Occurrence of mycotoxins in food, feed, and milk in two counties from different agro-ecological zones and with historical outbreak of aflatoxins and fumonisins poisonings in Kenya

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Received 19 February 2017; Revised 19 April 2017; Editorial decision 23 May 2017.

Abstract

Aflatoxins and fumonisins contaminate cereals during pre- and post-harvest periods. In this study, household or market maize, sorghum, millet, cow or goat milk, and animal feed samples collected from two counties (Makueni and Nandi) of Kenya and were analyzed for aflatoxins and fumonisins using competitive enzyme-linked immunosorbent assay and confirmation with high performance liquid chromatography. There was a significant difference ($P < 0.005$) in the levels of aflatoxins between the home grown and market-sourced maize, sorghum, and millet samples. In Makueni, 24.8% of home maize and 44.6% of the market maize samples exceeded the 10 ppb limit for aflatoxins. In all, 93% and 90% of the maize samples were contaminated with fumonisins and 34% and 6% exceeded the 2 ppm limit in Makueni and Nandi, respectively; 30% and 37% of homegrown sorghum and millet samples exceeded the 10 ppb limit for aflatoxin in Makueni and Nandi, respectively; and 89% and 81% of homegrown millet samples in Makueni and Nandi, respectively, were positive for fumonisins and 22% and 7% in Makueni and Nandi, respectively, exceeded the 2 ppm fumonisins limit. In total, 52% and 87% of the milk samples in Nandi and Makueni, respectively, were contaminated with aflatoxin M1 and 8% of the samples from Makueni exceeded the 50 ppt limit. There is an urgent need to build capacity among the households on cheap, practical, and effective technologies that would reduce the proportions of food samples contaminated with aflatoxins and fumonisins.

Key words: aflatoxins; fumonisins; contamination; cereals; agro-ecological zones; Kenya.

Introduction

Mycotoxins are a group of toxins produced by fungi. Nearly 70 000 fungal species have been reported and described, but it is estimated that nearly 1.5 million species might still exist (Bhat et al., 2010). Fungi colonize and utilize solid substrates by secreting enzymes to break down complex products. In most of the cases, the colonizing
fungi produce and secrete low-molecular-weight compounds generally referred to as ‘secondary metabolites’ or ‘mycotoxins’, which are usually not required for normal growth and survival. Mycotoxin production by fungi has been suggested as a response to fungi against pests or rodents and to agro-biocides (Moss and Frank, 1987; Wicklow et al., 1994).

Aflatoxins are produced by mainly Aspergillus flavus and A. parasiticus species. Aflatoxins were discovered in 1960 following deaths of turkey pullets in the UK (Blount, 1961). The cause was attributed to feed (Brazilian peanuts) contaminated with A. flavus. Aflatoxins have been estimated to contaminate about 25% of the world’s cereals (CAST, 2003). About 5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Strosnider et al., 2006). Eighteen different types of aflatoxins have been identified, wherein the major ones include aflatoxins B1, B2, G1, G2, and M1 (Bhat et al., 2010). The International Agency for Research on Cancer (IARC) has listed AFB1, AFB2, AFG1, and AFG2 as group 1 carcinogens (Chiavaro et al., 2001). Chronic exposure to aflatoxins has been associated with growth retardation, immunosuppression, and susceptibility to diseases (Caulfield et al., 2004a, 2004b; Williams et al., 2004).

Fumonisins are a group of mycotoxins produced by Fusarium verticillioides and F. proliferatum. Fumonisins were discovered in 1988 from a culture of F. verticillioides (Marasas, 2001) and occur naturally as FB1, FB2, FB3, and FB4. Of late, six different types of fumonisins (FA1, FA2, FB1, FB2, FB3, and FB4) have been reported (Bhat et al., 2010), wherein the ‘A’ series are amides and the ‘B’ series possesses a free amine (Gelderblom et al., 2010). Furthermore, FC1 has been reported in the ‘C’ series (Bhat et al., 2010). Fumonisins contaminate maize, sorghum, and millet and are known to inhibit ceramide synthase, an enzyme that increases the rate of cell death, blocks ceramide biosynthesis, which disrupts overall sphingolipid metabolism, and results in accumulation of sphinganine, a compound thought to be responsible for the toxicity of fumonisins (Voss et al., 2007). Fumonisins have been shown to cause equine leukoencephalomalacia (Diaz and Boermans, 1994), porcine pulmonary edema (Hashek et al., 2001), renal toxicity, hepatotoxicity, and hepatocarcinogenicity in rats and is a suspected risk factor for neural tube defects (Marasas et al., 2004). Consumption of maize contaminated with fumonisins has been correlated with esophageal cancer in areas of southern Africa and China (Li et al., 1998; Shephard et al., 2005).

The objective of this study was to establish the occurrence of aflatoxins and fumonisins in maize, sorghum, millet, and aflatoxin M1 (AFM1) in milk from two counties in Kenya, one with a history of acute aflatoxicosis (Makueni) and the other with high rates of esophageal cancer (Nandi) in humans.

Materials and Methods

Site and household selection

The counties Nandi and Makueni in Kenya were purposively selected based on history of human acute aflatoxicosis in Makueni (Lewis et al., 2005) and high incidences of esophageal cancer in Nandi (Wakhisi et al., 2005). The sublocations were identified by a team of researchers together with veterinarians, agriculture extension officers, and health officers at the county and ward offices. The selection criterion was based on dairy and maize production. In the first instance, the subcounties were selected that fitted the criteria based on the records at the county level. Three subcounties were selected in each county. At the subcounty headquarters, the wards were selected using the same criteria of dairy production and maize growing. The extension officers (veterinarians, agriculture, and public health) selected the sublocation that best fitted the criteria used to select the wards. The households in the sublocation were listed that fitted the criteria of having dairy animals, grew maize and/or sorghum, and barley growing. Makueni falls within the lower midland agro-ecological zones LM3, LM4, and LM5. It receives between 200 and 1200 mm of rainfall per year, which is unreliable at times with frequent droughts resulting in crop failures.

Sampling of cereals and milk

In each of the selected households, samples of maize, sorghum, millet, and milk were sought. If the household did not have maize, sorghum, millet, and milk, they indicated where they bought these and the research team subsequently purchased them and labeled them as being associated with those households. For milk, the team would leave money for the household to buy as part of their usual household supply from the same vendor or household supplying the milk.

Maize, sorghum, and millet in each household were sampled from the storage bags. If the total number of bags was less than 10, all bags were sampled. The sampling probe (spike) was used to sample at three levels, top, middle, and bottom, aiming at getting more than 1 kg of the sample when all subsamples were pooled. If the number of bags was more than 10, the formula of Mahuku et al. (2010) was used to calculate how many bags would be sampled:

$$M = \text{Sup} \left[ \sqrt[3]{N + 1} + 10 \right]$$

where $M$ is the maximum number of samples (starting from a minimum of 10), $N$ is the number of bags to be sampled, and $\text{Sup}$ is the supremum of the given set.

Market samples of maize, sorghum and millet, and milk were bought from markets and hammer mills serving the villages. About 500 ml of bulk cow and/or goat milk samples were collected in clean plastic bottles, transported in cool boxes, and stored frozen at −20°C until analyzed, usually within 3 months after collection.

Analysis of cereal grains and feeds for total aflatoxins

Sample preparation

Determination and quantification of total aflatoxin content in the cereal samples and feeds were done using competitive enzyme-linked immunosorbent assay (cELISA) (RIDASCREEN® test kit, r-Biopan, Germany). Sample preparation and analysis were carried out as per the manufacturer’s protocol. The final amount of aflatoxins present was determined using a software program supplied by the cELISA kit manufacturer, with 1.75 µg/kg as the limit of detection.
Analysis of maize, sorghum, millet, and feeds for fumonisins
The maize, sorghum/millet, and feed samples were analyzed using an ELISA test kit for fumonisins (RIDASCREEN® Fumonisins Art.: R3401, r-Biopharm) to quantify total fumonisins. The manufacturer’s recommended methodology was adopted. The test kit had the lowest detectable limit as 25 μg/kg.

Analysis of milk for Aflatoxin M1
Sample preparation
The analysis of milk for AFM1 was carried out using the competitive ELISA method according to the manufacturer’s instructions (r-biopharm) with modification adopted from EVIRA (Finnish Food Safety Authority) as described below. Analysis was done within 3 months of sample collection. About 5 ml of milk sample was warmed and centrifuged for 15 min at 1011 g. The upper cream layer was removed and 2.5 ml of the defatted milk transferred to a test tube and 5 ml of ethyl acetate (88.1 g/mol; melting point −83.6°C) added. This was vortexed for 1 min and the mixture centrifuged at 1011 g for 15 min at room temperature. About 3 ml of the ethyl acetate layer was transferred into a clean test tube and evaporated to dryness under a stream of nitrogen. The sample was diluted with 250 μl of the sample dilution buffer and 30 μl of 70% methanol:water, vortexed and analyzed for AFM1 using the competitive ELISA kit according to the manufacturer’s procedure. The limit of detection was 5 ng/L. Levels below this limit were estimated using a company program that extrapolated the quantitation levels.

Analysis of aflatoxins in cereals and feeds using high performance liquid chromatography
The high performance liquid chromatography (HPLC) conditions for analysis of total aflatoxins in cereals and feeds were as follows: injection volume 20 μl, flow rate 1 ml/min, runtime 35 min, fluorescence excitation at wavelength 363 nm, emission wavelength 440 nm. The mobile phase was acetonitrile:water (20:80), and the analytical column (C18) was a Waters nova pak (3.9 × 150 mm; 4.6 μm, detection limit of 0.01 ppb). The analysis was done using a Shimadzu (Japan) HPLC with a fluorescence detector model RF-2-A.

Imunoaffinity column preparation
A disposable syringe of 20 ml capacity was placed on the top of Immunoaffinity Column (IAC, AFLAPREP, r-Biopharm) with an adapter and the column connected to a vacuum manifold. Storing the column in toluene containing 10% dimethyldichlorosilane (layering with 10% dimethyldichlorosilane in toluene) round-bottom flask. The filtrate was evaporated to dryness using a rotavapor. The extract was diluted with 20 ml of phosphate-buffered saline (PBS, 0.13 M, pH 7.0) solution. The PBS extract was filtered into 20 ml syringe using a Whatman filter paper No. 4 and added to the IAC. The solution was allowed to drop at a rate of 1–3 ml/min. The IAC was washed with 15 ml of PBS buffer. To the column, 3 ml of methanol was added using a syringe. Methanol was left to stand in the column for a few seconds and then eluted. The eluate was dried completely by passing it through a stream of nitrogen. Into the dried sample, 200 μl of trifluoroacetic acid (TFA) was added, and then closed with a cap, vortexed, and let to stand at room temperature for 30 min. Into a brown vial, 800 μl of acetonitrile:water (30:70) was added and filtered with Whatman paper 4. The sample was then taken to run in the HPLC column following an elution order of G1, B1, G2, and B2. The peaks were identified as derivatives of TFA. The calibration curve was constructed, and the quantities were calculated as μg/kg.

Analysis of aflatoxins in milk using HPLC
The HPLC method was based on the ISO standard 14501:2007 as modified by the Finnish Food Safety Authority EVIRA (EVIRA 8556). The milk sample was warmed in a water bath between 35 and 37°C, centrifuged at 2065 g for 30 min and 50 ml of skimmed milk sample collected.

The barrel of a 50 ml disposable syringe was attached to the top of an immunoaffinity column (r-Biopharm) and connected to the 12-port vacuum manifold system (SUPELCO PREP®Y, Sigma-Aldrich Co. Ltd). In all, 50 ml of the test sample were placed into a 50 ml syringe and allowed to pass through the immunoaffinity column at the rate of 1–3 ml/min while controlling the volume flow by using the vacuum system. The column was washed with 10 ml of water by allowing it to pass through the column at a steady flow rate. The column was dried completely after washing. AFM1 was eluted slowly from the column into a conical tube by passing 4 ml of pure acetonitrile in about 60 s through the column using a 10 ml syringe. The sample was concentrated by drying in the water bath set at 30°C and under a stream of nitrogen. A final volume of 400 μl was made with 10% acetonitrile. The eluate was pumped at a constant flow rate of 0.5 ml/min through the HPLC column. The acetonitrile:water ratio of the HPLC eluate was adjusted (25%) to ensure an optimal separation of the AFM1 from other extract components.

The stability of the chromatographic system was checked by repeatedly injecting a fixed amount of AFM1 standard working solution until stable peak areas or heights were achieved. Consecutive injections did not differ by more than 5% in the peak area or peak height. The responses in retention time of the AFM1 peaks depend on the temperature; therefore, to compensate for drift in the detection system, a fixed amount of AFM1 standard working solution was injected at regular intervals.

Calibration curve of AFM1
Suitable volumes of the standard solutions containing 0.05, 0.10, 0.20, and 0.40 ng/ml of AFM1 were injected in sequence into the HPLC loop. A calibration graph was made by plotting the obtained peak area or peak height for each standard working solution against the mass of the AFM1 injected.

Statistical methods
Data were entered into an Excel spreadsheet 2009 and analysis done using IBM SPSS software version 19 (IBM Corp., 2010), for descriptive and quantitative data. Chi-square tests were used to check the associations between household characteristics and district of origin at P ≤ 0.05% level of significance. Statistical differences, for quantitative data, between two groups were done using independent samples t-test, whereas for more than two groups, F-tests were utilized.
Correlations were done to determine strength of association between the HPLC test results and ELISA.

Results

Aflatoxin levels in cereals

A total of 419 and 595 samples were collected from Nandi and Makueni counties, respectively. Table 1 summarizes the aflatoxin contamination of the three cereals in both counties. There was a significant difference in the means of aflatoxins levels between Makueni and Nandi for both homegrown and market purchased cereals (Table 1). More samples of maize, sorghum, and millet were contaminated in Makueni than Nandi. In Nandi, only sorghum samples (37.1%) exceeded the 10 ppb limit (Food and Agricultural Organization/World Health Organization, 1990, 1992) for food intended for human consumption, whereas in Makueni, both home and market samples for maize, sorghum, and millet exceeded this limit at different proportions (Table 1). The maize samples from Nandi, both home and market, recorded significantly (F = 19.88; P < 0.01) lower levels of aflatoxin contamination compared to Makueni. However, there were no significant differences between homegrown and market sourced maize within the two sites (Table 1). In Nandi, comparison was only possible for homegrown maize as market sorghum and millet samples were not available. Contamination levels of aflatoxin in sorghum and millet samples were not significantly different between the sites (sorghum F 0.83, P = 0.439; millet F 1.86, P = 0.164).

There was a significant difference between the contamination levels of maize in Makueni and Nandi irrespective of source (F = 22.316; P < 0.01). The means of aflatoxins levels in maize samples having more than 2 ppm, which is the limit for food intended for human consumption (FAO/WHO, 2014), were not statistically significant (t = 0.684, P = 0.495) between Nandi (2.72 ± 0.441 ppm) and Makueni (3.24 ± 0.293 ppm).

Fumonisins levels in sorghum and millet

In all, 257 sorghum and 65 millet samples from Makueni and Nandi, respectively, were analyzed for fumonisins by cELISA. Makueni constituted a majority (75.9%) of the sorghum samples, whereas 68.3% of the millet samples were from Nandi mainly homegrown. Overall, 97.7% and 84.1% of the sorghum and millet samples, respectively, were positive for fumonisins. The mean fumonisin levels in sorghum and millet collected from both sites were not statistically significant between and within the sites (Table 2). Overall, 61.5% (158/257) of the sorghum samples and 11.1% (7/63) of the millet samples had fumonisin levels exceeding the 2 ppm limit (FAO/WHO, 2014). There was no statistical difference (t = 0.576; P = 0.589) in the means of fumonisin levels in millet samples from Makueni and Nandi that exceeded 2 ppm, whereas Nandi recorded a significantly (t = 2.614, P = 0.010) higher mean fumonisin level (3.80 ± 0.15) above 2 ppm compared to Makueni (3.35 ± 0.08).

Table 1. Competitive ELISA results of total aflatoxins levels in maize, sorghum, and millet.

| Commodity | Site     | Source        | % Positive | % > 10 ppb | Mean ppb (95% CI)* | Range (ppb) | F-value | P-value |
|-----------|----------|---------------|------------|------------|--------------------|-------------|---------|---------|
| Maize     | Nandi    | Home (n = 272) | 68.3       | 0.0        | 0.98 (0.86, 1.10)  | 0.089–5.3   | 19.88   |         |
|           | Makueni  | Home (n = 325) | 80.4       | 24.8       | 24.82 (18.31, 31.33) | 0.054–279.2 | 0.00    |         |
|           | Nandi    | Market (n = 42) | 73.0       | 0.0        | 2.14 (0.95, 3.32)  | 0.26–20.9   |         |         |
|           | Makueni  | Market (n = 55) | 91.1       | 44.6       | 38.15 (20.54, 55.76) | 0.35–288.7  |         |         |
| Sorghum   | Nandi    | Home (n = 62)  | 66.1       | 37.1       | 24.52 (12.28, 36.76) | 0.149–210.1 | 0.83    |         |
|           | Makueni  | Home (n = 186) | 85.9       | 29.9       | 17.76 (11.86, 23.67) | 0.044–264.5 | 0.439   |         |
|           | Makueni  | Market (n = 9) | 100        | 33.3       | 9.47 (2.88, 16.06)  | 1.7–33.4   |         |         |
| Millet    | Nandi    | Home (n = 43)  | 92.3       | 0.0        | 1.64 (1.08, 2.20)   | 0.14–11.1   | 1.86    |         |
|           | Makueni  | Home (n = 18)  | 82.4       | 17.6       | 17.22 (0.00, 42.14) | 0.385–230.6 | 0.164   |         |
|           | Makueni  | Market (n = 2) | 100.0      | 50.0       | 7.90 (0.00, 20.64)  | 1.14–14.4  |         |         |

*Means with the same letters are not significantly different at α = 0.05

Table 2. Competitive ELISA results for total fumonisins levels in maize, sorghum, and millet.

| Site     | Sample source        | % Positive | % > 2 ppm | Mean (95% CI) (ppm)* | F-value | P-value |
|----------|----------------------|------------|-----------|----------------------|---------|---------|
| Maize    | Makueni Home (n = 285) | 91.9       | 28.9      | 1.31 (1.07, 1.55)    | 22.316  | 0.000   |
|          | Makueni Market (n = 49) | 94.2       | 38.2      | 2.14 (1.29, 2.99)    |         |         |
|          | Nandi Home (n = 219)   | 84.2       | 5.5       | 0.29 (0.10, 0.41)    |         |         |
|          | Nandi Market (n = 40)  | 95.2       | 7.1       | 0.34 (0.13, 0.53)    |         |         |
| Sorghum  | Makueni Home (n = 186) | 97.8       | 64.5      | 2.21 (1.97, 2.45)    | 0.042   | 0.959   |
|          | Makueni Market (n = 9) | 100.0      | 44.4      | 2.19 (0.93, 3.46)    |         |         |
|          | Nandi Home (n = 62)    | 96.8       | 54.8      | 2.28 (1.82, 2.74)    |         |         |
| Millet   | Makueni Home (n = 18)  | 88.9       | 22.2      | 1.22 (0.00, 2.50)    | 1.83    | 0.169   |
|          | Makueni Market (n = 2) | 100.0      | 0.0       | 0.24 (0.00, 0.55)    |         |         |
|          | Nandi Home (n = 43)    | 81.4       | 7.0       | 0.30 (0.00, 0.62)    |         |         |

*Means with same letters are not significantly different at α = 0.05
AFM1 levels in cow and goat milk

Overall, 529 milk samples from Makueni and Nandi were tested for AFM1 using competitive ELISA. Table 3 shows the levels obtained in various samples. Overall, 76.7% and 51.9% of the milk samples from Makueni and Nandi, respectively, were positive for aflatoxins. Only samples from Makueni (8.7% and 4.2% home and market, respectively) exceeded the Food and Agricultural Organization/World Health Organization (1990, 1992) limit of 0.05 µg/kg. There was a significant difference (t = 42.4; P = 0.00) in the levels of AFM1 in cow milk between Nandi and Makueni for the home produced milk. There was no statistical difference (t = 0.75; P = 0.39) between the levels of AFM1 [22.28 (16.89, 27.67); 16.0 (10.13, 21.87)] of home produced and market milk samples in Makueni. No market milk samples was obtained from Nandi.

Total aflatoxin levels in animal feeds

In all, 207 feed samples from Nandi were analyzed for total aflatoxin (B1, G1, B2, and G2) levels using cELISA. No feed samples was obtained from Makueni. Of the samples, 53.7%, 58.3%, and 59.6% were found positive for aflatoxins in Kipkarren, Kilibwoni, and Kaptumo (sampling sublocations in Nandi), respectively. Among these samples, 6.9%, 23.8%, and 23.5% in Kipkarren, Kilibwoni, and Kaptumo, respectively, exceeded the Food and Agricultural Organization/World Health Organization (1990, 1992) limit of 0.05 ppb set for feed destined for dairy animals. A majority of the feed samples from Nandi were mouldy (spoilt) maize. There was no significant difference (F = 0.61; P = 0.61) in the contamination levels between the three sublocations of Nandi county. The Kilibwoni sublocation had the highest average mean of 8.5 ppb (CI 95%, 0, 20.67) and Kaptumo had the sample with the highest level of total aflatoxins of 264 ppb. Generally, 56% of the feed samples was found positive and 14.6% exceeded the 5 ppb limit (Food and Agricultural Organization/World Health Organization, 1990, 1992). Although the overall average mean is 3.84 (0.55, 7.13) ppb, the number of samples exceeding the regulatory limit (Food and Agricultural Organization/World Health Organization, 1990, 1992) is high to raise concern.

Comparison between cELISA and HPLC

AFM1 in milk samples

In all, 109 randomly selected milk samples (positive and negative on cELISA) were analyzed by HPLC. Of these, 39 were goat milk mainly from Makueni. Of the cow milk samples, 53.8% and 13.8% were positive and 21.4 exceeded the Food and Agricultural Organization/World Health Organization (1990, 1992) limit in Makueni. As for goat milk, 61.5% and 41.7% of the samples were positive and exceeded the Food and Agricultural Organization/World Health Organization (1990, 1992) limit, respectively. There was a significant difference (P = 0.048) between the mean levels of AFM1 in cow milk from Makueni and Nandi. There was a significant difference (P < 0.05) in AFM1 levels between goat and cow milk samples obtained from Makueni. The correlation between the cELISA and HPLC results for cow and goat milk samples (64 and 39, respectively) was significantly different from zero (r = 0.46, P = 0.30 and r = 0.54, P = 0.00).

Fumonisins levels of selected samples using HPLC

Overall, 86 samples were tested for fumonisins with HPLC. Of these, 64 were cereals and 22 feed samples. FB1 was the most common toxin detected in 43% of the sample types. All the three fumonisins (FB1, FB2, and FB3) were detected in 34% of the maize samples. Overall (all sample types), FB1, FB2, and FB3 were detected in 24% and FB2 in 2.3% of the samples. No samples had FB3 alone. Other combinations were detected in about 2.3% of the samples each. In feeds, mainly FB1 (81.8%) was detected with minor proportions of FB1, FB2, and FB3 (13.6%).

Discussion

The results obtained in this study show that there is widespread contamination of cereals with aflatoxins in the Nandi and Makueni counties. The levels of contamination and number of samples contaminated with aflatoxins from Makueni were higher than from Nandi (Table 1). This could be attributed to the hot and dry weather conditions in Makueni that promote Aspergillus growth and subsequent aflatoxin production (Reddy et al., 2010). Makueni also experiences frequent drought periods, which increase the number of airborne fungal spores and plant stress, two factors that contribute to grain contamination pre-harvest (Payne, 1998). Another reason for high levels of contamination in Makueni is the presence of more toxigenic A. flavus S strain compared to the less toxigenic A. flavus L strain found in Nandi (Okoth et al., 2012).

The aflatoxin positive samples in maize, millet, and sorghum (Table 1) in both sites raise concern as they lead to chronic exposure to aflatoxins, as the grains constitute the staple diet in these two counties and the whole of Kenya, as well. The carcinogenicity of aflatoxin is due to their ability to produce altered forms of DNA adducts. In humans, epidemiological studies have shown that where there is a high incidence of hepatocellular carcinoma, there is an association between cancer incidence and the content of aflatoxins in the diet (Liu et al. 1988). Ingestion of low to moderate levels of aflatoxins has been associated with stunting in children below 5 years (Gong et al., 2004), teratogenic effects associated with congenital malformations, and depression of immune responses (Unnevehr and Grace, 2013).

The high contamination levels of aflatoxins in maize from Makueni concur with the results of earlier prevalence studies done in the area (Lewis et al., 2005; Muthomi et al., 2009). Maize samples tested for aflatoxins during the largest recorded outbreak of aflatoxicosis in the Eastern province showed contamination levels in the range of 1 to 46 400 ppb (Lewis et al., 2005). A study in the same

Table 3. Competitive ELISA results for Aflatoxin M1 levels in cow and goat milk samples.

| Site | Sources | % Positive | > 50 ppt | Range (ppt) | Mean (95% CI) | t-test | P-value |
|------|---------|------------|----------|------------|--------------|--------|---------|
| Cow milk | Makueni | Home (n = 185) | 87.0 | 8.7 | 0.002–273.8 | 22.28 (16.89, 27.67) | 42.4 | 0.00 |
| | Nandi | Home (n = 264) | 51.9 | 0.0 | 0.002–26 | 2.71 (1.92, 3.50) | 42.4 | 0.00 |
| Goats milk | Makueni | Market (n = 25) | 96.0 | 4.2 | 1.2–56.9 | 16.0 (0.13, 21.87) | 42.4 | 0.00 |
| | Nandi | Home (n = 54) | 92.6 | NA | 0.84–35.8 | 13.32 (10.87, 16.32) | 42.4 | 0.00 |
| | | Home (n = 1) | 100.0 | NA | | | | |
country by Mwihia et al., (2008) found contamination in 37 out of 104 (35.5%) locally grown maize samples exceeding the FAO/WHO recommended maximum limit of 10 ppb. Of those samples, 20.2% had aflatoxins levels above 100 ppb, with 10.6% having levels above 1000 ppb. Lewis et al. (2005) associated homegrown maize with the acute aflatoxicosis outbreak of 2004. In this study, the contamination of homegrown maize, sorghum, and millet samples was significantly different from market samples (Table 1). However, the levels of contamination reported here were lower than the levels reported in the previous studies (Lewis et al., 2005; Mwihia et al., 2008). This study, therefore, does not support the results reported by Lewis et al. (2005) that the number of homegrown maize samples contaminated with aflatoxins was higher than the number of market purchased maize samples. Market maize samples are source of exposure, as 91.1% and 73% from Makueni and Nandi, respectively, were contaminated with aflatoxins, whereas 44.6% of the samples from Makueni exceeded the FAO/WHO 10 ppb limit. In our opinion, the market samples had higher proportion and levels of contamination with aflatoxin than homegrown due to (a) aggregation of cereal stock from different sources resulting in increased levels of contamination; (b) additionally, Liu et al. (2006) reported that longer storage was associated with a significant increase in aflatoxin contamination. Market maize sold in Makueni County is mostly from other counties and may be contaminated at source. Makueni households (50%) cannot meet their household maize requirements from their homegrown maize. They rely on market purchased maize to meet these needs. Maize marketing in Kenya lacks traceability; therefore, it is difficult to trace the source of maize sold at the markets.

The average contamination level of 83% in the two counties was similar to the level found for market and maize products in Nairobi (Okoth and Kola, 2014). High proportions of aflatoxin-contaminated samples and high amounts of aflatoxin detected in samples from Makueni are consistent with the findings by Okoth et al. (2012), where all the isolated toxigenic strains from Makueni were of the S-type, whereas those from Nandi belonged to the L-type. The study further found quantitative differences in aflatoxin production in vitro between isolates and between strains with S strains producing relatively larger amounts of total aflatoxins, B toxins, and lower values for G toxins. This explains the findings reported in this study. There were significant differences in the adoption of good agricultural practices between farmers in Makueni and Nandi, which contribute to the contamination levels witnessed in this study (E. K. Kang’ethe, unpublished data).

These results imply that control of aflatoxin contamination of homegrown maize, millet, and sorghum is imperative in order to reduce exposure at household level. This is critical as the food products made from these cereals are used by the vulnerable groups (under 5 years and over 60 years old) considering the average per capita consumption of 0.28 and 0.607 vs. 0.171 and 0.48 kg/day of maize by these groups in Makueni and Nandi, respectively. The aflatoxin contamination levels of grains in Nandi and Makueni and per capita consumption reported by this study suggest an increased risk of chronic exposure to the residents. Aflatoxin-contaminated feeds are the source of exposure to livestock. In the study by Kang’ethe and Lang’a (2009) of animal feeds obtained from urban and peri-urban livestock farmers, 87% was contaminated with aflatoxins and 67% exceeded 5 ppb. In this study, 56% of the samples were contaminated and 14% exceeded the limit of 5 ppb (Food and Agricultural Organization/World Health Organization, 1992). Feeds from the rural farmers, which exceeded the 5 ppb limit, were four times less contaminated than those of their urban counterparts reported by Kang’ethe and Lang’a (2009). The main difference observed in this study was that the rural farmers relied less on commercial feeds but more on spoilt maize and pasture. However, the danger exists of greater exposure to rural households, if the spoilt maize is contaminated with the highly toxigenic A. flavus S strain found in Makueni (Okoth et al., 2012) than the less toxigenic L strain, which was more common in Nandi. With current climate change due to global warming, the conditions may change in Nandi prompting the L strains to produce more toxins than under the current climatic conditions. If this scenario changes, the feeds in Nandi would be a source of livestock exposure and consequently higher levels of milk samples would be contaminated with AFM1.

Over 70% of the milk samples (cow and goat) from both Nandi and Makueni counties were found positive for aflatoxin contamination. The figure is similar to that reported for urban milk by Kang’ethe and Lang’a (2009), who reported that 72% of the milk samples were positive for AFM1 from urban and peri-urban farmers. Elgerbi et al. (2004) reported a similar contamination level of 72% in North African milk with AFM1 range of 30–310 ppb. Other studies in Africa (Atanda et al. (2007) in Nigeria; Elzupir et al. (2009) in Sudan; Dutton et al. (2012) in South Africa; and Tchana et al. (2010) in Cameroon) have shown contamination of cow milk with AFM1 at different levels as well as samples that exceeded the regulatory limits set by FAO/WHO (1992) of 50 ppt. The mean level of AFM1 in rural Kenyan milk (cow and goat) ranged from 2.7 to 22.3 ppt for home produced milk from Nandi and Makueni, respectively, and 16 ppt for marketed milk in Makueni. These mean levels are lower than those reported for cow milk in South Africa of 120 ppt and Sudan of 2070 ppt (Elzupir et al., 2009; Dutton et al., 2012). The contamination of cow milk with AFM1 is important as the majority of children are weaned on to cow milk and maize-, sorghum-, and millet-based porridges fortified with milk, which could in addition be a major source of exposure for this vulnerable group.

There is increased advocacy for dairy goat keeping in Kenya as a less intensive means of dairying for rural communities and equally as a source of household milk. In Makueni, goat milk was common but from unimproved local galla goats. Of the samples taken in Makueni, the mean level of AFM1 was 13.3 ppt. This was lower than the levels found in cow milk. Bilandić et al. (2014) has reported higher mean values in Croatian goat milk than the ones reported in this study (2780–4800 ppt). This could be a reflection on the differences in the management of goats between Makueni and Croatia. In Makueni, goats are left to browse on the tree shrubs and are rarely supplemented with animal feeds. The source of AFM1 is mainly from spoilt cereal grains found unfit for human consumption in households that are given to the milking goats and not intentional supplementation, as would be the case in good dairy goat management practices. In this study, a significant positive correlation was observed between the farmers who fed their animals on spoilt maize and milk samples that were found to be positive for aflatoxin contamination (P < 0.05). Farmers who thought milk from animals fed on mouldy maize was safe were almost four times more likely to use spoilt maize to feed their animals.

The burden of disease due to aflatoxins in Kenya has been estimated at 8.5 and 4.9 liver cancer cases per 100 000 persons in men and women, respectively (Wu et al., 2011). The two counties have an estimated population of 949 298 and 920,445 for Makueni and Nandi, respectively, in 2015 (National Bureau of Statistics, 2012). In 2010, the World Health Organization published ‘The global burden of foodborne disease, according to WHO’, in which aflatoxin-associated Disability Adjusted Life Years (DALYs) is the sum of the
Years of Life Lost (YLL) due to premature mortality in the population and the Years Lost due to Disability (YLD) for people living with the health condition or its consequences: in the African region, which includes Kenya, it was estimated at 3 (range 1–8). This means that population in this region would lose on average 3 years due to death or disability due to aflatoxin exposure. Globally, aflatoxin food-associated DALY has been estimated at 636 863 (Havelaar et al., 2015). Lack of data from Kenya and regions within the considering Kenya has a high level of aflatoxin-contaminated cereals means that the County Governments cannot prioritize aflatoxin control and the population is continually at risk.

It is evident that maize from the two sites was contaminated with fumonisins. The proportion of maize testing positive in this study (93%) is similar to that reported by Warth et al. (2012) in Mozambique, where 92% of the samples were contaminated. Fandohan et al. (2005) reported higher levels (6–12 ppm) of fumonisins in maize from Burkina Faso than those reported in this study (3.99 ppm).

Chronic exposure to fumonisins is associated with maladies in humans and livestock. The levels reported here are consistent with chronic exposure and may contribute to the high incidences of esophageal cancer among the Nandi ethnic group as reported by Wakhisi et al. (2005). In the questionnaire administered during the sampling, we specifically asked whether it was known if people in the villages in the study sites were suffering from either aflatoxin poisoning or cancer of the throat/esophagus. In both study sites, 24 respondents (3 and 21 from Makueni and Nandi, respectively) said that they knew of people within their villages and those in surrounding areas who were or had suffered from cancer of the throat/esophagus. From Table 2, it is evident that a higher proportion of samples (maize, sorghum, and millet) from Makueni was contaminated with fumonisins than in Nandi. This trend is expected to lead to more esophageal cancer cases in Makueni due to higher exposure. However, the lower proportions of esophageal cancer cases reported in Makueni from anecdotial recall are attributable to low knowledge of the condition. In Nandi, the study by Wakhisi et al. (2005) increased awareness of the condition, whereas a similar study is lacking in Makueni.

Fumonisins have been reported to contaminate not only maize but also local beers brewed using contaminated sorghum in Botswana (Nkwe et al., 2005) and maize in South Africa (Shephard et al., 2005). In this study, 15% of the respondents indicated that mouldy spoiled maize was used to make local brews (changaa and busaa) and there is a risk of exposure to fumonisins from consumption of these maize-based local brews. Sorghum and millet were equally contaminated with fumonisins with a prevalence of 95%. The levels of fumonisins in sorghum and millet were comparable to those found in maize, thus failing to concur with the results of Leslie (2014) that sorghum was 1.8 times and pearl millet 12 times less risky than maize in regard to fumonisin contamination.

The contamination of feed samples with fumonisins mirrored the contamination of maize in the study sites. Feed samples that were from the households (mainly mouldy maize) were less contaminated than the commercial feeds obtained from the markets. The fact that none of these samples exceeded the 50 ppm level (FAO/WHO) indicated that fumonisins in feeds were not a major problem at the two study sites but considering the reproductive and production losses that are occasioned by fumonisins exposure in livestock; attempts should be made to control exposure. Farmers should be educated on the dangers of feeding animals with mouldy feeds that are not safe; noting that co-occurrence of aflatoxins and fumonisins in the maize, sorghum, and millets is common.

In this study, about 60% and 85% and 55% and 75% of market and home samples from Nandi and Makueni, respectively, were contaminated with both fumonisins and aflatoxins. The two toxins co-occur in the same crops. Aflatoxins are a known carcinogen causing point mutations in the tumor suppressor gene p53, leading to dysfunction of p53 protein/21-mediated tumor suppressing activity. Fumonisins, on the other hand, have their role in inhibiting ceramide synthase enzyme known to increase the rate of cell death and increase the accumulation of sphingosine bases that regulate cell proliferation, cell migration, vascularisation, and all processes involved in cancer progression (Watterson et al., 2003). The role of fumonisins in potentiating carcinogenesis associated with aflatoxins has been proposed (Pitt et al., 2012).

There was a good correlation between cELISA and HPLC with a kappa statistics of 1 indicating high correlation between the two tests. For market and home maize samples, kappa statistics was 0.223 indicating a medium correlation in the results of HPLC and cELISA. Sorghum and millet samples showed no correlation between the results posted by the two tests. This may be an indication of matrix effects in the analysis of sorghum and millet samples. cELISA and HPLC results of AFM1 in cow milk showed a positive correlation, which clearly indicates that the two tests were consistent in detecting samples with high and low levels of AFM1.

Conclusions

The frequent contamination of staple foods with aflatoxins and fumonisins found in the study sites implies that there should be capacity building targeting attitude and practice change at household level in rural communities. Aflatoxin and fumonisin control strategies should target proper practices of grain production and storage to prevent contamination with these mycotoxins in order to reduce exposure. Good agricultural practices have been shown to drastically reduce contamination during pre- and post-harvest of cereals. Therefore, such practices should be strongly recommended for the agro-ecological areas, where a high risk for mycotoxin contamination has been recorded.

Acknowledgements

The authors thank the Ministry of Foreign Affairs of Finland for providing financial assistance that enabled this study (MFA 24819801); the communities in Nandi and Makueni for allowing to carry out the study and Government officers in both counties for their facilitation. The views expressed in this paper are not necessarily those of the funding agency or institutions taking part in the study, but those of the authors.

Conflict of interest statement. The authors confirm that there is no conflict of interest with the publication of this paper.

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