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A pilot study to evaluate aflatoxin exposure in a rural Ugandan population

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Abstract

OBJECTIVES The fungal metabolite aflatoxin is a common contaminant of foodstuffs, especially when stored in damp conditions. In humans, high levels can result in acute hepatic necrosis and death, while chronic exposure is carcinogenic. We conducted a pilot study nested within an existing population cohort (the General Population Cohort), to assess exposure to aflatoxin, among people living in rural south-western Uganda.

METHODS Sera from 100 adults and 96 children under 3 years of age (85 male, 111 female) were tested for aflatoxin–albumin adduct (AF-alb), using an ELISA assay. Socio-demographic and dietary data were obtained for all participants; HIV serostatus was available for 90 adults and liver function tests (LFTs) for 99.

RESULTS Every adult and all but four children had detectable AF-alb adduct, including five babies reported to be exclusively breastfed. Levels ranged from 0 to 237.7 pg/mg albumin and did not differ significantly between men and women, by age or by HIV serostatus; 25% had levels above 15.1 pg/mg albumin. There was evidence of heterogeneity between villages ($P = 0.003$); those closest to trading centres had higher levels. Adults who consumed more Matooke (bananas) had lower levels of AF-alb adduct ($P = 0.02$) than adults who did not, possibly because their diet contained fewer aflatoxin-contaminated foods such as *posho* (made from maize). Children who consumed soya, which is not grown locally, had levels of AF-alb adduct that were almost twice as high as those who did not eat soya ($P = 0.04$).

CONCLUSIONS Exposure to aflatoxin is ubiquitous among the rural Ugandans studied, with a significant number of people having relatively high levels. Sources of exposure need to be better understood to instigate practical and sustainable interventions.

keywords aflatoxin, general population cohort, rural, Uganda

Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi. Contamination of crops most commonly occurs during harvest and storage, when damp warm conditions allow the fungi to proliferate. Pre-harvest contamination of crops has also been reported. They are highly toxic and have been designated by the International Agency for Research on Cancer (IARC) as human carcinogens (Class 1: carcinogenic to humans). High levels of exposure can cause acute hepatic necrosis and death, while chronic exposure can cause carcinoma of the liver and possibly also growth impairment in children and compromised immunity (IARC 2002; Wild & Gong 2010).

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Aflatoxins can be measured directly in foodstuffs, and human exposure can be assessed at the individual level by measuring aflatoxin–albumin (AF-alb) adducts in blood. Foods for consumption by humans and domestic farm animals are routinely monitored and strictly regulated for aflatoxin contamination as part of standard food safety practices in most developed countries. In many low- and middle-income countries, regulations, even if present, are less frequently enforced and food shortages can exacerbate exposure to heavily contaminated products. Despite aflatoxin having a well-characterised detrimental effect on human health, understanding of human exposure and prevention has not been prioritised in resource poor settings.

Aflatoxin contamination of foodstuffs in Uganda – especially maize and groundnuts, as well as processed infant foods – has been reported, although levels in humans have not previously been systematically assessed (Kaaya & Warren 2005). The incidence of liver cancer remains high in Uganda, although the relative contribution of aflatoxin, hepatitis B and C, alcohol and other factors remains unclear (Parkin *et al.* 2010). We therefore conducted a pilot study nested within a longstanding population-based cohort – the General Population Cohort (GPC) – to investigate, for the first time, aflatoxin exposure among adults and children living in rural Uganda.

Methods

This pilot cross-sectional survey of aflatoxin exposure among adults and children living in rural Uganda was conducted within the context of an existing population-based cohort study – the GPC. It was originally established in 1989, by the UK Medical Research Council and the Uganda Virus Research Institute, in Kalungu District, south-western Uganda, to examine prevalence, incidence, risk factors, and trends of infection with the human immunodeficiency virus in a rural African population (Nunn *et al.* 1994). More recently, research activity has broadened to include the epidemiology and genetics of other communicable and of non-communicable diseases, including cancer, cardio-vascular disease and diabetes (Asiki *et al.* 2013).

In brief, the GPC is a community-based open cohort study of residents of neighbouring villages within one half of a subcounty, lying about 40 km from the shores of Lake Victoria. The population is scattered across the countryside in villages defined by administrative boundaries, with a few concentrated in small trading centres. Agriculture is the main economic activity with rain-fed, smallholder farms. Data on agricultural practice within the GPC area were collected in 2009 from 200 randomly selected households, as part of a study funded by the Food and Agriculture Organization (Taylor *et al.* 2011). This allowed examination of the proportion of households growing staple crops. Many households indicated growing fruit, beans, maize, cassava, vegetables, coffee, bananas, herbs, sweet potatoes, bananas, yam, sugarcane, groundnuts and pumpkins. There are different varieties of bananas, and the most common ones are *Matooke, Mbidde*, *Gonja* and sweet bananas. *Matooke* is the main staple food for the majority of households, while *Mbidde* is mainly used for brewing alcohol. The majority of these crops are grown for subsistence (household consumption) as well as for generating cash, and a number of them are a potential source of aflatoxin. Some farmers rear goats, pigs and cattle. Non-farming sources of income include petty trade in fish, coffee, food crops and locally brewed alcohol.

A population of approximately 10 000 people in a cluster of 15 villages was studied from 1989 to 1999. In 2000, the GPC was expanded to cover a further 10 villages. The cohort is dynamic with new births, deaths and migration reported at each round of follow-up, and the population under survey includes approximately 22 000 people. Data are collected through an annual census, questionnaire and serological survey. Details of sexual behaviour, medical, socio-demographic and geographical factors are recorded. Blood specimens are obtained at each annual survey. Serum is tested for HIV-1, and the remainder is stored at −80 °C in freezers in Entebbe. Seroprevalence of HIV has remained relatively stable in this population, with about 8% of participants infected.

In October 2011 (the rainy season), as part of survey round 22, additional serum samples and dietary information were collected from selected individuals living in four villages, to obtain aflatoxin exposure data, with a view to informing the design of larger and more comprehensive studies of mycotoxins in the future. A sample of 100 adults, aged 18 years and older, and 96 children younger than 3 years (distributed equally across the four villages) was identified using a multistage sampling method. At the first stage, four villages were selected (of 25) with probability proportional to size, leading to a self-weighting sample – of the 25 villages, numbers 3, 9, 15 and 20 were selected, because they are distributed across the GPC study area. At the second stage, a random sample of about 25 adults and 25 children was selected from within each village. Dietary information was collected from the adults (or from the parent or guardian of a child) using a food frequency questionnaire asking about the most common foods consumed, together with frequency of consumption per day or per week in the preceding 30 days. The questionnaire for children

obtained information on exclusive breast feeding and, where relevant, foods introduced during weaning. Indeed, the reason for inclusion of children under the age of 3 years was to examine the impact of different weaning practices on aflatoxin exposure levels. Liver function test results were available for 99 adults, as part of the round 22 medical survey.

From each participant, 2–3 ml of sera were collected in 4.5 ml SST vacutainers, stored in cool boxes and transported on the same day to the laboratory in Entebbe, where each sample was aliquoted on arrival and frozen at −80 °C. All samples were then shipped in one batch, on dry ice, to the University of Leeds, UK, where they were tested for aflatoxin–albumin (AF-alb) adducts – a well-validated aflatoxin exposure biomarker, which indicates exposure to aflatoxin over the preceding 2–3 months – using an ELISA assay. The assay, previously described by Chapot and Wild (1991), comprises four steps: albumin extraction, pronase digestion, adduct purification using a C18 cartridge and a competitive ELISA measurement of AF-alb adduct using a polyclonal anti-aflatoxin–albumin antibody. AF-alb was quantified against a standard curve, and quality controls (three positive and one negative) were analysed alongside each batch of samples. For the purposes of quantification and analysis, the detection limit was 3 pg/mg albumin; a value of 1.5 was assigned to any sample in which AF-alb was detected, but at levels below 3 pg/mg.

Data were transcribed onto case report forms (CRFs), double-entered and managed in MS Access. Analyses were conducted using Stata 11 (Stata Corporation, College Station, USA). Box plots were used to graphically examine the distribution of raw aflatoxin levels by socio-demographic and dietary variables. Because aflatoxin levels showed skewed distributions, results were $\log_{10}$-transformed and geometric mean levels and 95% confidence intervals (CI) were calculated. Analysis of variance (ANOVA) was used to compare mean log-transformed aflatoxin levels between socio-demographic and diet groups. We used linear regression to assess the association of aflatoxin level, as a continuous covariate, with laboratory liver function tests as the outcome. Fractional polynomials were used to examine the shape of the relationship of log-transformed aflatoxin levels with each liver function test, using a set of defined powers [−2, −1, −0.5, 0.5, 1, 2 and ln(x)] and a maximum of two power terms in the model. The differences in model deviances were compared; the linear model was used if the improvement in fit was not statistically significant at $P < 0.05$.

Ethical approval for this study was granted by the Uganda Virus Research Institute Scientific Ethics Committee and the Uganda National Council for Science and Technology.

Results

Sera from 100 adults (age range 18–89 years) and 96 children (age range 0–3 years) were tested; there were 85 males and 111 females. AF-alb levels ranged from 0 to 237.7 pg/mg albumin; 75% of the participants had levels above 7.1 pg/mg albumin, 50% had levels above 10.3 pg/mg albumin, and 25% had levels above 15.1 pg/mg albumin. Every adult and all but four children had detectable AF-alb. The individual with a level of 237.7 pg/mg albumin was an outlier (an apparently healthy adult female, who was followed up in the MRC clinic), the next highest level being 92.8 pg/mg albumin. In Figure 1, the distribution of raw AF-alb levels by socio-demographic and dietary variables is displayed for adults and children in the form of box and whisker plots. For the sake of clarity, the individual with the highest AF-alb level of 237.7 pg/mg albumin was excluded. In general, with the exception of village, there was relatively little heterogeneity in levels by each of the variables examined.

Table 1 shows geometric mean levels of AF-alb by age group (adults and children), sex, village and, among adults, HIV serostatus. No significant differences were detected by age group and sex. There was evidence of heterogeneity between the villages ($P = 0.003$): the two closest to the trading centres had higher levels. Ten of the 90 adults for whom an HIV test result was available were seropositive, but geometric mean levels of AF-alb were not different from those who were HIV seronegative ($P = 0.79$). There was no evidence of an association between aflatoxin level and any of the liver function tests examined (alanine transferase; aspartate transferase; AST/ALT ratio; alkaline phosphatase; gamma GT; bilirubin), although the numbers with abnormal levels were fewer than 10 for each marker (data not shown).

Among adults, diet was relatively homogeneous and few specific risk factors for high levels of AF-alb were identified (Table 2). However, those who reported eating *Matooke* (banana) had about half the level of AF-alb of those people who did not eat it ($P = 0.02$). As *Matooke* is a staple foodstuff in the area, those who do not eat it consume more of other products. Indeed, those who do not eat *Matooke* are more likely to report eating fresh cassava (64% *vs.* 35%, $P = 0.24$), dried cassava (27% *vs.* 10%, $P = 0.10$), fresh *posho* (82% *vs.* 28%, $P = 0.003$) and beans (55% *vs.* 26%, $P = 0.13$). At the time the blood specimens were obtained, few adults were

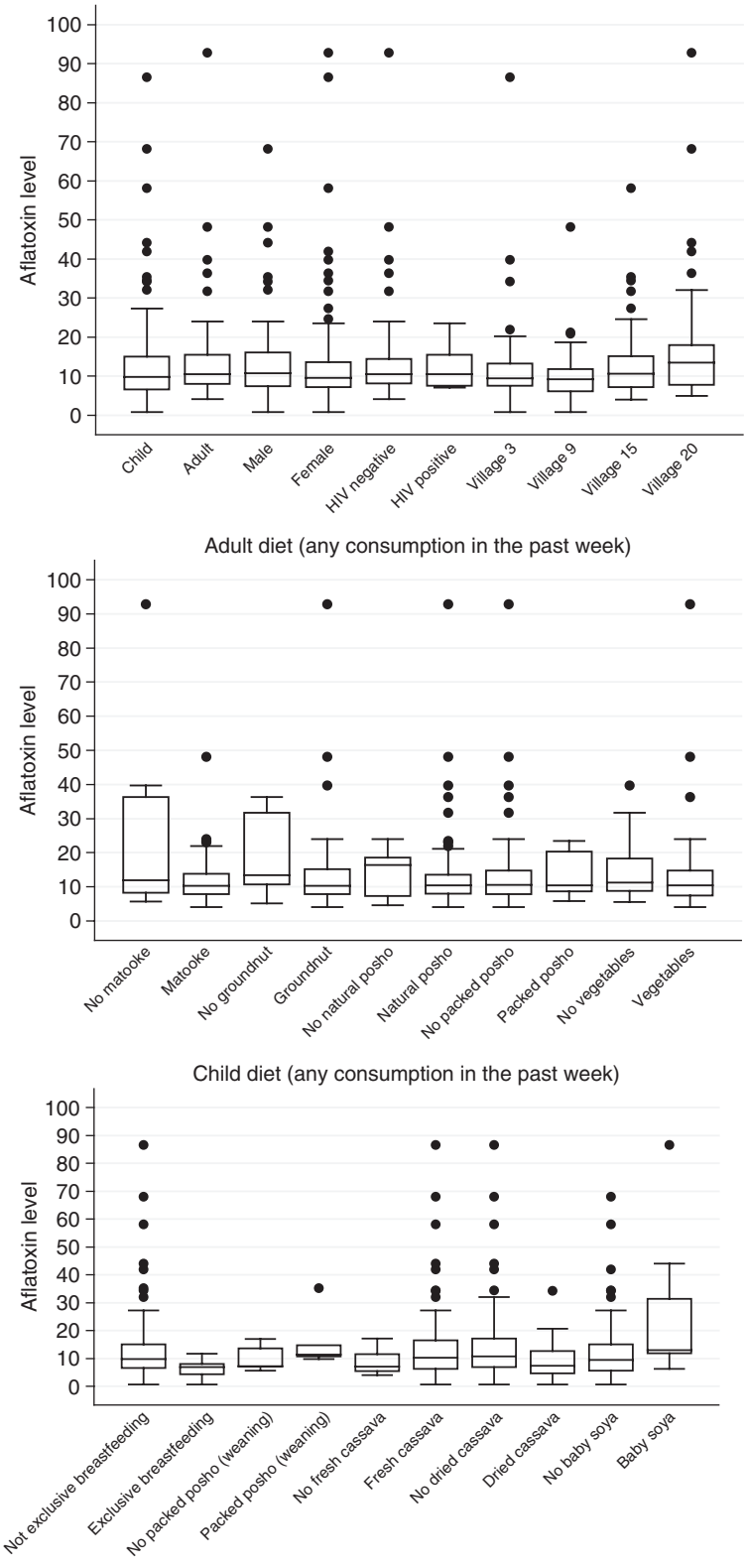

Figure 1 Aflatoxin levels by demographic factors (top), and diet in the past 30 days (adults, middle; children, bottom). The central line represents the median; boxes represent 75th and 25th centiles; whiskers represent upper and lower adjacent values, and dots represent outside values.

Table 1 Aflatoxin levels by socio-demographic factors and HIV serostatus

Variable	Geometric mean (95% CI)	*N*	*P*-value*
Age group			
Child	9.7 (8.2–11.5)	96	0.11
Adult	11.5 (10.2–13.0)	100	
Sex			
Male	10.5 (8.9–12.3)	85	0.88
Female	10.6 (9.3–12.2)	111	
Village number			
3	9.4 (7.5–11.8)	48	0.003
9	8.3 (6.7–10.2)	48	
15	11.4 (9.6–13.4)	50	
20	13.9 (11.1–17.3)	50	
HIV status			
Negative	11.5 (10.1–13.1)	80	0.79
Positive	10.9 (8.0–14.7)	10	

**P*-value from ANOVA.

eating maize, as it had not ripened yet, although groundnut consumption was more common (Table 1).

All five children reported to be exclusively breastfed at the time of the survey had detectable AF-alb, although the levels were less than half those of children who were not exclusively breastfed ($P = 0.03$), but the numbers are too small to draw reliable conclusions. Among the 14 children being weaned, there was some evidence that mean AF-alb levels were higher in those being given packed *posho* or maize ($P = 0.09$ and $P = 0.06$, respectively). Weaned children who consumed soya, which is not grown locally, had geometric mean levels of AF-alb that were almost twice as high as those who did not eat soya ($P = 0.04$). Consumption of dried cassava was associated with lower mean AF-alb levels ($P = 0.03$).

Discussion

The environmental conditions in Uganda are ideal for *Aspergillus* growth both during crop cultivation and during subsequent storage. Contamination by aflatoxin of staple foods – maize (Kaaya *et al.* 2001, 2007), cassava (Sebunya & Yourtee 1990; Kaaya & Eboku 2010), groundnuts (Lopez & Crawford 1967; Kaaya *et al.* 2006) and locally manufactured baby foods (including baby soya and rice porridge) (Kaaya & Warren 2005) – has been reported. Bananas, particularly *Matooke*, are not a major source of exposure – hence, in all likelihood, the lower AF-alb levels in people consuming *Matooke* may reflect lower consumption of potentially aflatoxin-contaminated foodstuffs. While it is known that foodstuffs in Uganda can be contaminated by aflatoxins, this is the first well-planned study of human exposure except a small pilot study among 30 children during the mid-1970s (Wild *et al.* 1990).

AF-alb adduct was near-ubiquitous among the rural Ugandans studied here, with levels similar to those reported in Guinea (Turner *et al.* 2003), but lower than levels reported in other sub-Saharan African countries including Tanzania, The Gambia and Kenya (Turner *et al.* 2003; Gong *et al.* 2012; Shirima *et al.* 2013). However, caution should be exercised in drawing conclusions about country comparisons in levels of aflatoxin exposure, given the limited size of the studies, the lack of comparability of design and the possible impact of seasonality on findings. In our study, samples were collected in October – during the rainy season. At this time, maize has not ripened – a fact reflected in the relatively low levels of consumption reported here. The staple food was *Matooke* – associated with lower AF-alb – and so the levels of aflatoxin reported may be lower than would be found at other times of the year.

There is evidence from Uganda to show that levels of aflatoxin in foodstuffs increase along the supply chain, being lowest in the areas where the food is grown and highest in urban centres (Kaaya *et al.* 2007). Therefore, the levels of AF-alb adduct might be expected to be lower among people living in a rural area (such as those studied here), than among people living in urban centres, where a higher proportion of the food consumed will have been transported and stored. This has significant implications for many countries as they undergo nutritional and economic transition, with population growth in urban centres and an increasing reliance on consumption of food that has been transported and stored for prolonged periods. In these data, it is notable that AF-alb levels were higher among those living in villages closest to the trading centre. It is possible that individuals from those villages consume more purchased food, which may have been stored for longer and hence have had more opportunity to become contaminated with aflatoxin. However, it is unclear whether the food sold at trading centres is locally produced or commercially grown and imported from elsewhere. Indeed, we have few details of the pattern of import and export of food from the study area, nor on how this might change with season or in response to other factors.

Among adults, diet was homogeneous and no specific dietary factors were associated with significantly higher levels of AF-alb adducts; consumption of *Matooke* was associated with lower levels. It is notable that maize consumption, a major source of aflatoxin exposure in many countries in sub-Saharan Africa, was low in this study, but it was not in season at the time the study was conducted. Among children under the age of 3 years, all

Table 2 Aflatoxin levels by dietary factors

	Geometric mean (95% CI)	*N*	*P*-value*
Adult diet			
Meals per day			
≤2	10.8 (9.0–12.9)	42	0.64
3	12.0 (10.0–14.4)	52	
>3	12.8 (9.5–17.2)	6	
Matooke (times/week)			
None	17.2 (9.5–31.2)	11	0.02
1	9.3 (7.5–11.5)	15	
2	9.8 (8.6–11.2)	28	
3	14.3 (9.9–20.7)	20	
≥4	11.0 (8.8–13.6)	26	
Fresh cassava (times/week)			
None	12.0 (9.1–15.9)	17	0.96
1	11.8 (8.7–16.0)	12	
2	11.4 (9.2–14.1)	18	
3	12.4 (7.7–19.9)	15	
≥4	10.9 (8.8–13.5)	38	
Groundnuts (times/week)			
None	14.7 (7.9–27.1)	7	0.23
1	9.0 (7.3–11.0)	15	
2	10.5 (8.1–13.8)	23	
3	13.3 (9.6–18.2)	27	
≥4	11.6 (9.8–13.6)	28	
Vegetables (times/week)			
None	15.3 (9.8–24.0)	17	0.13
1	11.0 (9.2–13.2)	20	
2	13.0 (9.0–18.7)	18	
3	10.0 (7.0–14.1)	12	
≥4	10.0 (8.5–11.8)	33	
Fruit (times/week)			
None	13.8 (10.0–19.1)	13	0.36
1	10.5 (8.7–12.6)	28	
2	13.9 (9.3–20.6)	19	
3	10.5 (7.7–14.4)	10	
≥4	10.6 (8.4–13.4)	30	
Local posho (times/week)			
None	12.3 (8.0–18.9)	10	0.36
1–3	9.8 (8.3–11.5)	31	
4–6	11.9 (9.3–15.4)	25	
≥7	12.7 (9.9–16.3)	34	
Beans (times/week)			
≤2	11.7 (9.6–14.3)	20	0.92
3–6	11.7 (9.7–14.0)	51	
≥7	11.1 (8.6–14.2)	29	
Sugar (spoons/day)			
≤1	12.1 (9.4–15.6)	35	0.75
2	10.9 (9.1–13.0)	38	
≥3	11.6 (9.4–14.2)	27	
Salt (spoons/day)			
≤1	10.5 (8.9–12.4)	40	0.03
2	13.4 (11.0–16.2)	49	
≥3	8.3 (6.5–10.6)	11	
Meat (times/week)			
None	11.9 (10.0–14.2)	47	0.85
1	10.6 (9.0–12.4)	24	
2	12.0 (7.7–18.7)	18	
≥3	10.9 (7.3–16.3)	11	
Packed posho (any in week)			
No	11.1 (9.9–12.4)	90	0.09
Yes	15.7 (7.2–34.1)	10	
Dried cassava (any in week)			
No	11.4 (10.0–13.0)	88	0.71
Yes	12.2 (8.0–18.7)	12	
Maize (any in week)			
No	11.4 (10.1–12.9)	92	0.71
Yes	12.4 (6.9–22.4)	8	
Millet (any in week)			
No	11.4 (10.0–13.0)	86	0.67
Yes	12.3 (8.6–17.4)	14	
Beer (any in week)			
No	11.6 (10.2–13.3)	83	0.65
Yes	10.8 (8.1–14.5)	17	
Waragi (any in week)			
No	11.4 (9.9–13.0)	82	0.69
Yes	12.1 (9.3–15.8)	18	
Potatoes (any in week)			
No	10.3 (9.2–11.6)	67	0.33
Yes	11.8 (9.1–15.4)	33	
Child diet			
Exclusive breastfeeding			
No	10.2 (8.7–12.1)	89	0.03
Yes	4.6 (1.2–17.6)	5	
Completely weaned			
No	10.7 (8.1–14.2)	14	0.82
Yes	10.2 (8.4–12.3)	75	
Age of weaning			
≤6 m	10.6 (8.9–12.8)	72	0.41
>6 m	8.9 (6.0–13.3)	16	
Diet during weaning			
Any local posho			
No	8.2 (2.7–25.2)	3	0.30
Yes	11.5 (8.3–16.0)	11	
Any packed posho			
No	9.1 (6.7–12.4)	9	0.09
Yes	14.4 (7.6–27.6)	5	
Any fresh cassava			
No	10.1 (6.6–15.4)	6	0.70
Yes	11.2 (7.0–17.9)	8	
Any beans			
No	9.8 (0.01–19.1)	2	0.81
Yes	10.8 (8.03–14.7)	12	
Any vegetables			
No	11.3 (2.6–49.1)	3	0.85
Yes	10.6 (7.6–14.6)	11	

Table 2 (*Continued*)

	Geometric mean (95% CI)	*N*	*P*-value*
Any fruit			
No	8.2 (3.7–18.0)	4	0.20
Yes	11.9 (8.6–16.6)	10	
Any meat			
No	8.8 (5.4–14.4)	6	0.21
Yes	12.4 (8.3–18.5)	8	
Any maize			
No	9.7 (7.63–12.3)	12	0.06
Yes	19.5 (0.01–36.9)	2	
Diet in children completely weaned			
Any packed posho			
No	9.7 (7.9–12.0)	52	0.45
Yes	11.2 (7.5–16.7)	23	
Any fresh cassava			
No	7.8 (4.5–13.4)	6	0.46
Yes	10.4 (8.5–12.7)	69	
Any dried cassava			
No	11.9 (9.8–14.5)	56	0.03
Yes	6.4 (4.1–9.8)	19	
Any vegetables			
No	12.5 (5.0–31.6)	6	0.51
Yes	10.0 (8.2–12.1)	69	
Any fruit			
No	6.0 (0.02–19.5)	2	0.41
Yes	10.3 (8.5–12.5)	73	
Any meat			
No	13.6 (6.4–28.5)	8	0.30
Yes	9.8 (8.1–12.0)	67	
Any cow's milk			
No	16.6 (3.6–76.1)	3	0.31
Yes	10.0 (8.2–12.1)	72	
Any baby soya			
No	9.5 (7.8–11.5)	67	0.04
Yes	17.9 (8.9–36.2)	8	
Any maize			
No	10.1 (8.3–12.3)	46	0.60
Yes	10.2 (6.9–15.2)	29	
Any millet			
No	9.5 (7.6–12.0)	55	0.46
Yes	12.1 (8.8–16.8)	20	

**P*-value from ANOVA.

but four had detectable AF-alb, at levels similar to adults. All five babies reported to be exclusively breastfeeding had detectable levels, suggesting the possibility of exposure through breast milk, although levels were lower than among those who were mixed feeding. Among completely weaned children given soya, higher levels of AF-alb adduct were detected than in children not eating it – it is not grown locally and would have been purchased from trading centres.

Aflatoxins are known carcinogens and cause growth faltering and immune suppression in animals (IARC 2002). In humans, hepatocellular carcinoma (HCC) is a particular problem, especially in combination with chronic hepatitis B virus (HBV) infection and, to a lesser extent, with hepatitis C virus (HCV) (IARC 2002). HCC is one of the most common cancers in Uganda (Parkin *et al.* 2010), and chronic infection with HBV has a prevalence of between 8 and 10% and infection with HCV, about 2% (Sero-behavioural survey, Uganda 2006; Pirillo *et al.* 2007). Recently unpublished data from the GPC suggest a similar prevalence in the population studied here. Chen *et al.* (2013) reported that in China, a reduction in exposure to aflatoxin was associated with a decline in incidence of HCC, even when HBV prevalence was unchanged, offering alternative approaches to primary prevention. However, the relative contribution of aflatoxin to the aetiology of HCC in Uganda is unclear.

Many African children are highly exposed to aflatoxins from their diet, and consistent correlations between child stunting and exposure have been identified. However, the possible effects of confounding by other factors could not be entirely ruled out as an explanation for these findings (Gong *et al.* 2002, 2004). Furthermore, a study among children in the Gambia has shown a decreased salivary IgA level in association with high levels of exposure to aflatoxin, which might suggest systemic suppression of immune status, as well as specific suppression of immunity within the digestive system (Turner *et al.* 2003). Further evidence was found to support the immune function modulation by aflatoxins in the Ghanaian normal population and among HIV patients (Jiang *et al.* 2005, 2008; Wild & Gong 2010). However, whether the impaired growth and suppressed immune function are associated with increased susceptibility and progression of infectious disease, including HIV, remains unknown.

In conclusion, exposure to aflatoxin is ubiquitous among the rural Ugandans studied here, but more precise dietary studies are needed to identify the major sources of exposure. Potentially, there are a number of ways to reduce human consumption of aflatoxin, both pre- and post-harvest. *Aspergillus* spp. infects crops as they grow, and aflatoxin accumulates after crop harvest when storage conditions are inadequate. Therefore, interventions that focus on timing of harvest, crop drying and storage, processing and cooking may reduce aflatoxin levels (Wild & Gong 2010).

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