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# Outbreak of an Acute Aflatoxicosis in Kenya in 2004: Identification of the Causal Agent[▽]

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**Maize contaminated with aflatoxins has been implicated in deadly epidemics in Kenya three times since 1981, but the fungi contaminating the maize with aflatoxins have not been characterized. Here we associate the S strain of *Aspergillus flavus* with lethal aflatoxicoses that took more than 125 lives in 2004.**

The 2004 outbreak of acute aflatoxicosis in Kenya was one of the most severe episodes of human aflatoxin poisoning in history. A total of 317 cases were reported by 20 July 2004, with a case fatality rate of 39% (1, 26). This epidemic resulted from ingestion of contaminated maize (22). However, identities of the fungi causing the contamination remain unclear.

Aflatoxins are carcinogenic metabolites produced by several *Aspergillus* species (4, 28). Aflatoxin-producing fungi vary widely in many characteristics, including virulence for crops and aflatoxin-producing capacity (10). *A. flavus* and *A. parasiticus* are most commonly implicated as causal agents of aflatoxin contamination. *A. flavus* has two morphotypes, the typical or L strain (sclerotia of >400 μm in diameter) and the S strain (sclerotia of <400 μm in diameter) (10, 18). S-strain isolates produce more aflatoxins than L-strain isolates, on average (10). Many L-strain isolates produce no aflatoxins ("atoxigenic") (7). All members of *A. flavus* lack the ability to synthesize G aflatoxins due to a 0.8- to 1.5-kb deletion in the 28-gene aflatoxin biosynthesis cluster (15). In contrast to cases in the United States, studies conducted in West Africa found that an unnamed taxon (sometimes called strain $S_{BG}$) is commonly implicated in contamination events (12). Strain $S_{BG}$ is morphologically similar to the S strain of *A. flavus*, but DNA-based phylogenies reveal strain $S_{BG}$ to be a distinct species ancestral to both *A. flavus* and *A. parasiticus* (14, 16). In order to determine the primary causal agent(s) of the 2004 contamination events in Kenya, we considered both fungal aflatoxin-producing potential and frequency of occurrence in the contaminated crop (7).

Representative maize samples were collected from major agricultural markets and storage facilities of the most affected Kenyan districts by the National Public Health Laboratory Services in Nairobi, Kenya, during the 2004 outbreak (24). Samples were screened for aflatoxin content, and only B aflatoxins were detected (22, 24). Subsamples ($n$ = 104; average weight = 87.5 g; range of contamination = 0.27 to 4,400 ppb total aflatoxin) were imported to the United States from the National Public Health Laboratory Services for fungal analyses. Fungi were isolated from the maize by using the dilution plate technique on modified rose Bengal agar (8). Isolates were classified into species and strains by observing colony characteristics and sclerotial and conidial morphologies after subculturing on 5/2 agar (5% V8 juice; 2% agar; pH 5.2) (10). Isolations were repeated two to four times to verify results. Isolates from each sample were collected from at least two isolations. Quantities of *Aspergillus* section Flavi isolates in maize were expressed as the numbers of CFU per mg (19). A total of 1,232 isolates (10 to 18 per sample) were recovered from the maize, saved, and stored at 4°C. *A. flavus* was recovered from all samples (97.9% of isolates); 15 samples also contained *A. parasiticus* (2.1% of isolates). Other aflatoxin producers were not detected. All *A. flavus* isolates were assigned to either the L strain or the S strain, 28.2% and 71.8%, respectively (10). Both simple linear and quadratic regression analyses ($b_0 + b_1x$ and $b_0 + b_1x + b_2x^2$, respectively) were performed for aflatoxin content as a function of S-strain incidence, *A. parasiticus* incidence, or *A. flavus* quantity (CFU/mg) using SAS 8.0 software (SAS Institute, Cary, NC). Maize aflatoxin content and S-strain incidence were highly correlated. When corn samples were sorted into groups based on aflatoxin content, the incidence of the S strain increased with average maize aflatoxin content from 69% in samples with <20 ppb total aflatoxins to 94% in samples with >1,000 ppb (Table 1; Fig. 1). Only S-strain isolates were recovered from five of six samples with >1,000 ppb (the sixth sample was 66.7% S strain). *A. parasiticus* was not recovered from any sample with >260 ppb, and its incidence was not correlated with aflatoxin content (Table 1).

Aflatoxin production by representative *A. flavus* isolates (26 S-strain isolates; 26 L-strain isolates) was measured to assess variance in aflatoxin production among strains. Fermentations were carried out in the medium of Mateles and Adye (23) with 22.5 mM urea as the sole nitrogen source exactly as described previously (6, 12). This medium allows detection of the strain $S_{BG}$ phenotype. Reference isolates from the United States (*A. flavus* S strain AF70 and L strain AF13) and West Africa (strain $S_{BG}$ isolates BN008R and BN038G) were included for comparison. Fermentations were replicated three times. S-strain isolates produced more (mean = 356.46 μg aflatoxin

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TABLE 1. Quantity of *Aspergillus* section Flavi and incidences of *A. parasiticus* and the S strain of *A. flavus* in Kenyan maize containing various concentrations of aflatoxins[a,b,c,d,e,f]

| Region of isolation | Amt of aflatoxin (ppb) | No. of samples[g] | S (%) | AP (%) | No. of CFU/mg |
|---|---|---|---|---|---|
| Machakos District | >1,000 | 1 | 100 | 0 | 41.9 |
| | 301 to 1,000 | 3 | 93 | 0 | 5.9 |
| | 21 to 300 | 10 | 70 | 13 | 5 |
| | 2 to 19 | 3 | 67 | 3 | 0.1 |
| | 0.5 to 2 | 3 | 66 | 2 | 1.3 |
| Total | | 20 | 75 | 7 | 5.5 |
| Makueni District | >1,000 | 3 | 89 | 0 | 15.4 |
| | 301 to 1,000 | 4 | 75 | 0 | 3 |
| | 21 to 300 | 19 | 73 | 2 | 2 |
| | 2 to 19 | 3 | 71 | 2 | 2.9 |
| | 0.5 to 2 | 8 | 67 | 0 | 1.5 |
| Total | | 37 | 73 | 1 | 3.2 |
| Kitui District | >1,000 | 2 | 100 | 0 | 56.6 |
| | 301 to 1,000 | 4 | 86 | 0 | 14.9 |
| | 21 to 300 | 15 | 70 | 0 | 3.3 |
| | 2 to 19 | 6 | 58 | 1 | 4.9 |
| | 0.5 to 2 | 11 | 75 | 4 | 1.5 |
| Total | | 38 | 73 | 1 | 7.1 |
| All samples | >1,000 | 6 | 94 | 0 | 33.6 |
| | 301 to 1,000 | 12 | 84 | 0 | 7.5 |
| | 21 to 300 | 49 | 68 | 3 | 3.2 |
| | 2 to 19 | 12 | 63 | 2 | 3.2 |
| | 0.5 to 2 | 24 | 69 | 2 | 1.4 |
| Total | | 103 | 71 | 2 | 5 |

[a] S (%), percentage of *Aspergillus* section Flavi isolates belonging to the S strain of *A. flavus*; AP (%), percentage of *Aspergillus* section Flavi isolates belonging to *Aspergillus parasiticus*; no. of CFU/mg, no. of CFU of *Aspergillus* section Flavi per mg of maize.

[b] Trends are as follows. For Machakos District, S (%), positive; AP (%), negative; no. of CFU/mg, positive. For Makueni District, S (%), positive; AP (%), negative; no. of CFU/mg, positive. For Kitui District, S (%), positive; AP (%), negative; no. of CFU/mg, positive. For all samples, S (%), positive; AP (%), negative; no. of CFU/mg, positive.

[c] Linear $r^2$ values are as follows. For Machakos District, S (%), 0.89; AP (%), 0.229; no. of CFU/mg, 0.897. For Makueni District, S (%), 0.95; AP (%), 0.279; no. of CFU/mg, 0.905. For Kitui District, S (%), 0.774; AP (%), 0.215; no. of CFU/mg, 0.991. For all samples, S (%), 0.889; AP (%), 0.595; no. of CFU/mg, 0.965.

[d] Linear *P* values are as follows. For Machakos District, S (%), 0.016; AP (%), 0.415; no. of CFU/mg, 0.0144. For Makueni District, S (%), 0.0048; AP (%), 0.3598; no. of CFU/mg, 0.0127. For Kitui District, S (%), 0.0492; AP (%), 0.4314; no. of CFU/mg, 0.0004. For all samples, S (%), 0.0163; AP (%), 0.1268; no. of CFU/mg, 0.0028.

[e] Quadratic $r^2$ values are as follows. For Machakos District, S (%), 0.975; AP (%), 0.741; no. of CFU/mg, 0.932. For Makueni District, S (%), 0.956; AP (%), 0.279; no. of CFU/mg, 0.979. For Kitui District, S (%), 0.979; AP (%), 0.341; no. of CFU/mg, 0.991. For all samples, S (%), 0.988; AP (%), 0.703; no. of CFU/mg, 0.989.

[f] Quadratic *P* values are as follows. For Machakos District, S (%), 0.0245; AP (%), 0.2593; no. of CFU/mg, 0.0682. For Makueni District, S (%), 0.0436; AP (%), 0.359; no. of CFU/mg, 0.021. For Kitui District, S (%), 0.0202; AP (%), 0.6591; no. of CFU/mg, 0.0088. For all samples, S (%), 0.0119; AP (%), 0.297; no. of CFU/mg, 0.0102.

[g] Ten to fifteen individuals were isolated from each sample and examined.

$B_1$/g mycelium) aflatoxins than L-strain isolates (mean = 37.55 μg aflatoxin $B_1$/g mycelium). Fifty percent of the L-strain isolates ($n$ = 13) were atoxigenic. Similar disparities in aflatoxin production by S- and L-strain isolates have been reported from other continents (21, 25, 27).

An additional 100 *A. flavus* S-strain isolates were screened in similar fermentations. The examined isolates produced only B aflatoxins (mean = 488.95 μg aflatoxin $B_1$/g mycelium); this excludes the possibility that any of the tested isolates belong to strain $S_{BG}$, previously reported from West Africa (6).

To further investigate the potential of Kenyan S-strain isolates to contaminate maize, 20 S-strain isolates were inoculated onto living maize kernels surface sterilized in hot water (80°C, 45 s). Kernels were adjusted to 25% moisture and incubated for 7 days (31°C), and aflatoxin was quantified as described previously (5). Inoculated maize developed 95,000 ppb to 212,000 ppb aflatoxin $B_1$. G aflatoxins were not detected.

Characterization of causal agents is an important initial step for development of management procedures. Attribution of specific etiologies to aflatoxin contamination episodes is complicated by variability in aflatoxin-producing capacity among species, strains, and isolates (11). The maize contamination event that led to the 2004 outbreak of aflatoxicoses in Kenya is a particularly important contamination episode, because it led to deaths of more than 100 people. Results of the current study suggest that the Kenyan outbreak was caused by the S strain of *A. flavus*.

This is supported by the following. (i) The S strain, which was not previously found in Africa (2, 12), was repeatedly isolated from all 104 maize samples from affected districts. Communities of aflatoxin-producing fungi associated with highly contaminated maize were invariably dominated by the S strain of *A. flavus*, which occurred in the most toxic Kenyan maize at proportions greater than those previously observed on any crop from any location (20, 25). (ii) S-strain isolates from the Kenyan maize consistently produced large amounts of aflatoxins in both liquid medium and living maize. (iii) Only S-strain isolates were recovered from five out of six samples with >1,000 ppb total aflatoxin. (iv) The S-strain incidence was strongly correlated with maize aflatoxin content. (v) The inci-

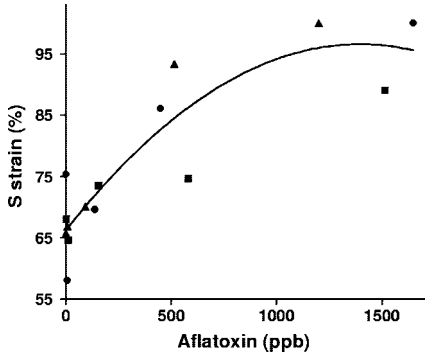


FIG. 1. Incidence of the S strain of *Aspergillus flavus* increased with aflatoxin content in maize samples collected in Kenya during 2004. Samples from each of three districts were sorted into five groups by aflatoxin content (■ = Makueni district; ▲ = Machakos district; ● = Kitui district). Significance of relationships and coefficients of determination are given in Table 1.

dence of no other aflatoxin-producing fungus was correlated with contamination.

Identification of factors leading to S-strain dominance in semiarid regions of Kenya may result in management procedures effective in both Kenya and other regions where the S strain is an important etiologic agent of aflatoxin contamination.

Currently, atoxigenic *A. flavus* L-strain isolates are used to competitively exclude aflatoxin producers during crop infection and thereby limit contamination in U.S. agriculture (9, 13). Such atoxigenic strains are highly effective against the S strain (17). Deployment of similar technologies in Africa could provide a promising strategy for prevention of future aflatoxicoses in East Africa while enhancing export possibilities for maize (3).

Representative isolates (A1168, A1169, A1170, and A1171) have been deposited at the Fungal Genetics Stock Center, St. Louis, MO.

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