

Exposure of Rural and Urban Populations in KwaZulu Natal, South Africa, to Fumonisin B₁ in Maize

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We surveyed households in rural and urban areas of KwaZulu Natal, South Africa, to assess the exposure of the inhabitants to fumonisin B₁ (FB₁), a mycotoxin produced by *Fusarium verticillioides*. In southern African regions maize, used as a staple food by the population, is prone to *F. verticillioides* infection. Furthermore, high levels of FB₁ in maize have been associated with esophageal cancer in South Africa. We assessed exposure of the population to FB₁ at three levels, namely, by analyzing stored maize, plate-ready food, and feces. The positions of participating households in the rural area were recorded using geographic information systems (GIS) for ease and accuracy of follow-up. Of the 50 rural maize samples examined, 32% had levels of FB₁ ranging from 0.1–22.2 mg/kg, whereas 29% of the 28 cooked maize (phutu) samples contained FB₁ ranging from 0.1–0.4 mg/kg. The incidence and levels of FB₁ in feces were 33% and 0.5–39.0 mg/kg, respectively. Of the 49 urban maize samples analyzed 6.1% had a range of 0.2–0.5 mg/kg FB₁, whereas 3 of 44 fecal samples (6%) ranged between 0.6 and 16.2 mg/kg. No FB₁ was detected in urban phutu samples. Because these levels are lower than those published from regions in South Africa with high incidence of esophageal cancer, it may be concluded that the risk of esophageal cancer from FB₁ exposure is lower in the KwaZulu Natal region. **Key words:** esophageal cancer, fumonisin B₁, *Fusarium verticillioides*, high performance liquid chromatography (HPLC), maize. *Environ Health Perspect* 109:253–256 (2001). [Online 1 March 2001] <http://ehpnet1.niehs.nih.gov/docs/2001/109p253-256chelule/abstract.html>

Certain mycotoxins have been associated with disease conditions among rural populations around the world, for example, aflatoxin B₁ and liver cancer (1). More recently, fumonisin B₁ (FB₁) has been associated with the etiology of esophageal cancer in South Africa (2), and this has been supported by immunolocalization of FB₁ in esophageal cancer tissue (3). *Fusarium verticillioides* (mating type A), a producer of FB₁, has been identified as a major fungal contaminant on maize, especially in the homegrown crops intended for human consumption (4). The degree of maize infection is highly dependent on environmental factors, such as average temperatures below 22°C and frequent rain during the first 2 weeks after silking, which increase the risk of colonization by *Gibberella fujikuroi*.

Because maize is the staple diet of the South African rural population, there is an increased risk of consumption of fumonisins. Studies carried out in four districts of Transkei, South Africa, linked high esophageal cancer rates in Butterworth and Kentani to the consumption of maize contaminated with elevated levels of FB₁ (117.3 mg/kg in 1989) as compared to lower levels in the control areas (Bizana and Lusikisiki; 11.3 mg/kg) (5). *F. verticillioides* was found to be the most dominant fungus infecting commercially produced maize in South Africa (6). In this case, the urban population may not be spared from FB₁ exposure.

We wanted to know whether other rural and urban populations of KwaZulu Natal,

South Africa, are exposed to FB₁ at the same level as in the Transkei. Our study was carried out in Durban Metro and in villages of Mphise and Ngcolosi, a rural area near Kranskop, KwaZulu Natal, to determine the level of FB₁ in raw (stored) and cooked food. The analysis of fecal samples was also carried out as a short-term marker for exposure to FB₁ (7).

The use of geographic information systems (GIS) in health has been applied to epidemiologic investigations in South Africa, including malaria (8,9). In this study, we used GIS to locate and plot the rural dwellings for identification and follow-up sampling.

Materials and Methods

Chemicals. All chemicals, unless otherwise specified, were of Analar Grade. SPE SAX and C₁₈ cartridges containing 500 mg sorbent (10 mL capacity, Varian Bond-Elut) were purchased from Analytichem (Harbour City, CA, USA). For the mobile phase, we used methanol:0.1 M sodium dihydrogen phosphate (80:20, v/v) adjusted to pH 3.4 with orthophosphoric acid. o-Phthaldialdehyde (OPA) reagent was prepared by dissolving 40 mg OPA in 1 mL methanol and diluting with 5 mL 0.1 M sodium tetraborate and mercaptoethanol (50 µL). Solvents used included acetonitrile/water (1:1, v/v), butanol, acetic acid, and methanol, all obtained from BDH Chemicals, Poole, England. o-Phosphoric acid (concentration > 85%) was obtained from BDH Chemicals,

Poole, England. We obtained the FB₁ standard (10 mg in sealed glass vials) from PROMEC, Cape Town, South Africa. The FB₁ standard was dissolved in acetonitrile/water (1:1v/v) to give 1 mg/mL and 50 µg/mL FB₁ working solutions.

Ethical approval. We obtained ethical permission for the study from the Ethics Committee of the University of Natal (Nelson R. Mandela Medical School, H194/97). We also obtained informed consent from the clients before they were recruited into the study.

Collection of samples. We identified two villages in Tugela Valley (Mphise and Ngcolosi) for inclusion in the study. Recruitment of families into the study started by systematic random sampling (choosing every fifth of those who volunteered to take part in the study). We visited the recruited volunteers in their households and collected samples of processed foods [cooked, milled maize (phutu), fermented maize meal (ama-hewu), and local homebrew (isizulu, alcoholic beverage)] and unprocessed stored cereals. On the day after processed food was collected, feces samples were collected from the same households and stored at –20°C pending analysis. We mapped the study area using GIS.

We carried out a similar study within the Durban metropolitan area, where households were visited (all subjects in the study were black). The households varied from formal to squatter (shack) settlements. Food and fecal samples were collected and analyzed as in the rural study. We included the Durban metropolitan area because of its urban character; people from this region consumed a more varied diet. Where maize

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is consumed in urban areas, it is subject to food safety regulation and is more likely to be mycotoxin-free.

Data analysis. We analyzed the questionnaire survey data using Excel (Microsoft, Redmond, WA, USA) and SPSS statistical packages (SPSS, Chicago, IL, USA). We used Mapinfo professional software (Mapinfo Corporation, Troy, NY, USA) to process geographic data and display household locations on maps.

Extraction and clean-up of FB₁ from cereal samples. Ground cereal (maize and sorghum) samples (100 g) were mixed thoroughly and a 25 g sample was extracted with 100 mL methanol/water (3:1 v/v) for 1 hr in a homogenizer (10). Liquid foods, isizulu and amahewu, were homogenized, and 50 mL of each was extracted with an equal volume of methanol/water (3:1 v/v). Phutu samples (25 g) were extracted using 100 mL 50% aqueous acetonitrile (adjusted to pH 2.7 using 0.1M HCl), as described above (11). The aqueous acetonitrile extracts were filtered, and 10 mL aliquots were carefully dried by rotary evaporation under low heat (60°C). These samples were then reconstituted in 10 mL of methanol/water (3:1 v/v). The pH was adjusted to 6.5 and cleaned using strong anion exchange (SAX) solid phase cartridges, previously conditioned by washing with 5 mL methanol and 5 mL methanol/water (3:1 v/v). The columns were then washed with 5 mL methanol/water (3:1 v/v) and then 3 mL methanol, and FB₁ was eluted at the rate of 1 mL/min with 1% acetic acid in methanol (10 mL). The eluates were dried under a stream of nitrogen at 60°C and stored at 4–8°C until further analysis. The methanol/water extracts were filtered, the pH adjusted to 6.5, and 10 mL aliquots were applied to SAX.

Recovery and determination of FB₁ from cereal samples. Four samples of maize meal (25 g) containing no detectable FB₁ were placed in 250 mL conical flasks, thoroughly mixed with 10 mL methanol containing 100 µg FB₁, and left to dry overnight. Two of the spiked samples were used to prepare phutu, a common traditional Zulu dish, by adding boiling water (20 mL) and a pinch of sodium chloride. The mixture was stirred to

Table 1. Results of FB₁ recoveries from 25 g of cooked and uncooked maize-based food spiked with a known amount (100 µg) of FB₁.

Sample	FB ₁ recovered ^c	Percent recovery
Phutu ^a	89 µg (± 3.4)	89
Phutu ^b	36 µg (± 2.9)	36
Maize meal	97 µg (± 1.3)	97

Recovery was performed after extraction with 50% aqueous acetonitrile.

^aModified solvent, pH 2.7. ^bUnmodified solvent pH.

^cRecovery is a mean of two results.

an even paste using a glass rod and heated to 93°C with stirring. The temperature was then reduced to about 70°C and the samples left to simmer for 15 min. After cooling, the cooked samples and the uncooked spiked samples were each extracted with 100 mL 50% aqueous acetonitrile, pH 2.7. The samples were continuously homogenized for 1 hr, filtered, and purified (10).

Analysis of FB₁ in fecal samples. Briefly, feces were lyophilized and then ground to a fine powder (7). A fraction (1.5 g) of the sample was extracted three times by vortexing for 1 min in a capped tube with 15 mL 0.1M EDTA, pH 5.2. The mixture was centrifuged at 2,000g for 10 min at 4°C and the supernatant was removed; the extraction was repeated twice. The supernatants were combined, acidified to pH 2.9–3.2 with 5M HCl, and centrifuged at 4,000g for 10 min. A supernatant aliquot of 10 mL was applied to a Bond-Elut C₁₈ cartridge previously conditioned with 5 mL methanol and 5 mL water. The sorbent was first washed with 5 mL water, followed by 5 mL methanol/water (1:3, v/v) and finally with 3 mL methanol/water (1:1, v/v). Fumonisin B₁ was eluted with 15 mL methanol and the solvent was evaporated under a stream of nitrogen at 60°C.

Quantitation by HPLC. We quantitated FB₁ by HPLC (10) using a Spectra Physics SCM400 system with a P2000 manual injector pump, a Nova-Pak 4 mm C₁₈ reversed phase analytical column (150 × 3.9 mm i.d.), and a Spectra FL2000 fluorescent detector (all from Waters, Milford, MA, USA). Detector excitation and emission wavelengths were set at 335 and 440 nm, respectively. The detection limit of the method was 50 ng/g FB₁ (signal/noise ratio of 5:1). The calibration curve was constructed to quantify up to 100

µg/g ($R^2 = 0.9983$). Briefly, a sample (25 µL) and FB₁ standard (50 µg/mL) were pipetted into a tube, and 225 µL of OPA was added and mixed. An aliquot of 20 µL of derivatized sample or standard was injected into the column within 1 min of adding OPA. The mobile phase, methanol/sodium dihydrogen phosphate (80:20, v/v), was run isocratically at the rate of 1 mL/min. We identified FB₁ by its constant retention time and deduced its quantities by comparing the peak areas of the standards to those of the samples.

Results and Discussion

Analysis of food. To determine the amount of FB₁ being ingested by the subjects in this study, we analyzed plate-ready food as well as raw stored maize. We performed an experiment to assess the best possible way to recover FB₁ because we anticipated that preparation and cooking could affect FB₁ recovery levels. When maize meal was spiked with known amounts of FB₁ and cooked in a traditional way to produce phutu, we found that the pH of the material being extracted was critical. If the pH was not modified, only 36% of FB₁ was recoverable, whereas at pH 2.7 the recovery improved to 89% (Table 1). It is important to adjust this extract to pH 6.5 as soon as possible, to avoid FB₁ hydrolysis and for the subsequent cleanup. However, when FB₁-spiked samples were cooked, extraction with unmodified solvent (50% aqueous acetonitrile) gave 35.9% recovery, whereas the modified extraction solvent (with pH 2.7) gave a recovery of over 85% FB₁ (Table 1).

Table 2 summarizes the results of food analysis for FB₁. Of the 47 maize samples analyzed from the rural area, 15 (32%) contained FB₁ (0.1–22.2 mg/kg; average = 2.2 mg/kg), whereas 3 out of 49 (6%) of the

Table 2. Analysis of cereals and feces for FB₁ from the rural group and the urban control group using HPLC.

Sample/region	Samples analyzed	No. of positives for FB ₁	Range (mg/kg)	Percent positives
Maize				
Rural*	47	15	0.1–22.2* (2.2)	31.9
Urban*	49	3	0.2–0.5* (0.3)	6.1
Sorghum				
Rural	13	ND	NA	NA
Urban	NS	NA	NA	NA
Amahewu				
Rural	14	ND	NA	NA
Urban	NS	NA	NA	NA
Isizulu				
Rural	11	ND	NA	NA
Urban	NA	NA	NA	NA
Phutu				
Rural	28	8	0.1–0.4 (0.3)	28.6
Urban	39	ND	NA	NA
Feces				
Rural	40	13	0.5–39.0* (9.0)	32.5
Urban	44	3	0.6–16.2* (6.8)	6

Abbreviations: NA, not analyzed; NS, not sampled; ND, not detected (below the detection limit of 50 ng/g). Sample means are shown in parentheses.

*The mean concentration of FB₁ was significantly different ($p = 0.014$).

maize samples from the urban area contained detectable levels (0.2–0.5 mg/kg; average = 0.3 mg/kg). The mean concentration of FB₁ in the rural maize was significantly different from that of the urban group ($p = 0.014$). This difference in FB₁ distribution is noteworthy because it shows that the rural populace is at a 6 times higher risk of FB₁-associated disorders than the urban group (Fisher's exact test, $\chi^2 = 6.03$). We observed that 29% of the cooked maize meal samples (phutu) from the rural area contained FB₁ at a range of 0.1–0.5 mg/kg (average = 0.3 mg/kg), whereas the urban samples had no detectable levels. This may reflect the FB₁ incidence in maize. As was shown in the recovery experiments, the preparation of phutu does not appreciably degrade FB₁. The high end of the range of > 22.2 mg/kg in stored maize is of some concern, but is not as high as the level found in regions of Transkei with a high incidence of esophageal cancer (117 mg/kg) (5).

Fermented maize (amahewu), homebrew (isizulu), and unprocessed sorghum did not contain detectable levels of FB₁ from either region studied. Although *G. fujikuroi* (anamorph, *F. moniliforme*), mating type A, is the main contaminant of maize, sorghum often gets infected by members of mating type F, which are not producers of fumonisins (12). This could explain in part why we did not detect FB₁ in unprocessed sorghum. Although a limited amount of FB₁ is degraded during alcoholic fermentation of contaminated maize (13,14), isizulu (alcoholic fermented beverage) had no detectable levels of FB₁. This is possible because isizulu is prepared using maize as a starter, but sorghum is the main component. Amahewu is made by boiling maize meal to soft porridge, which is then left to ferment overnight. This is a nonalcoholic (lactate) fermentation process that has been shown to degrade a number of mycotoxins (15). It is possible that FB₁, if it present in this product, could have also been degraded. From these results, it seems that the rural population should be advised to grow and use sorghum rather than maize. These communities can also be encouraged to consume traditional fermented products instead of unfermented maize-derived dishes, especially after our findings in this study have been confirmed.

Fecal analysis. Of the 40 rural fecal samples analyzed, 13 (33%) were positive for FB₁ (range = 0.5–39.0 mg/kg; average = 9.0 mg/kg) (Table 2), whereas only 3 out of 44 (7%) urban samples were positive (range = 0.6–16.2 mg/kg; average = 6.8 mg/kg). The mean concentration of FB₁ in the samples of the rural feces was significantly different to that from the urban area ($p = 0.014$). These results reflect the incidence of FB₁ in the maize that was consumed and is important

in showing that analysis of feces is a useful short-term marker for FB₁ exposure, as the fecal samples were taken 24 hr after maize consumption. It is tempting to correlate the range of FB₁ (0.1–22.2 mg/kg) intake from maize in the rural population with the level found in feces samples (0.5–39.0 mg/kg), but clearly, other factors are also of importance here. These include personal habits, amount of food consumed, body weight, gut flora, and frequency of bowel movement. However, it does not seem unreasonable to assume, as a working rule, that in the rural population after 24 hr of ingestion a similar magnitude of FB₁ will be found in the dried feces. A time-course study of the period after the ingestion of contaminated maize is required to evaluate whether feces is a practical

marker of FB₁ exposure. In general, Fisher's exact test showed that the rural population is 6 times more at risk ($\chi^2 = 6.03$) of exposure than the urban population. This is possible because members of the rural population are routinely exposed to FB₁ from their diet (7).

GIS. In the case of the rural population, the various households were pinpointed using GIS and their locations are presented in Figure 1. This proved highly valuable because it avoided ambiguity and ensured that repeated visits and follow-up were easy, even by different workers in the field.

Conclusions

This study has shown that the rural communities are at a much higher risk of exposure to FB₁ from consuming maize than the urban

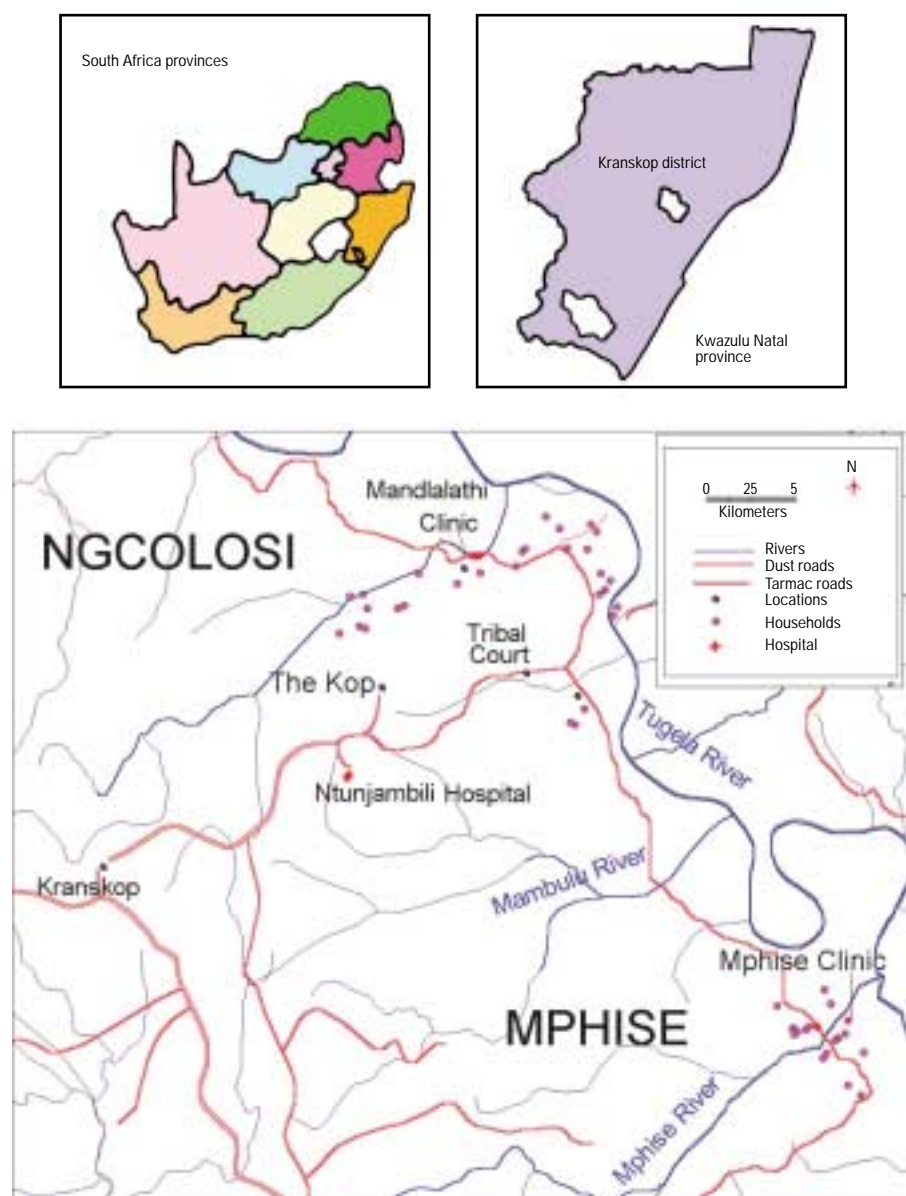


Figure 1. Plot of dwellings in the study area derived from GIS (courtesy of Geographic Information Systems, South Africa, for provision of Magisterial Districts map data).

communities. However, this risk is minimized when nonalcoholic fermented maize such as amahewu is consumed. Degradation of FB₁ in this lactate fermentation process is not well understood and warrants further investigation. There is evidence that sorghum and its products are less prone to FB₁ contamination; furthermore, the drier areas such as Tugela Valley seem to favor its cultivation. It would be wise to encourage rural communities to grow and consume sorghum. It must, however, be noted that the sample size in this study was small. Another survey with a larger sample size, covering other areas of KwaZulu Natal, should be carried out to give a more comprehensive conclusion.

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