were significantly ($p = 0.001$) higher in the semi-urban population (0.31 ± 0.09 ng mL$^{-1}$) compared to the rural population (0.24 ± 0.07 ng mL$^{-1}$). There were, however, no significant differences in mean urinary AFM$_1$ levels of males and females, and among children, adolescents and adults. This study indicates high aflatoxin exposure to the extent of public health concerns in the studied populations. Thus, more efforts are required for aflatoxin exposure monitoring and control in high-risk regions.

Introduction
Aflatoxins are toxic secondary metabolic products of Aspergillus origin. They are produced in crops during planting, harvesting and post-harvest handling such as drying, storage, transportation and food processing (Ayalew et al. 2016). Contamination of crops and processed foods by aflatoxins is a continuous challenge in developing countries where poor agricultural practices and inadequate postharvest conditions are commonplace (Williams et al. 2004; IARC 2015). In Nigeria, a tropical country with hot and humid climate, aflatoxins have been widely reported in a variety of agricultural crops such as maize, peanuts (groundnuts), rice, sorghum, melon seeds (Makun et al. 2009, 2010; Adetunji et al. 2014; Ezekiel et al. 2016; Oyedele et al. 2017), and their ready-to-eat products such as maize- and peanut-based snacks, kunu-zaki, ogi, ogiri and roasted peanuts (Bankole et al. 2005; Ezekiel et al. 2012, 2013, 2015; Kayode et al. 2013; Afolabi et al. 2015; Okeke et al. 2015; Adedeji et al. 2017).

Consumption of aflatoxin-contaminated foods has been associated with several health challenges such as growth retardation in children, hepatocellular carcinoma, gastrointestinal dysfunction, immune system toxicity, and deaths across Africa (Gong et al. 2002, 2016; Turner et al. 2003, 2012; Lewis et al. 2005; Probst et al. 2007; Turner 2013; Yard et al. 2013; IARC (International Agency for Research on Cancer) 2015; All Africa 2016; Outbreak News Today 2017). However, the degree of morbidity and mortality depends on several factors including the toxin levels in the food, extent of consumption of the contaminated foods, age and developmental stage of exposed individuals (Williams et al. 2004; IARC (International Agency for Research on Cancer) 2015). Among the known types of aflatoxins, aflatoxin B$_1$ (AFB$_1$) is the most toxic and is classified as a category 1 human carcinogen (IARC 2002).
AFB1, when ingested by humans via contaminated foods, can be metabolised into several metabolites which serve as biomarkers of exposure (Routledge and Gong 2011; Leong et al. 2012; Warth et al. 2016). The metabolites include aflatoxin albumin adduct (AF-alb) in blood (Wild and Turner 2002; Turner et al. 2007), aflatoxin N7-guanine in urine (Groopman et al. 1993), and aflatoxin M1 (AFM1) in urine (Wild and Turner 2002) and breast milk (Degen et al. 2013). In the urine, AFM1 is more commonly detected than the aflatoxin-guanine complex (Abia et al. 2013; Ezekiel et al. 2014; Ali et al. 2016, 2017; Schwartzbord et al. 2016; Chen et al. 2017). Urine is often preferred to blood for aflatoxin biomonitoring in high-risk regions due to its non-invasiveness, ease of collection and usefulness in providing data on recent (within 48 h) daily exposures (Zhu et al. 1987; Groopman et al. 1992; Chen et al. 2017).

Common analytical platforms for aflatoxin monitoring in human urine include the Enzyme-linked immuno sorbent assay (ELISA) (Groopman et al. 1993; Ali et al. 2016), high performance liquid chromatography (Groopman et al. 1993; Ali et al. 2017) and liquid chromatography tandem mass spectrometry (Rubert et al. 2011; Solfrizzo et al. 2011; Warth et al. 2012). The ELISA, however, remains a low-cost, easily accessible, and high throughput technique available for urine biomonitoring in developing countries such as Nigeria where resources for high-end techniques are scarce. Recently, Chen et al. (2017) used the ELISA to prove a strong correlation between aflatoxin levels in blood and urine.

Despite the widespread contamination of aflatoxins in crops and processed foods in Nigeria, only few reports are available for aflatoxin monitoring by biological fluids: blood (Oluwafemi et al. 2012; Mcmillian et al. 2018), breast milk (Adejumo et al. 2013) and urine (Ezekiel et al. 2014). Considering the need for continuous monitoring of daily exposures in high-risk (tropical) regions, and the paucity of data for comparison of aflatoxin exposures among rural dwellers and urban/semi-urban populations, this study aimed to assess aflatoxin exposures in two population cohorts (rural and semi-urban) in Ogun State, Nigeria using the urinary ELISA method.

Materials and methods

Study area

The study was carried out in two communities (Ilumafon and Ilishan Remo) in Ogun State, Nigeria. The study locations were purposely selected for convenience of sampling. Ilumafon is a rural settlement comprising about 200 dwellers who subsist on agriculture, growing own crops for income. Crops grown and consumed by the rural dwellers of Ilumafon include cassava, cocoa, oil palm and maize. Ilishan Remo is a semi-urban community of about 10,000 residents due to the presence of a tertiary education institution within the community. The residents were made up of students, civil servants, farmers and marketers. Farming and production of own food is considered a secondary source of income among Ilishan Remo residents, and foods are usually purchased from local markets and stores within the community.

Study design and study population

A pilot, cross-sectional study made up of 84 participants (38 males and 46 females; age: 2–65 years) was conducted in the selected locations between December 2016 and January 2017. The participants were informed about the purpose of the research, and based on their willingness to participate in the study, 84 individuals were randomly selected. Each participant signed an informed consent; parents gave consent for their children. The age categories reported by Ezekiel et al. (2014) (children: ≤8 years; adolescents: ≤19 years; adults: ≥20 years) were adopted, and the categories had 13, 24 and 47 participants, respectively. Of the 84 participants, 45 were recruited at rural community (Ilumafon), while 39 were from semi-urban area (Ilishan Remo). A well-structured questionnaire was administered to each participant by trained interviewers to obtain their demographic information, socio-economic status and dietary preferences. Translation to Yoruba language was necessary for the dwellers of the rural community. The study was approved by the Babcock University Health Research Ethics Committee (BUHREC154/17).
**Sample collection**

A first morning urine sample (20 mL) was collected from each recruited participants (n = 84). Urine samples were collected prior to consumption of food or water for the day. The urine samples were immediately transferred to the laboratory and then frozen until aflatoxin analysis. Prior to analysis, urine samples were brought to ambient temperature and centrifuged at 5000 rpm for 5 min to obtain a clear supernatant.

**Determination of aflatoxin M₁ in urine by ELISA**

AFM₁ in the urine samples was assayed by a quantitative ELISA kit assay (Helica Biosystems, Inc. AFLMO1U) according to manufacturer’s instructions. Briefly, all reagents and urine samples were allowed to reach ambient temperature. Aliquots of both the AFM₁ standards and samples (urine supernatant) were diluted 1:20 with distilled water. Assay buffer (200 µL) was dispensed into each mixing well, and 100 µL of each diluted standard and sample was added to the appropriate mixing well containing the assay buffer. The mixture (100 µL) in each mixing well was transferred to a corresponding antibody coated microtiter well which was then incubated at ambient temperature for 1 h. After incubation, the content of each microwell was discarded, and the microwells were washed three times with PBS-Tween wash buffer and dried. Aliquots (100 µL) of a conjugate and a substrate were added consecutively with a step of incubation at room temperature for 15 min after each solution was added. A stop solution (100 µL) was then added, and the optical densities (OD) of the reaction solution in the microtiter plates were read at 450 nm using a Rayto (RT-2100C) Microplate reader (Rayto Life and Analytical Sciences Co. Ltd, Shenzhen, China).

The corresponding aflatoxin concentration in each well was estimated from standard curve plotted using AFM₁ standard solutions (0–4 ng mL⁻¹) and taking into account the inverse proportionate level of the absorption intensity to AFM₁ concentration in the samples. Blank urines were spiked at three concentration levels (0.8, 1.5 and 4 ng mL⁻¹) of AFM₁ standard in an assay validation test in order to determine the recovery (range: 84–106%) and limit of detection (0.06 ng mL⁻¹).

**Statistical analysis**

Data was analysed by SPSS 17.0 for Windows (SPSS Inc., IL, USA). Questionnaire data were analysed by simple descriptive statistics, while one-way ANOVA and Student’s t-test were used to test for significance (α = 0.05) in aflatoxin data for age groups, sex and location.

**Results and discussions**

**Demographic characteristics and dietary preferences of the participants within the cohorts**

The demographic characteristics and dietary preferences of the 84 participants recruited into this

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rural</th>
<th>Sub-urban</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>45</td>
<td>39</td>
<td>84</td>
</tr>
<tr>
<td>Male</td>
<td>16(35.6)</td>
<td>22(56.4)</td>
<td>38(45.2)</td>
</tr>
<tr>
<td>Female</td>
<td>29(64.4)</td>
<td>17(43.6)</td>
<td>46(54.8)</td>
</tr>
<tr>
<td>Age (n (%); mean±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (age range: 2–7 years)</td>
<td>8(17.8); 4.6 ± 1.3</td>
<td>5(12.8); 4.8 ± 2.3</td>
<td>13(15.5); 4.7 ± 1.7</td>
</tr>
<tr>
<td>Adolescent (age range: 9–19 years)</td>
<td>11(24.4); 12.1 ± 2.9</td>
<td>13(33.3); 18.5 ± 1.1</td>
<td>24(28.6); 15.8 ± 3.7</td>
</tr>
<tr>
<td>Adult (age range: 20–65 years)</td>
<td>26(57.8); 39.6 ± 12.1</td>
<td>21(53.9); 25.6 ± 6.2</td>
<td>47(55.9); 33.3 ± 12.1</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local traders of foodstuffs</td>
<td>18(40.0)</td>
<td>-</td>
<td>18(21.4)</td>
</tr>
<tr>
<td>Farmers</td>
<td>21(46.7)</td>
<td>-</td>
<td>21(25.0)</td>
</tr>
<tr>
<td>Civil servants</td>
<td>6(13.3)</td>
<td>6(15.4)</td>
<td>12(14.3)</td>
</tr>
<tr>
<td>Student</td>
<td>-</td>
<td>33(84.6)</td>
<td>33(39.3)</td>
</tr>
<tr>
<td>Dietary preferences per day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuber-based foods</td>
<td>33(73.3)</td>
<td>6 (15.4)</td>
<td>39(46.4)</td>
</tr>
<tr>
<td>Cereal/nut-based foods</td>
<td>12(26.7)</td>
<td>33(84.6)</td>
<td>45(53.6)</td>
</tr>
<tr>
<td>Combined/equal preference</td>
<td>7(15.6)</td>
<td>8(20.5)</td>
<td>15(17.9)</td>
</tr>
</tbody>
</table>

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FOOD ADDITIVES & CONTAMINANTS: PART A

3
Aflatoxin biomonitoring study are presented in Table 1. About 45% of the participants were males, while 55% were females. In the rural setting (Ilumafon, n = 45), a higher percentage of participants 64.4% were females while the male participants had a higher percentage 56.4% in the semi-urban community (Ilishan-Remo). The mean ages (years) of participants within the rural setting (Ilumafon) were 4.6, 12.1 and 39.6 for children, adolescents and adults, while children, adolescents and adults in the semi-urban community (Ilishan Remo) had mean ages of 4.8, 18.5 and 25.6, respectively. In the two locations, adults were the most recruited, followed by adolescents and children. Participants from the rural community (Ilumafon) were farmers (47%), small-scale traders (40%) and civil servants (13%), whilst the participants from the semi-urban community (Ilishan Remo) were students (85%) and civil servants (15%). With respect to dietary preferences, participants from the rural setting (Ilumafon) consumed more tuber-based foods (73%), while those from the semi-urban community (Ilishan Remo) preferred cereal-based foods 33(85%). Overall, dietary preferences of the 84 participants were 54%, 46% and 18% for cereal-based foods, tuber-based foods, and a combination of both types of foods, respectively.

**Distribution of aflatoxin M₁ levels in urine of the two cohort groups**

The levels of AFM₁ found in urines of rural dwellers and semi-urban residents in Ogun state, Nigeria, are presented in Table 2. AFM₁ was detected in 98% (range: 0.11–0.39 ng mL⁻¹) of the urine samples collected from the rural setting (Ilumafon), whilst all of the urine samples from the semi-urban community (Ilishan Remo) contained detectable levels (range: 0.06–0.51 ng mL⁻¹) of AFM₁. The mean AFM₁ level (0.31 ± 0.07 ng mL⁻¹) in the urines from the semi-urban community (Ilishan Remo) was significantly (p = 0.001) higher than the mean levels (0.24 ± 0.07 ng mL⁻¹) quantified in urines from participants living in the rural environment (Ilumafon). On the overall, 83 (99%) out of the 84 urine samples contained detectable AFM₁ levels, with mean of 0.27 ± 0.08 ng mL⁻¹ (range: 0.06–0.51 ng mL⁻¹; median: 0.27 ng mL⁻¹).

Aflatoxin biomonitoring studies, especially those that utilise the urinary AFM₁ biomarkers, are increasing in number and span different continents due to the need to provide exposure data from all food sources (Abia et al. 2013; Polychronaki et al. 2008; Warth et al. 2012, 2014; Ezekiel et al. 2014; Kouadio et al. 2014; Ali et al. 2016, 2017; Schwartzbord et al. 2016; Ayelign et al. 2017; Chen et al. 2017). Our study adds to the growing body of evidence on this subject. In this study, the urinary AFM₁ mean values reported for both cohorts were higher than the mean values of 54 ± 15 pg mL⁻¹ and 99 ± 71 pg mL⁻¹ reported by Ali et al. (2016) in Bangladeshi adults from urban and rural settings, respectively. Our study further presents a higher overall mean value for AFM₁ in urines compared to the overall means reported in the urines of individuals in Egypt (5.5 pg mL⁻¹, Polychronaki et al. 2008), Guinea (97 pg mL⁻¹, Polychronaki et al. 2008) and Ethiopia (0.0064 ng mL⁻¹, Ayelign et al. 2017). The overall mean value in our present paper is, however, relatively similar to the means reported in urine of individuals from northern Nigeria (0.3 µg L⁻¹, Ezekiel et al. 2014), Cameroon (0.33 ng mL⁻¹, Ediage et al. 2013) and Thailand (0.33 µg L⁻¹, Warth et al. 2014). Furthermore, the prevalence of aflatoxin exposure found in our study is higher than the prevalence (<50%) of aflatoxin exposure via urinary AFM₁ reported for any population; although more recently, a re-analysis of 120 urine samples from Nigeria, by a more sensitive method, which earlier was reported to have 14.2% prevalence of urinary AFM₁ (Ezekiel et al. 2014), has now been shown to increase to 70% prevalence (Šarkanj et al. 2018). This confirms that aflatoxins exposure is very high in Nigeria.

### Table 2. Aflatoxin M₁ levels in urines of rural and semi-urban dwellers in Ogun state, Nigeria.

<table>
<thead>
<tr>
<th>Aflatoxin levels (ng mL⁻¹) by location</th>
<th>Rural (n = 45)</th>
<th>Semi-urban (n = 39)</th>
<th>Overall (n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage positive</td>
<td>97.7</td>
<td>100</td>
<td>98.8</td>
</tr>
<tr>
<td>Range</td>
<td>0.11–0.39</td>
<td>0.06–0.51</td>
<td>0.06–0.51</td>
</tr>
<tr>
<td>Mean ± standard dev.</td>
<td>0.24±0.07</td>
<td>0.31±0.09</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>Median</td>
<td>0.24</td>
<td>0.32</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Means with different superscript alphabets are significantly different (α = 0.05).
Comparing the urinary AFM\textsubscript{1} levels in the two cohorts, we found significantly higher mean values from the semi-urban community (Ilishan Remo) than from the rural setting (Ilumafon). This finding, however, does not agree with Ali et al. (2016), who reported higher AFM\textsubscript{1} levels in urines of adult rural dwellers than in urines from adult urban dwellers in Bangladesh. It should, however, be noted that the samples size of 39 individuals from a population of about 10,000 resident in the semi-urban community (Ilishan Remo) is not representative unlike the sampled rural village (Ilumafon). The disparity in the reported AFM\textsubscript{1} levels from the two cohorts could be attributed to the occupation and dietary preferences of the participants in the groups that we studied. There are evidences that suggest higher aflatoxin exposures in populations that depend on cereal- and nut-based foods, which are more prone to aflatoxin contamination, than in those with diversified diets (IARC (International Agency for Research on Cancer) 2015; Watson et al. 2015). In our study, participants from the rural setting (Ilumafon) were primarily farmers and traders of locally produced foodstuffs, whilst the semi-urban (Ilishan Remo) participants made up of students and civil servants. This implies that most of the rural (Ilumafon) dwellers consume their own crops, mainly tuber-based foods, which are less prone to aflatoxin contamination (Abass et al. 2017), whilst the semi-urban (Ilishan Remo) residents who were predominantly students and civil servants tend to consume more cereal (e.g. maize)- and nut (peanut)-based foods of local and industrial origin. This may have contributed to a lower exposure level within this cohort compared to the semi-urban cohort. Furthermore, handling (e.g. transportation lag from farm to markets, storage duration and conditions) of crops used to make the cereals which the participants from the semi-urban community depended on may also have been a contributor to the higher exposure levels observed in this cohort compared to the rural dwellers who did not need to store their crops for months before consumption. Aflatoxin contamination of crops is mostly a storage problem requiring urgent interventions (Shephard et al. 2003; Ezekiel et al. 2017; Mishairabgwi et al. 2017).

With respect to age and sex (Table 3), no significant differences were recorded for AFM\textsubscript{1} levels in the urine samples of male versus female participants, and among children, adolescents and adults, although the male participants had higher mean urinary AFM\textsubscript{1} levels than the females, and the urines from children and adolescent age groups recorded higher AFM\textsubscript{1} levels than the adult group. The non-significant findings for variations in AFM\textsubscript{1} levels of male and female participants recorded in our study agree with the reports of Edege et al. (2013) and Ali et al. (2017). On the other hand, the dietary requirements (e.g. higher food consumption per body weight) and rapid metabolism of the children and adolescents (Kostelanska et al. 2010; Lombard 2014) compared to adults may have contributed to the higher urinary AFM\textsubscript{1} levels detected for the children and adolescents groups than in the adults, although statistical analysis did not reveal significant differences between the groups.

**Conclusion**

Aflatoxin contamination of foods and subsequent human exposure remains a food safety and public health challenge in Nigeria. For the first time, we have compared aflatoxin exposure in two cohorts distinguished by type of residential location (rural and semi-urban) which influenced their dietary preferences. Exposure was higher among the semi-urban (Ilishan Remo) population than within the rural (Ilumafon) dwellers. More efforts

<table>
<thead>
<tr>
<th>Table 3. Distribution of aflatoxin M\textsubscript{1} levels in the urines of rural and semi-urban dwellers in Ogun state, Nigeria, grouped by age and sex.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin levels (ng mL\textsuperscript{-1}) by sex</td>
</tr>
<tr>
<td>Male (n = 38)</td>
</tr>
<tr>
<td>Percentage positive</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Mean ± standard dev.</td>
</tr>
<tr>
<td>Median</td>
</tr>
</tbody>
</table>
should, however, be put in place to control the quality of foods consumed in Nigeria, especially among the younger age groups in order to minimise chronic health effects from toxin exposure over a long period. For the semi-urban residents, dietary diversity is highly recommended coupled with sourcing of high-quality grains. Educational modules need to be developed in partnership with farmers, agricultural extension workers, traditional leaders, and other significant members of the community on proper storage, processing and handling of agricultural products. In view of the limited sample size per cohort, further surveillance and intervention studies with larger sample sizes and across multiple seasons are required.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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