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Case–Control Study of an Acute Aflatoxicosis Outbreak, Kenya, 2004

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OBJECTIVES: During January–June 2004, an aflatoxicosis outbreak in eastern Kenya resulted in 317 cases and 125 deaths. We conducted a case–control study to identify risk factors for contamination of implicated maize and, for the first time, quantitated biomarkers associated with acute aflatoxicosis.

RESULTS: Homegrown (not commercial) maize kernels from case households had higher concentrations of aflatoxins than did kernels from control households [geometric mean (GM) = 354.53 ppb vs. 44.14 ppb; p = 0.04]. Serum adduct concentrations were associated with time from jaundice to death [adjusted hazard ratio = 1.3; 95% confidence interval (CI), 1.04–1.6]. Case patients had positive hepatitis B titer [odds ratio (OR) = 9.8; 95% CI, 1.5–63.1] more often than controls. Case patients stored wet maize [OR = 3.5; 95% CI, 1.2–10.3] inside their homes [OR = 12.0; 95% CI, 1.5–95.7] rather than in granaries more often than did controls.

CONCLUSION: Aflatoxin concentrations in maize, serum aflatoxin B1–lysine adduct concentrations, and positive hepatitis B surface antigen titer were all associated with case status.

RELEVANCE: The novel methods and risk factors described may help health officials prevent future outbreaks of aflatoxicosis.

2004. The descriptive epidemiology investigation found that a large number of patients with presumed aflatoxicosis had sought treatment at Makindu Sub-District Hospital (Makueni District) during 18 May—7 June and at Mutomo Mission Hospital (Kitui Districts) during 28 May—9 June. We did not restrict cases to live case patients or to case patients from which KEMRI had obtained blood samples because we did not want to introduce bias in our assessment of risk factors associated with disease.

To select 40 patients that met our case definition, we reviewed hospital records for the relevant time period and identified 19 case patients admitted to Makindu Sub-District Hospital and 21 case patients admitted to Mutomo Mission Hospital. All of the 29 case patients were alive at the time of the investigation, and all of the families of 11 deceased case patients verbally consented to participate in the study.

Selection of controls. We randomly selected two controls from each case patient’s village because the descriptive epidemiology investigation suggested that these individuals would share similar soil, microclimate, and farming practices. Because the descriptive epidemiology investigation did not find a significant association among sex, case status, and case fatality, we did not match cases and controls by sex. To choose each control, we spun a bottle in front of the village elder’s home and walked to the fifth house in the direction indicated by the bottle (or to the third house in sparsely populated areas). At the selected household, we identified all residents who had slept in the house the night before, and we used a random number list to select one of these household residents. We excluded infants who were solely breast-feeding because they would not have been directly exposed to aflatoxin B1 found in maize. If selected individuals were not at their homes, we attempted to reach them wherever they were. All controls verbally consented to participate in the study.

Survey instrument. A literature review and the descriptive epidemiology investigation allowed development of hypotheses about the relationship between aflatoxicosis and methods of handling maize. We developed a questionnaire to elicit information about maize and protein consumption, the quality of homegrown and purchased maize products, maize storage and cooking practices, and associated illness and death of family members and pet dogs. All questions related to the relevant exposure period, which was designated as 1 month before the onset of case patients’ illness or 1 month before controls heard about the outbreak.

Teams piloted the questionnaire on hospitalized patients who had presumed aflatoxicosis in Thika District. Local public health officials translated the questionnaire, which was written in English, into Kikamba and Kiswahili as needed. Teams carried measuring cups to obtain standardized information on maize food portions consumed by participants.

Food sample collection. We obtained samples of maize products from participants to quantify personal exposure to aflatoxins. We collected samples from case households if they had maize in storage from the month before individuals developed aflatoxicosis (median date of symptom onset, 20 May 2004). We collected samples from control households if they had maize in storage from the month before hearing about the outbreak (median date of first hearing about the outbreak, 19 May 2004). We used metal cups to obtain multiple samples from different areas of the maize containers. These samples were combined in a paper bag to obtain 1 kg of maize for analysis. Collected maize products were replaced with commercial maize meal.

Blood sample collection. We obtained blood samples from participants to quantify their exposure to aflatoxins in the preceding month. With the exception of six case patients from whom KEMRI had banked blood in May, we collected approximately 5–10 mL of venous blood in a Vacutainer tube with gel separators from all participants. All blood samples were transported on ice to KEMRI for serum separation.

Laboratory analysis. We analyzed maize samples using the VICAM AflaTest (VICAM, Watertown, MA, USA) immunoaffinity fluorometric method that quantitated total aflatoxin concentrations. Ground maize (50 g) that passed through a no. 20 sieve was mixed with 100 mL of a methanol:water mixture (80:20) with 5 g sodium chloride. The twice-filtered mixture (2 mL) was then passed through the immunoaffinity column at a rate of 1–2 drops/sec. The columns were washed with water, and the aflatoxins were recovered using 1 mL methanol. The methanol extract was read using a calibrated Vicam Series-4 Fluorometer set at 360 nm excitation and 450 nm emission. This method had an aflatoxin recovery of ≥85% and a detection limit of 1 ppb (VICAM 2001).

The Centers for Disease Control and Prevention (CDC) analyzed the serum specimens for aflatoxin B1–lysine albumin adducts using high-performance liquid chromatography (HPLC) and isotope dilution tandem mass spectrometry (McCoy et al. 2005). After enzymatic hydrolysis of serum albumin, aflatoxin B1–lysine adducts were extracted using solid-phase cartridges and separated using isocratic reversed-phase chromatography. We used positive ion electrospray with selected reaction monitoring mass spectrometry to measure aflatoxin B1–lysine adducts and its corresponding D3-labeled internal standard.

We measured total serum albumin using a bromocresol purple binding assay and a microplate reader. The limit of detection of aflatoxin B1–lysine albumin adducts was 0.0003 ng/mg. The CDC also analyzed all remaining sera for hepatitis B surface antigen using ETT-MAK-2 PLUS enzyme immunoassay kits from DiaSorin (DiaSorin, Stillwater, MN).

Data management and analysis. Data were analyzed using SAS, version 8.02 (SAS Institute, Cary, NC). We used conditional logistic regression to calculate odds ratios (ORs) between case status and participants’ methods of harvesting, storing, and preparing maize. We also used conditional logistic regression models to explore the relationship between case status, maize and protein consumption, aflatoxin concentrations in maize, aflatoxin B1–lysine adduct concentrations, and hepatitis B surface antigen titers in serum. We restricted mixed linear regression models to controls because we wanted to investigate the relationship between serum aflatoxin concentrations and methods of harvesting, storing, and preparing maize, daily maize and protein consumption, and total aflatoxin concentrations in maize using a sample that more closely resembled the general population. We also used Cox proportional hazards models to explore the relationship between the number of days case patients survived after the onset of jaundice and aflatoxin concentrations in maize, aflatoxin B1–lysine adducts concentrations in serum, hepatitis B surface antigen titers, and reported maize and protein consumption. Calculations were adjusted for age, sex, and participant’s district.

Results

Demographic information. With few exceptions, case patients (n = 40) and controls (n = 80) had similar demographic characteristics (Table 1). Half of the participants lived in the Makueni District and the other half lived in the Kitui District. The mean age of case patients was similar to that of controls (22.5 years (range, 1.3–80.0 years) vs. 26 years (range, 0.5–75.0 years), respectively). When
compared with controls, more of the case patients were male (62.5% vs. 33.8%, respectively; p = 0.003). Case patients were also more likely than controls to report having family members with acute jaundice during the 2 months before the study (37.5% vs. 3.8%; p < 0.001). As of 9 August, 18 of the 40 case patients (7 additional case patients since completion of our study) had died of acute liver failure.

**Food consumption and maize aflatoxin analysis.** Eating contaminated homegrown maize kernels was the primary risk factor for developing aflatoxicosis. On average, maize samples were collected 33 days (range, 8–112 days) after case-patients’ onset of symptoms. Homegrown maize kernels from case households had significantly higher aflatoxin concentrations than kernels sampled from control households (geometric mean (GM) = 354.5 ppb vs. 44.1 ppb, respectively; p = 0.04; Figure 1). Eating homegrown maize kernels was significantly associated with case status (adjusted OR = 3.0; 95% confidence interval (CI), 1.01–8.8). Owning “bad” homegrown maize kernels (maize with colored flecks, discoloration, unusual odor, or signs of mold) was found to be a risk factor for aflatoxicosis (adjusted OR = 5.9; 95% CI, 1.9–18.2). Case patients who fed their dogs household food (adjusted OR = 15.2; 95% CI, 2.1–180) were more likely than controls to report having household aflatoxin B1–lysine adduct concentrations in their serum than did controls (p = 0.04; Figure 1). Eating contaminated homegrown maize kernels from case households had significantly higher aflatoxin concentrations than those who did not (GM = 17.4 ppb vs. 142.2 ppb; p = 0.05). We did not find an association between case status, the type of container used to store maize (plastic burlap, plastic bucket, woven basket, clay pot, gourd, or sisal), the use of soda and pesticides in the storage area, or the culling of maize kernels that appeared moldy.

**Discussion.**

**Food consumption and aflatoxin analyses.** This is the first investigation to quantify the association among environmental contamination, a history of exposure, biomarker concentrations, and acute aflatoxicosis. The results of our case-control study suggest that consumption of contaminated maize kernels placed people in this region of Kenya at risk for life-threatening aflatoxicosis (case-fatality rate of 39%). Through systematic sampling of maize and serum from participants, we found a strong association between aflatoxin concentrations in homegrown maize, serum B1–albumin adducts, hepatitis B surface antigen titers, and case status.

The aflatoxin concentrations measured from the maize of case patients was comparable with those measured in other acute aflatoxicosis outbreaks. The aflatoxin B1–lysine adduct concentrations measured in the serum of case patients were the highest ever reported. This is the first study to quantify aflatoxin B1–lysine adduct concentrations in the serum of case patients during an outbreak of acute aflatoxicosis; a critical step in the elucidation of the clinically relevant action levels for aflatoxin exposure. We associated these serum aflatoxin B1–lysine adduct concentrations with the risk for life-threatening acute aflatoxicosis.

**Figure 1.** Frequency of total maize aflatoxin concentrations for participants.

**Figure 2.** Frequency of serum aflatoxin B1–lysine albumin adduct concentrations for participants.
We found an association between aflatoxin concentrations in maize and aflatoxin $B_1$–lysine adduct concentrations in serum from controls. The GM aflatoxin $B_1$–lysine adducts concentration in serum from controls is higher than the majority of concentrations documented in population-based studies from countries with a high incidence of liver cancer (Wild et al. 1990). It is unclear why some controls with high aflatoxin $B_1$–lysine adduct concentrations did not manifest symptoms of acute hepatitis during the time of the investigation. The concentrations found in controls were not associated with acute symptoms and may have represented chronic exposure to aflatoxins. Chronic exposure to aflatoxins is associated with impaired immunity, malnutrition, and liver cancer (the third most common cause of death from cancer in Africa) (Parkin et al. 2003; Williams et al. 2004). People chronically exposed to elevated concentrations of aflatoxins are three times more likely to develop hepatocellular carcinoma.

We also found an independent association between hepatitis B surface antigen titers and case status. Although people with hepatitis B (which is endemic in Kenya) who are chronically exposed to aflatoxins may be more likely to develop hepatocellular carcinoma, this is the first study to quantify the association between hepatitis B, aflatoxin adducts, and acute hepatitis (Keenleyside et al. 1977; Qian et al. 1994). Further research is needed to determine if the high incidence of liver cancer in eastern Kenya is attributable to chronic asymptomatic exposure to aflatoxins. In addition, clinicians working in areas where aflatoxicosis is endemic should consider obtaining a dietary history for aflatoxin exposure from cases patients with symptoms of acute hepatitis and positive hepatitis B titers.

Risk factors. Our case–control study quantified ORs for suspected risk factors described in previous aflatoxicosis outbreaks. As in a 1974 outbreak in India (Krishnamachari et al. 1975b), we found that males were more likely to die from aflatoxicosis, in spite of eating similar quantities of maize as females. We found that acute aflatoxicosis manifests in family clusters, as reported in a 1988 outbreak in Malaysia (Lye et al. 1995). Sharing contaminated food and genetic polymorphisms of cytochrome P450 enzymes may place families at risk for aflatoxicosis (Chen et al. 2000). As reported by Ngindu (1982) in a 1981 outbreak in Kenya, we found that, more often than controls, case patients reported dog deaths before developing aflatoxicosis. In the future, reports of deaths in dogs may warn public health officials of a potential aflatoxin contamination of the food supply.

Food preparation and storage analysis. Although maize is traditionally stored in granaries, storage inside homes occurs during periods of food shortage; this may have facilitated the contamination of maize with aflatoxins. The rainy season (from March through May) accounts for 80% of annual food production [Food and Agriculture Organization (FAO) 2000]. In 2004, an early and insuffi cient rainy season caused a food shortage of 156,000 metric tons of maize (Associated Press 2004). Some participants reported storing maize inside their homes to ensure it would not be stolen during the food shortage. Drought conditions stress maize plants and render them susceptible to contamination by Aspergillus spp. (Wilson and Payne 1994). The warm environment inside these windowless homes and storage of maize on the dirt floor may have promoted fungal growth in wet maize kernels.

Our case–control study suggests that traditional methods of drying and storing maize in elevated granaries were protective against aflatoxicosis. Traditional granaries are raised structures that are well ventilated, and they promote the drying of grain (FAO 1998). The granaries’ elevated platforms isolate the maize from spores and insects on the ground. We also found that storing maize mixed with ash was associated with lower concentrations of aflatoxin than storing maize without ash. Ash acts as a physical barrier against insects and helps keep maize dry.

Limitations. Our case–control study was limited by its retrospective design. It is possible that case patients (or the family members of deceased case patients) may have recalled the amount, source, and quality of maize that was consumed differently than did controls. The aflatoxin concentrations measured in sampled maize may have differed from those consumed by case patients before they became ill with aflatoxicosis. We may not have found an association between the number of portions of maize consumed and case status due to the limited accuracy of the food questionnaires. In addition, it is possible that some case patients developed jaundice as a result of undiagnosed medical conditions unrelated to aflatoxicosis. This potential misclassification would have weakened any demonstrable associations.

Conclusion
Aflatoxins and other mycotoxins contaminate 25% of agricultural crops worldwide and are a source of morbidity and mortality throughout Africa, Asia, and Latin America (Smith et al. 1994). To prevent future aflatoxicosis outbreaks, it is necessary to explore public health interventions that promote effective production, storage, and processing of homegrown and commercial maize. In addition, surveillance that monitors aflatoxin concentrations in food and incidence of acute jaundice in humans may prevent widespread outbreaks of acute aflatoxicosis (Trucksess and Wood 1994). In the future, serum aflatoxin $B_1$–albumin adducts may be used to diagnose acute aflatoxicosis and monitor interventions aimed at reducing aflatoxin exposure (Kensler et al. 1999). Although short-term interventions such as food replacement mitigate the loss of life during outbreaks, it is necessary to develop long-term, culturally appropriate strategies to prevent aflatoxicosis.

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The American Plastics Council respectfully requests that *EHP* address the misinformation that appeared in these articles and which is available on the *EHP* website.

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Editor’s note: The following erratum was published in the January 2006 issue (Environ Health Perspect 114:A21):

In the October articles “Children’s Centers Study Kids and Chemicals” [Environ Health Perspect 113:A664–A668 (2005)] and “Are EDCs Blurring Issues of Gender?” [Environ Health Perspect 113:A670–A677 (2005)], photographs and their captions erroneously imply that plastic drink bottles contain ortho-phthalates. Plastic drink bottles sold in the United States are made from polyethylene terephthalate and do not contain ortho-phthalates. Also, at the end of the EDCs article, references are made to plastic wrap and Saran Wrap. For clarification, neither plastic wrap nor Saran Wrap contains ortho-phthalates. *EHP* regrets these errors.

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**ERRATA**

Azziz-Baumgartner et al. noticed two errors in “Case–Control Study of an Acute Aflatoxicosis Outbreak—Kenya?” [Environ Health Perspect 113:1779–1783]. The units in Figure 2 and Table 2 should be nanograms per milligram instead of micrograms per milligram. The errors were introduced when new figures and tables were generated during the final revision of the paper. The authors apologize for these errors.

In the article by Feist et al. [Environ Health Perspect 113:1675–1682], the units were incorrect in several figures and tables: “Lipid (µg/g)” should be “µg/g lipid” in Tables 1 and 2 and in the y-axes of Figures 2 and 3A–C. Also, on the y-axes in Figure 5A–D, “dL” should be “mL.” *EHP* regrets these errors.

In the January Focus article “In Katrina’s Wake” [Environ Health Perspect 114:A32–A39 (2005)], Hurricane Katrina was identified as a Category 4 storm, reflecting statements from the National Hurricane Center as of press time. The National Hurricane Center has since reported that Katrina was actually a Category 3 storm at the time of landfall.