



An initial characterization of aflatoxin B1 contamination of maize sold in the principal retail markets of Kigali, Rwanda



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ABSTRACT

Food security considerations have shifted in recent years, with the recognition that available food should also be nutritious and safe. There is a growing evidence base for contamination of maize and other crops by fungal toxins in the tropics and sub-tropics. As an initial snapshot of contamination by one of these toxins in Rwanda, Aflatoxin B1 (AFB1) was analyzed in 684 samples of maize flour collected in seven principal retail markets of Kigali and in 21 samples of animal feed from seven feed vendors. Two rounds of sample collections were carried out, the first in September 2014 and the second in January 2015. A questionnaire given to vendors was used to determine if gender and education level of vendors, origin of maize and awareness of aflatoxins had any significant effect on AFB1 level in collected samples. Enzyme-Linked Immunosorbent Assay (ELISA) and Immuno-affinity fluorimetry were used to analyze samples. Only markets had a significant effect on AFB1 level; for the two collections, differences were inconsistent among markets. In the first round, market means of AFB1 varied between $8.0 \pm 5.57 \mu\text{g/kg}$ and $24.7 \pm 23.74 \mu\text{g/kg}$ and for the second round, between $10.4 \pm 8.4 \mu\text{g/kg}$ and $25.7 \pm 25.85 \mu\text{g/kg}$. In most animal feed samples AFB1 was $>100 \mu\text{g/kg}$. None of the vendors interviewed was aware of the risk of mycotoxin contamination in their maize-based flours and feed. Limits set by the United States Food and Drug Administration ($20 \mu\text{g/kg}$) for total aflatoxins and European Commission ($2 \mu\text{g/kg}$) for AFB1 for maize flour imports, were varied between 2–35% and 66–100% of samples, respectively. The implications of this study for human and animal health in Rwanda suggest that expanded surveys are needed to understand the scope of contamination, given the influence of environment and other factors on aflatoxin accumulation. Available options to mitigate and monitor aflatoxin contamination can be further deployed to reduce contamination.

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1. Introduction

The Government of Rwanda through its Economic Development and Poverty Reduction Strategy (EDPRS) has used crop intensification programs (Minecofin, 2014) to triple maize production from 144,481 tons (t) in 2008 to 573,038 t in 2013 (NISR, 2014). However, to the best of our knowledge, there is no published record of the

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amount of stored maize in Rwanda that is potentially contaminated by aflatoxigenic fungi or by aflatoxin. Such contamination is a common problem in much of sub-Saharan Africa and globally, and has implications for human and animal health, as well as international trade (Wagacha & Muthomi, 2008). *Aspergillus flavus* and *A. parasiticus* are the major species responsible for aflatoxin production, with other *Aspergillus* species such as *A. nomius* and *A. arachidicola* also capable of its production (Perrone, Gallo, & Logrieco, 2014). As yet, toxigenic species and aflatoxins have yet to be characterized and reported in Rwanda.

There are four naturally occurring aflatoxins (AFs) viz AFB1, AFB2, AFG1 and AFG2 (D'Mello, Placinta, & Macdonald 1999; Pitt,

2014); AFB₁, a metabolite of AFB₁, is also found in dairy products (Prandini et al., 2009). Of the types, AFB₁ has been reported to be the most dangerous mutagen and carcinogen (Francis, Shetty, & Bhattacharya, 1989; Anitha et al. 2014; Doi, & Uetsuka, 2014; IARC, 2015), and where aflatoxins are present in foods, generally exceeds half the total amount present (Matumba et al., 2014). For these reasons, regulatory limits for aflatoxins include AFB₁, and a number of analytical methods have been developed to measure its concentration or that of total aflatoxins (Matumba et al., 2014).

Given the scope of contamination reported, aflatoxins currently pose serious health problems in many parts of Africa (Gnonlonfin et al. 2013; Probst, Bandyopadhyay, & Cotty, 2014; Wagacha & Muthomi, 2008) and other parts of the tropics and sub-tropics. For example, an aflatoxicosis outbreak in Kenya in 2004 caused 125 deaths (Lewis et al., 2005). Chronic dietary exposure to aflatoxins is common in the east African region (Ek, Ka, & Kang, 2009; Ismail, Taligoola, & Ssebukyu, 2004; Owaga, Muga, Mumbo, & Aila, 2011; Rushunju, Laswai, Ngowi, & Katalambula, 2013) and in some cases, this is associated with the development of tumors (Awadelkarim, Mariani-Costantini, & Elwali, 2012; Elzupir & Alamer, 2014; Peers & Linsell, 1973). Stunting levels in the region are also high by comparison globally, and aflatoxin exposure has been associated with stunting (Asiki et al., 2014; IARC, 2015; Lombard, 2014).

The dearth of information for Rwanda on levels of contamination of maize by fungi and their toxins is possibly associated with a lack of appreciation of the current risks of exposure (Zain, 2011) and the factors that contribute to exposure to those risks. Aside from biophysical factors themselves (e.g., pre-, peri- and post-harvest practices, crop species and genotype, fungal populations, climatic conditions), three other factors have been previously reported to significantly influence levels of aflatoxins in food commodities: levels of education and awareness, and gender. Jolly et al. (2006) noted that Ghanaian participants in their study with primary education only or less were 2.3 times more likely to have AFB₁ albumin-adduct (AFB₁ that is covalently bound in peripheral blood albumin) in their blood than those with secondary education. Another study found that women were more concerned with creating an awareness of the risks of AFs and the benefit of reducing their levels whereas men were most concerned about the cost of reducing AF levels (Jolly, Bayard, Awuah, Fialor, & Williams, 2009). Low level of knowledge on fungal and AF contamination has been found to be positively correlated with high levels of AFs in the population by Sabran, Redzwan, Jamaluddin, Rosita, Mutalib, Sokhini, and Aqilah (2012).

In the absence of published data on the prevalence of aflatoxins in Rwanda, the first objective of this study was undertaken to determine the contamination levels of AFB₁ in maize sold in markets in Kigali. The second objective was to establish whether there was any association between AFB₁ levels in maize flour intended for human consumption and animal feeds, and the gender and education level of vendors, the origin of the maize, and awareness of AFs and other mycotoxins. Concentrations of AFB₁ obtained were compared to published maximum allowable limits for human and animal consumption. Characterizing the scope of contamination, and understanding potential social drivers of risk can help inform strategic and effective piloting and scaling of interventions to mitigate this challenge in Rwanda.

2. Materials and methods

2.1. Sampling

2.1.1. Sampling maize intended for human consumption

Sample collections from Kigali markets were carried out in

September 2014 (Round I) and January 2015 (Round II). A total of 474 samples from 158 vendors and 210 samples from 70 vendors were collected randomly in the first and second round, respectively. Seven principal retail markets (Nyamirambo, Kimisagara, Gikondo, Kimironko, Kicukiro, Nyarugenge and Nyabugogo) located in three districts (Kigali, Gasabo, Kicukiro and Nyarugenge) were selected. As they are open markets, vendors both inside and outside the formal market buildings were considered.

From each vendor, three 1 kg samples of maize flour were collected from each of three randomly selected bags. Because of the likelihood of a heterogeneous fungal and toxin distribution in each bag (Campbell, Whitaker, Pohland, Dickens, & Park, 1986), each sample consisted of ten 100 g sub-samples collected from different parts of the bag. Each 1 kg sample was thoroughly mixed and a 100 g sub-sample used for analysis. In Round I, a questionnaire was used to record the gender and education level of each vendor (primary only or primary and secondary education), the origin of the maize (domestic or imported) and any awareness by the vendor of aflatoxins and other mycotoxins. Round II collections were undertaken to verify whether market differences identified in Round I were a repeatable phenomenon attributable to specific markets. For Round II, ten maize flour vendors were randomly selected from each of the seven markets. Samples were collected as for the Round I but only the origin of maize was recorded.

2.1.2. Sampling for maize intended for animal feed

Three animal feed vendors were selected in Nyarugenge District, two in Kicukiro District and two in Gasabo District which yielded 11, five and four samples respectively. The same methodology described above was used to gather the samples. The limited number of samples was because there were relatively fewer feed vendors. These animal feed samples were maize-based but contained groundnut and other ingredients.

2.2. AFB₁ analysis

All samples were first analyzed using the competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique (Catalog # 941BAFLO1B1-96, Helica Biosystems, Santa Ana, CA, USA) which allows quantification between 1.0–20.0 and 5.0–100 µg/kg AFB₁ with a 5 and 25 dilution factor respectively. Samples with AFB₁ >100 µg/kg were analyzed using immuno-affinity column fluorometry with a VICAM[®] fluorometer (Series-4EX, Source Scientific LLC, USA) which enables quantification between 2.0 and 300 µg/kg AFB₁. For this study, maize intended for human consumption only required the ELISA method (AFB₁ <100 µg/kg); however for animal feeds, most samples had AFB₁ >100 µg/kg.

2.2.1. ELISA

The aforementioned AFB₁ ELISA Quantitative Kit was used. Either 5.0 g (5× dilutions) or 1.0 g (25× dilution) of the maize flour sample was mixed with 25 ml methanol (Sigma-Aldrich St. Louis, MO, USA):water (Milli-Q Water Purifier, Millipore, Bedford, MA, USA) (7:3 v/v) for 5 min at high speed (250 rpm) in a shaker (New Brunswick Scientific, Edison, NJ, USA). All standards and sample extracts were analyzed in duplicate micro-wells, according to manufacturer instructions. The micro-wells were measured optically by a micro-plate reader with an absorbance filter of optical density 450 nm. A logit regression equation generated from standard ODs and corresponding standard concentrations was used to calculate the AFB₁ concentration in sample extracts. The final concentration was adjusted according to the dilution factor; the limit of detection was 1.0 µg/kg and 5.0 µg/kg for the 5× and 25× dilutions, respectively.

a.) Method validation

Prior to sample analysis by ELISA, the method was validated for ensuring data quality. Spike recoveries and the Coefficient of Variation (CV) were calculated for each AFB1 standard concentration used respectively (0, 0.20, 0.50, 1, 2 and 4 µg/kg) with the acceptable level of CV set at 5%. Certified corn reference material obtained from the Office of the Texas State Chemist (OTC) were used to assess the accuracy of aflatoxin prediction.

b.) Quality control

In-house analytical method performance characteristics developed for assessing the accuracy, precision and linearity for each ELISA plate were performed to screen for the integrity of data generated. The linearity of calibration curve was assessed by calculating the regression coefficient (r^2). The minimum acceptable level for the r^2 was set at 0.98. The accuracy of the method was assessed in each ELISA plate by using three different known concentrations of certified ground corn samples of aflatoxins (OTC-Aflatoxin Proficiency Testing in Eastern and Central Africa program) for varying sample dilutions and quantification ranges. The three concentrations used are 5 µg/kg ($\pm 40\%$), 40 µg/kg ($\pm 34\%$) and 273 µg/kg ($\pm 20\%$). ELISA plates whose determined aflatoxin concentration of the reference material was off the range were repeated. One sample was randomly selected and analyzed twice per plate, and plates with greater than 10% relative percent difference were repeated.

2.2.2. Immuno-affinity fluorometry

Extracts were obtained by mixing 5.0 g sample with 0.5 g salt (NaCl) and 25.0 ml methanol:water (8:2; v/v) and shaking the mixture for 5 min at 250 rpm before filtering the extract (Catalog No 1001 125 Filter Paper, 125 mm Ø, Whatman®, GE Healthcare UK Ltd, Buckinghamshire, UK). Subsequently, 2.0 ml filtered extract was diluted with 8.0 ml distilled water. The diluted extract was then filtered through a 1.5 µm glass micro-fiber filter (VICAM, Sweden); 2.0 ml of filtered diluted extract was then passed completely through an AflaTest® column at a rate of 1–2 drops s^{-1} . Columns were washed twice with 5.0 ml distilled and deionized water (ddH₂O). The aflatoxin elution was completed by passing 1.0 ml HPLC grade absolute methanol through the column and eluate collected into a glass cuvette. The latter was mixed with 0.5 ml HPLC grade absolute methanol (Sigma-Aldrich, Switzerland). Then 1.0 ml Aflatest developer was mixed by vortexing with the eluate. After 60 s, total aflatoxin (AFB1 + AFB2 + AFG1 + AFG2) (µg/kg) concentration was read on a VICAM® fluorometer calibrated using mycotoxin standards supplied by the manufacturer. The final concentration was adjusted for sample dilution.

2.3. Statistical analysis

AFB1 concentrations in different samples and associated socio-demographic factors collected during interview with vendors were entered into SPSS (IBM, PASW Statistics 16.0, USA) and analyzed with R software. The analysis was performed on the log transformed level of aflatoxin to reduce the heterogeneity in the variances among markets and rounds and skewness of the distribution among samples (Campbell et al., 1986; Maestroni & Cannavan, 2011). A linear mixed model was used to calculate the association between the socio-demographic factors considered (gender of vendor, market origin of maize and awareness of aflatoxins) and the level of AFB1 in samples using Analysis of Variance (ANOVA). Since socio-demographic data was collected for Round 1 only, analysis these terms could only be tested for Round 1 data.

The chi-square test was applied to calculate the association between markets and two rounds of maize collection using the Analysis of Deviance (ANDEV). Mean, standard deviation and median were calculated for samples according to the markets. No p-values are associated with the ANOVA because of the ambiguity of the definition of the degrees of degrees of freedom (df) for the F distribution of each of the variables investigated; however since the number of samples was in the hundreds, 100 was used for the denominator. The Sums Squares (SS) was used to determine which components contribute most to the variance in aflatoxin levels.

AFB1 concentrations obtained were compared to different multiple maximum allowable limits set by different countries and organizations, and the percentage of maize samples above these limits was estimated. The European Commission (EC) has fixed the maximum levels for AFB1 and total aflatoxins at 2 µg/kg and 4 µg/kg in maize, respectively (EC, 2006). In maize that is intended for human consumption, 10 µg/kg is the maximum level of total aflatoxins allowed by the Kenyan Government and the United Nations World Food Programme (WFP) (IFPRI, 2011), and 20 µg/kg by the Food and Drug Administration (FDA, United States of America) (Zain, 2011). For animal feed, 100 µg/kg total aflatoxins is currently the regulatory limit set by the FDA for corn and peanut products intended for breeding beef cattle, breeding swine or mature poultry (e.g. laying hens) (FDA, 2016).

3. Results

3.1. Markets

There were significant differences in AFB1 levels between markets in Round I with a >95% contribution to the overall variation (Table 1). However there was also a significant interaction between Rounds (Table 2), indicating that the differences between markets were not the same in each Round. In Round I, the highest mean level, 24.7 ± 23.7 µg/kg, was found at Nyarugenge and the lowest, 8.0 ± 5.6 µg/kg at Kimisagara; in Round II, the highest mean level, 25.7 ± 25.9 µg/kg, was found at Nyamirambo, and the lowest, 10.2 ± 8.4 µg/kg, at Nyarugenge (Table 3). Maximum levels did not exceed 98.6 µg/kg and 116.9 µg/kg for the Round I and Round II respectively.

3.2. Socioeconomic factors, origin of flour and vendor awareness

There was no discernable effect of gender, level of education or origin of flour on AFB1 level (Table 1; Fig. 1a, b, c). The only imported maize flour encountered during sample collection was from Uganda, of which 100 samples were collected. All vendors stated that they were unaware that their maize flour may be contaminated with either aflatoxins or other mycotoxins. Mean levels of AFB1 for all three variables was approximately 8–10 µg/kg.

3.3. Animal feed

There were no differences in levels of AFB1 between markets located in three different districts (Fig. 2). Mean levels varied between 100.4 and 168.6 µg/kg, and maximum levels were 265 µg/kg.

3.4. Proportion of maize flour samples above legal limits

For all samples intended for human food consumption, the percentage range above each limit considered vary between 66 and 100%, 28–87% and 2–35% for EU (2 µg/kg), Kenya-WFP (10 µg/kg) and USA FDA (20 µg/kg) limits, respectively (Table 4). For samples for animal feed, 75% had >100 µg/kg AFB1, the FDA limit.

Table 1
ANOVA table showing contribution to variation in AFB1 by each socio-demographic factor during the first round.

Socio-demographic factor	Df	Sum squares (SS)	Mean squares (MQ)	F value	Contribution SS (%)
Market	6	3.681	0.6135	7.5317	99.24776
Origin of maize	1	0.0056	0.00563	0.0691	0.150988
Gender of vendors	1	0.0059	0.00592	0.0727	0.159077
Education level of vendors	1	0.0164	0.0164	0.2014	0.44218
Total		3.7089	0.64145		

Table 2
Variance components and market differences analysis.

	Df	Sums squares (SS)	Mean squares (MQ)	F value	% SS
Market	6	2.424	0.404	5.544	0.455
Round	1	0.481	0.481	6.595	0.090
Market-Round	6	2.422	0.404	5.539	0.455
Total		5.327	1.288		

4. Discussion

Contamination of samples collected in Kigali markets commonly exceeded legal limits, and there was a lack of awareness amongst vendors that their maize flours as they had never heard of aflatoxins. The factors that may be contributing to the current situation in Rwanda form the basis of this discussion.

Although there were significant differences in AFB1 levels between markets, there was no consistent pattern in the two Rounds of sampling. That the maize flour of domestic origin was contaminated was anticipated because although Rwanda experiences a montane and therefore less humid tropical climate than is often the case in other maize-growing parts of sub-Saharan Africa, it remains favorable for aflatoxigenic fungi growth (Clay & Dejaegher, 1987; Medina, Rodriguez, & Magan, 2014). However, similar levels of contamination were found in samples indicated by vendors of having originated from Uganda. Their precise origin remains unknown, although only small differences have been found in levels of aflatoxins in maize kernels collected from humid and dry zones in Uganda in one study (Kaaya & Kyamuhangire, 2006). However, the levels of AFB1 observed in Kigali markets were lower than those found during recognized outbreaks elsewhere, such as in Kenya (reported levels can exceed 1000 µg/kg) (Lewis et al., 2005; Mutiga et al., 2014; Mwihi et al., 2008; Ndung et al., 2013). Pre- and post-harvest conditions, including storage, play major roles in aflatoxin production, and how this affected the samples collected in this study is unknown.

There were no discernable effects of any of the socioeconomic factors tested. This may be linked to the limited time and the conditions under which vendors store the maize flour before it is sold having a minimal effect on levels of aflatoxins compared to the

opportunities for contamination to develop during on-farm storage after harvest (Kaaya & Kyamuhangire, 2006; Villers, 2014). All vendors had a basic formal education, and in Kigali, the minimum requirement before starting any business is to be literate. In contrast, levels of education in rural areas of Rwanda remain very low (Chatikobo, Manzi, Kagarama, Rwemarika, & Umunezero, 2009), and in spite of technology transfer schemes for farmers (Odeyemi, 2003), their awareness of the dangers of aflatoxin contamination is likely to be low. Kumar and Popat (2010) have shown that extension staff and traders with basic levels of education can have a good understanding of concerns about aflatoxin contamination.

However, vendors were unaware of mycotoxins and their consequences, a finding similar to that for a groundnut value and supply chain in Malawi where awareness of aflatoxins was low amongst all value-chain actors (Matumba, Van Poucke, Monjerezi, Njumbe Ediage, & De Saeger, 2015). An awareness amongst farmers has been shown to reduce the level of aflatoxins in maize through good agricultural practices and proper handling (Muthomi, Mureithi, Chemining, Gathumbi, & Mutit, 2012). That traders in Kigali markets had no knowledge of aflatoxins suggests that creating an awareness and capacity to reduce levels in maize and other products is urgently needed for a range of stakeholders in Rwanda. Of course, the ultimate objective of reducing aflatoxin accumulation pre- and post-harvest also requires farmer education and deployment of interventions with actors across the value chain.

Compared to samples for human consumption, those for animal feeds were highly contaminated, with mean maximum levels of aflatoxins between 4 and 6 times greater (100–250 µg/kg). These results are consistent with those reported from Kenya for animal feed, 52–556 µg/kg, though as in this study, few samples (27 and 21 in the respective studies) were collected (Rodrigues, Handl, & Binder, 2011). Similarly in Ethiopia, 26.2% of feed samples contained AFB1 at levels exceeding 100 µg/kg (Gizachew, Szonyi, Tegegne, Hanson, & Grace, 2016). There is a linear correlation between AFB1 in feed consumed by dairy animals and AFM1 excreted in milk (Golge, 2014), in which it remains stable following pasteurization and ultra-high temperature treatments (Bilandžić et al., 2015). The detection of AFM1 in poultry products following consumption of aflatoxin-contaminated feed has negative

Table 3
Analysis of aflatoxin levels in maize intended for human consumption, by market across the two collections.

Markets	Round I						Round II									
	Number of vendors	Number of samples	Mean (µg/kg)	SD ^a	Median (µg/kg)	Wald confidence interval (95%)			Number of vendors	Number of samples	Mean (µg/kg)	SD ^a	Median (µg/kg)	Wald confidence interval (95%)		
						Est. Mean ^b	2.5%	97.5%						Est. Mean ^b	2.5%	97.5%
Nyabugogo	26	78	10.55	18.15	3.53	3.89	2.89	5.45	10	29	21.41	19.10	18.77	12.61	9.77	22
Kimisagara	29	87	8.00	5.57	6.93	5.75	4.33	7.91	10	30	21.31	14.06	16.38	18.29	10.35	22.89
Gikondo	28	84	11.74	14.79	7.28	7.18	5.38	9.93	10	30	10.40	10.71	8.70	7.86	4.37	9.69
Nyamirambo	15	45	17.33	16.09	10.43	9.32	6.29	14.53	10	30	25.69	25.85	22.25	18.21	16.44	39.68
Kicukiro	25	75	17.79	13.48	12.57	13.53	9.98	18.36	10	30	12.38	8.12	11.73	10.31	5.55	12.47
Kimironko	14	42	14.93	7.31	13.98	11.59	7.72	19.09	10	30	11.64	5.77	10.72	10.48	7.15	17.47
Nyarugenge	21	63	24.71	23.74	14.70	15.42	11.06	22.44	10	30	10.24	8.40	6.85	7.68	4.07	9.33

^a SD: Standard Deviation.

^b Est. Mean: Estimated Mean.

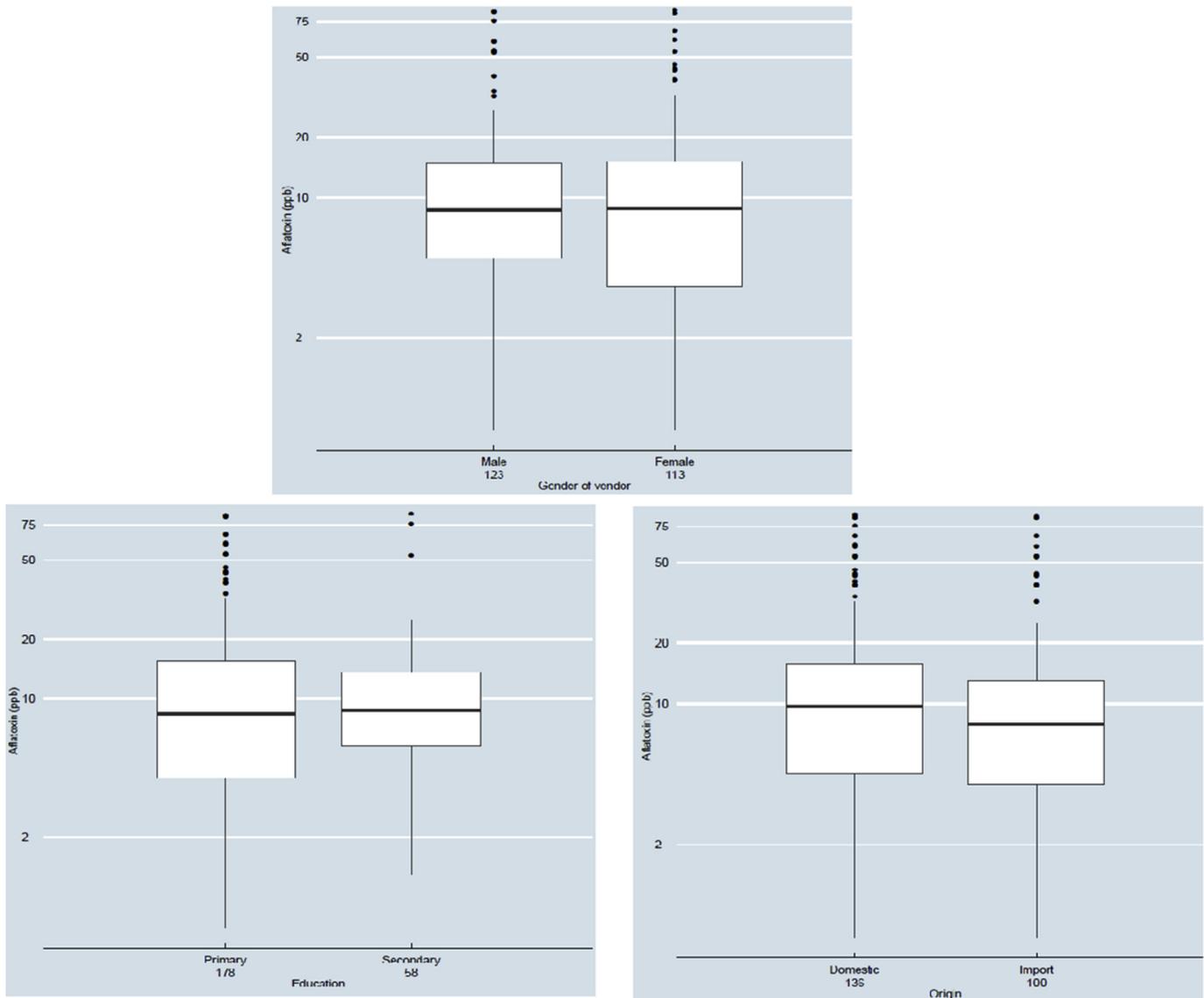


Fig. 1. The relationship between aflatoxin level and gender (a), level of education (b) and origin of flour (c).

implications for public (Iqbal, Nisar, Asi, & Jinap, 2014) and livestock health (Gündüz & Oznurolu, 2014). So in spite of the small sample size, these findings suggest that a greater awareness of the presence of aflatoxins in animal feed in Rwanda is required, coupled with piloting and scaling of known and adapted interventions to reduce risk of aflatoxin contamination.

The percentage range of maize flour samples for human consumption above EC regulation varied in our study, between 66 and 100%; this is the most stringent limit for both AFB1 and total aflatoxins, 2 and 4 $\mu\text{g}/\text{kg}$ respectively (EC, 2006). However, 28–87% of samples had higher levels than the US FDA regulatory limit of 20 $\mu\text{g}/\text{kg}$ for total aflatoxins (Luo et al., 2011). This situation inevitably prejudices Rwandan products on international markets; cereal-based products have already been rejected at European borders for non-compliance (EU, 2015). Developed countries offer a lucrative and high-value export market and a source of income for developing countries. Failure to meet food safety standards is often related to limited resources and institutional constraints. Sanitary and Phytosanitary Standards (SPS) can vary but should be seen as non-tariff barriers (Li & Beghin, 2012) that can help persuade exporting countries to upgrade quality standards not only to meet

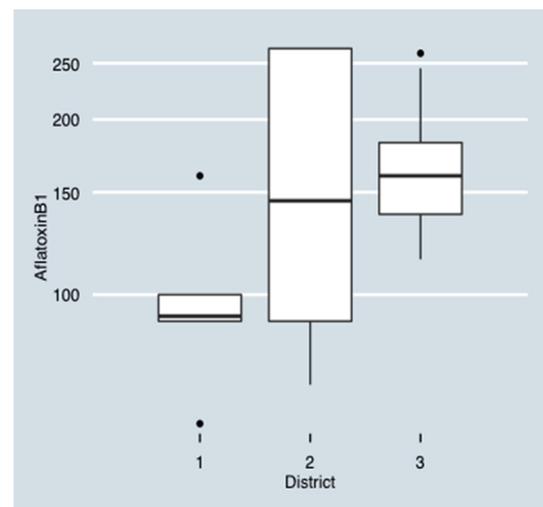


Fig. 2. Aflatoxin levels in maize-based animal feed blends in three districts: Gasabo (1), Kicukiro (2) and Nyarugenge (3).

Table 4

Proportion of maize flour (food) samples above the European Union, Kenya-WFP and USA FDA regulations by collection round, and probability (based on chi-square test) that the relative proportions in affected maize among the markets between rounds 1 and 2 and the interaction of round and market.

Markets	>2 µg/kg		>10 µg/kg		>20 µg/kg	
	Round I (%)	Round II (%)	Round I (%)	Round II (%)	Round I (%)	Round II (%)
Nyabugogo	66.7	89.7	28.2	62.1	10.3	34.5
Kimisagara	85.1	100	28.7	86.7	2.3	23.3
Gikondo	89.3	96.7	33.3	30	8.3	6.7
Nyamirambo	91.1	100	37.8	80	24.4	30
Kicukiro	100	96.7	62.7	63.3	21.3	6.7
Kimironko	97.6	100	59.5	53.3	19	6.7
Nyarugenge	98.4	96.7	79.4	30	30.2	10
Percentage range above each limit	66–100		28–87		2–35	
	P-Value (Chi-square)					
Market	<0.0001		<0.0001		0.0008	
Round	<0.0001		0.002		0.589	
Market × Round	0.0926		<0.0001		<0.0001	

the requirements of their export markets but also food safety in their local markets (Jongwanich, 2009; Neeliah, Neeliah, & Goburdhun, 2013).

While aflatoxin levels are notoriously difficult to fully characterize in a single study, given that they can change drastically depending on the harvest season, these results highlight challenges and opportunities to improve food safety in Rwanda. Further surveys and testing of interventions can help further characterize the dynamics and scope of this challenge in Rwanda, and resolve the best available options to address it. In parallel, ongoing regional and continental discussions, including the East African Community's recently developed Roadmap to address aflatoxin contamination outlines priorities and a range of interventions that have been effective elsewhere. Integration of appropriate Good Agricultural Practices, including proper tillage, fertilizer application (Mutiga et al., 2014; Tubajika, Mascagni, Damann, & Russin, 1999), use of appropriate varieties (Bhatnagar-Mathur, Sunkara, Bhatnagar-Panwar, Waliyar, & Sharma, 2015), proper harvesting (eg, not placing harvested maize on the soil), and proper drying and storage, can be effective at reducing aflatoxin levels in maize and other crops. Furthermore, biological control using Aflasafe is being piloted and scaled out in various sub-Saharan African countries, and could be considered for application in Rwanda (Bhatnagar-Mathur et al., 2015). The disparate amounts of maize above the limit for different official standards further highlights the need for a better understanding of what the appropriate level is in a Rwandan and sub-Saharan African context. While the spectre of aflatoxin contamination is a challenge to address, strategic adaptation and deployment of appropriate interventions can help secure a safe harvest.

5. Conclusion

The percentage range above each limit considered vary between 66 and 100%, 28–87% and 2–35% for EU (2 µg/kg), Kenya-WFP (10 µg/kg) and USA FDA (20 µg/kg) limits, respectively while 75% of animal feed samples had >100 µg/kg AFB1, the FDA limit. All vendors declared that they are unaware of aflatoxins and their consequences. These findings reveal the need to both enforce and update existing SPS relating aflatoxins in Rwanda, and for education programs to raise awareness amongst stakeholders and their capacity to reduce aflatoxin risk. Consideration should also be to more widespread testing of food products, e.g. milk and eggs that are likely to be contaminated because of the widespread use of maize feed and the potential negative consequences on public health. Follow up studies are necessary to more extensively assess the scope and dynamics of aflatoxin contamination across multiple

years, and to identify and deploy effective and sustainable interventions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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