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Determination of multi-mycotoxin occurrence in maize based porridges from selected regions of Tanzania by liquid chromatography tandem mass spectrometry (LC-MS/MS), a longitudinal study

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ABSTRACT

Residents of certain areas of Tanzania are exposed to mycotoxins through the consumption of contaminated maize based foods. In this study, 101 maize based porridge samples were collected from villages of Nyabula, Kikelelwa and Kigwa located in different agro-ecological zones of Tanzania. The samples were collected at three time points (time point 1, during maize harvest; time point 2, 6 months after harvest; time point 3, 12 months after harvest) over a 1-year period. Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to detect and quantify 9 mycotoxins: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), deoxynivalenol (DON), ochratoxin A (OTA) and zearaleneone (ZEN) in the samples following a QuEChERS extraction method. Eighty two percent of samples were co-contaminated with more than one group of mycotoxins. Fumonisins ($FB_1 + FB_2$) had the highest percentage occurrence in all 101 samples (100%) whereas OTA had the lowest (5%). For all three villages the mean concentration of FB_1 was lowest in samples taken from time point 2. Conversely, In Kigwa village there was a distinct trend that AFB_1 mean concentration was highest in samples taken from time point 2. DON concentration did not differ greatly between time points but the percentage occurrence varied between villages, most notably in Kigwa where 0% of samples tested positive. ZEN occurrence and mean concentration was highest in Kikelelwa. The results suggest that mycotoxin contamination in maize can vary based on season and agro-ecological zones. The high occurrence of multiple mycotoxins found in maize porridge, a common weaning food in Tanzania, presents a potential increase in the risk of exposure and significant health implications in children.

1. Introduction

Mycotoxins are naturally occurring toxic secondary metabolites produced by filamentous fungi which can contaminate many kinds of agricultural products. Toxigenic fungi are capable of growing under a wide range of atmospheric conditions depending on the species and they can contaminate crops during pre-harvest, immediate post-harvest, storage, transport and processing (Bennett & Klich, 2003). Mycotoxins have been shown to contaminate a wide range of agricultural products including: cereals, nuts, fruit, spices and wine (Abia et al., 2013; Serra, Braga, & Venâncio, 2005; Van de

Abbreviations: AFB_1, AFB_2, AFG_1, AFG_2, aflatoxin B_1, B_2, G_1, G_2; DON, deoxynivalenol; FB_1, FB_2, fumonisin B_1, B_2; IARC, International Agency for Research on Cancer; LOD, LOQ, limit of detection, quantification; OTA, ochratoxin A; QuEChERS, Quick, Easy, Cheap, Effective, Rugged, Safe; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; ZEN, zearalenone.

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Perre et al., 2014; Yogendrarajah, Van Poucke, De Meulenaer, & De Saeger, 2013). In the case of aflatoxins, they have also been detected in milk produced by cows that have consumed contaminated feed (Huang et al., 2013). Due to the ubiquitous presence of mycotoxins in both food and feed supply chains, and their association with various toxicological risks in both humans and animals, they have become a major economic and health concern.

More particularly, aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), deoxynivalenol (DON), ochratoxin A (OTA) and zearaleneone (ZEN) have all been recognised global health, agriculture and trade concerns due to the high occurrence and associated health impacts of these mycotoxins that has been found around the world. However, due to socio-economic and environmental factors, developing countries tend to be more severely affected by the threat of mycotoxins, especially aflatoxins, than developed countries (Wild & Gong, 2010; Williams et al., 2004).

The growing recognition of the threat of mycotoxins has stimulated scientific research to better understand how exposure and toxicity impact human health. The toxicity varies among different types of mycotoxins but many have shown the capacity to be acutely toxic, carcinogenic, mutagenic and immunosuppressive (Bakırdere et al., 2012). The most fatal human aflatoxicosis outbreak occurred in Kenya, 2004, with 317 recorded cases of acute hepatitis and 125 deaths (Nyikal et al., 2004). Public health officials discovered that this was linked to the consumption of AFB_1-contaminated maize and a case control study found that maize in case households had higher concentrations of aflatoxins compared to that of the maize from control households (Azziz-Baumgartner et al., 2005). The International Agency for Research on Cancer (IARC) classifies naturally occurring mixtures of aflatoxins ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) as Group 1 carcinogens (International Agency for Research on Cancer, 2002). Previous studies have also provided evidence that aflatoxins may cause immune suppression as a result of decreased protein synthesis, changes in enzymatic activity and changes in metabolism or cell cycles (Jiang et al., 2005, 2008). A study in West African children reported a strong inverse correlation between the exposure of aflatoxin and body height increase (Gong et al., 2004). Fumonisins, a group of mycotoxins produced by fungal species belonging to the *Fusarium* genus, have also been shown to have detrimental health effects. Fumonisins are widely distributed around the world and have been classified as possible carcinogens (Joint FAO/WHO Expert Committee on Food Additives, 2011). A study carried out in South Africa was able to demonstrate a positive correlation between fumonisin exposure and high incidences of human oesophageal cancer (Marasas, 2001). Fumonisin exposure has also been linked with increased occurrences of neural tube defects (Missmer et al., 2006). DON, another mycotoxin produced by fungal species within the *Fusarium* genus, has not yet been associated with any long-term health impacts in humans but animals with low dose chronic exposure to DON have shown that decreased growth and feed intake (Forsell, Witt, Tai, Jensen, & Pestka, 1986; Rotter, Thompson, Lessard, Trenholm, & Tryphonas, 1994). OTA, produced by *Aspergillus ochraceus*, is a common mycotoxin and a possible human carcinogen. Studies in animals have shown that it has the potential to be carcinogenic, immunosuppressive and neurotoxic (Álavarez, Gil, Ezpeleta, García-Jalón, & López de Cerain, 2004; Lioi, Santoro, Barbieri, Salzano, & Ursini, 2004; Schaaf et al., 2002). ZEN is produced by several fungi in the *Fusarium* genus including: *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium crookwellense*. It has estrogenic effects in pigs (Jiang et al., 2011) and suggested that it can trigger central precocious pubertal in human females (Massart, Meucci, Saggese, & Soldani, 2007).

Due to the toxic effect of mycotoxins in humans and animals it is important to develop analytical methods to detect them in food in order to facilitate their control and regulation. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is an effective method of detection for mycotoxin analysis. In recent years, studies have demonstrated LC-MS/MS methods capable of sub ppb detection for multiple mycotoxins in maize (Frenich, Vidal, Romero-Gonzalez, & Aguilera-Luiz, 2009; Liao et al., 2013; Malachová, Sulyok, Beltrán, Berthiller, & Krska, 2014; Zachariasova et al., 2014). Analytical methods for multiple mycotoxins should be selective for their target analytes, sensitive enough to detect toxins at relatively low concentrations and efficient to ensure rapid and reliable analysis.

Recently a mycotoxin study carried out in Tanzania examined the extent of dietary exposure of AFB_1, FB_1 and DON through the quantification of their respective biomarkers in serum and urine (Shirima et al., 2013; Srey, Kimanya, Routledge, Shirima, & Gong, 2014). The study found that young children in Tanzania are chronically exposed to AFB_1, FB_1 and DON through their diet. Urinary FB_1 was found to be negatively associated with length for age Z-scores whilst the negative association between AF-Alb and child growth did not reach statistical significance. In a recent study in Tanzania, maize kernels were sampled from three districts and multi-mycotoxins were measured by LC-MS method (Kamala et al., 2015). The study reported high occurrence of AFB_1 (50%) and FB_1 (73%). The food cooking process is known to have varying impact on mycotoxin levels, therefore measuring the levels of mycotoxins in cooked food can provide more close estimates of exposure than in maize flour.

This paper utilises a recently developed multi-mycotoxin detection method to determine the extent of multi-mycotoxin contamination in the maize porridge, in order to build upon mycotoxin occurrence and exposure data from previous studies; and to compare with the exposure biomarker data where possible.

2. Materials and methods

2.1. Reagents and chemicals

Acetonitrile (LC-MS grade), ammonium hydroxide (≥25% in water), dimethyl sulfoxide (≥99.9%), formic acid (≥98%), magnesium sulfate (anhydrous, ≥99.5%), methanol (LC-MS grade), mycotoxin standards (AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB_2, DON, ZEN and OTA), sodium chloride (≥99.0%) and Whatman® Puradisc 4 syringe filters (0.2 μm, PTFE) were all acquired from Sigma-Aldrich (Poole, United Kingdom). Each mycotoxin standard was separately dissolved in acetonitrile (0.2 mg/ml solution) and stored at −20 °C. Bondesil C_{18} was acquired from Agilent Technologies (Waldbronn, Germany).

2.2. Study design and sampling

Cooked maize porridge samples were collected from households across three rural villages in Tanzania: Nyabula (Iringa region), Kikelelwa (Kilimanjaro region) and Kigwa (Tabora region), which are from different agro-ecological zones. The samples were collected at three time points over the period of a year: Time point 1 (June/July 2010, a maize harvesting season), time point 2 (January 2011, six months after maize was harvested) and time point 3 (June/July 2011, another maize harvesting season 12 months after time point 1). The cooked porridge samples were dried after collection. A total of 101 samples; 10 samples from each village at time points 1 and 2, 14 samples from Nyabula and Kikelelwa villages at time point 3 and 13 samples from Kigwa village at time point 3 were randomly selected. The samples were oven dried and kept frozen at −80 °C until extraction for UPLC-MS/MS analysis. A blank maize flour