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Fumonisin Contamination and *Fusarium* Incidence in Corn from Santa Catarina, Brazil

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In Brazil, the southern region has the highest incidence of esophageal cancer and also the highest production and consumption of corn (*Zea mays*) products. Corn samples intended for human consumption from the western, northern, and southern regions of the state of Santa Catarina, southern Brazil, had mean total fumonisin B (B_1, B_2, and B_3) levels of 3.2, 3.4, and 1.7 mg/kg, respectively. *Fusarium verticillioides*, the predominant fungus in the corn samples, had mean incidences (percent of kernels infected) of 14, 11, and 18% for the three regions, respectively. Additional corn samples intended for animal feed from the southern region had a mean total fumonisin level of 1.5 mg/kg and a mean *F. verticillioides* incidence of 10%. The fumonisin levels in corn from the state of Santa Catarina, Brazil, were similar to the high levels determined in other high esophageal cancer incidence regions of the world.

KEYWORDS: Fumonisin; *Fusarium verticillioides*; corn (*Zea mays*); Brazil; esophageal cancer

INTRODUCTION

Fumonisins, produced predominantly by *Fusarium verticillioides* (Sacc.) Nirenberg (formerly known as *F. moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg, occur widely around the world in corn (*Zea mays*) (*1*). The major naturally occurring fumonisin analogues in corn are fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) (see **Figure 1**). The contamination of corn with fumonisins is of concern as these mycotoxins cause various animal diseases and occur in corn and corn-based products intended for human consumption (*1*). In addition, high levels of fumonisins have been found in naturally contaminated corn from areas where high incidences of esophageal cancer occur, for example, the Centane District, Transkei region of South Africa; Cixian County, Hebei Province, China; and Mazandaran Province, Iran (*2*−*5*). On the basis of current data, the International Agency for Research on Cancer has classified FB_1 to be possibly carcinogenic to humans (group 2B carcinogen) (*6*).

Brazil is the third largest producer of corn in the world, of which the southern region is the highest producer and consumer of corn-based products. A considerable portion of this corn crop is produced by small farmers, and nearly 25% of the harvest is consumed on these farms (*7*). The states of Santa Catarina, Paraná, and Rio Grande do Sul in southern Brazil have the highest incidences of esophageal cancer in the country, with an age-standardized incidence rate (ASIR) of 18 per 100000 (*8*). The situation in southern Brazil is similar to that in other rural areas, where high esophageal cancer incidence and high corn consumption co-occur (*2*−*4*). In a survey conducted in Florianópolis in Santa Catarina, Brazil, esophageal cancer patients accounted for 134 of 2495 total cancer cases registered, mostly originating from the southern and western regions of Santa Catarina. These regions are also the main corn-producing areas of Santa Catarina, and these populations consume corn-based products as their staple diet (*9*).

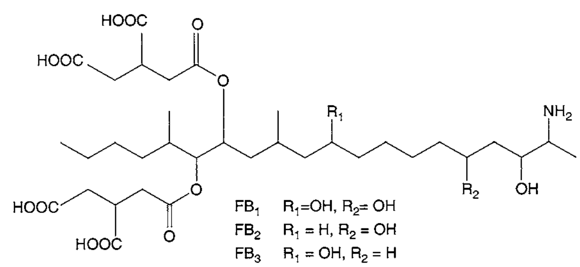

Figure 1. Chemical structures of fumonisins B_1, B_2, and B_3 (FB_1, FB_2, and FB_3).

The first report on the natural occurrence of fumonisins in corn in Brazil was from feed samples associated with outbreaks of confirmed and suspected mycotoxicoses in various animal species collected from farms in the state of Paraná. These samples were contaminated with mean levels of 8.9 mg/kg FB_1

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Table 1. Fumonisin Levels in Corn Samples from the State of Santa Catarina, Southern Brazil

		fumonisin level[a] (mg/kg)				
					total fumonisins	
region	*n*	FB_1	FB_2	FB_3	mean	range
west	39	2.06 ± 2.04	0.79 ± 0.80	0.36 ± 0.33	3.21 ± 3.09	0.02–18.74
north	17	2.24 ± 1.01	0.91 ± 0.63	0.30 ± 0.17	3.42 ± 1.75	1.01–7.73
south (intended for human consumption)	20	1.28 ± 2.14	0.35 ± 0.69	0.10 ± 0.17	1.73 ± 2.99	0.14–13.07
combined (intended for human consumption)	76	1.89 ± 1.90	0.70 ± 0.76	0.28 ± 0.28	2.87 ± 2.87	0.02–18.74
south (intended for animal feed)	14	1.05 ± 0.85	0.37 ± 0.50	0.11 ± 0.11	1.53 ± 1.36	0.15–4.79

[a] Values are means ± standard deviation. Differences between the means in corn intended for human consumption from all three regions are not statistically significant ($p > 0.05$). Differences between the means in animal feed and the corn intended for human consumption from all three regions are not statistically significant ($p > 0.05$).

and 2.8 mg/kg FB_2 (*10*). The first report on the occurrence of fumonisins in Brazilian corn-based food products, acquired from markets in Campinas, São Paulo, showed 35 of 72 products to be contaminated with FB_1, with a mean level of 0.4 mg/kg. However, an additional nine cornmeal samples, also acquired from these markets, were all contaminated and showed a much higher mean FB_1 level of 2.3 mg/kg (*11*). The highest natural fumonisin contamination in corn in Brazil was from freshly harvested samples from the state of São Paulo that had mean levels of 16.4 mg/kg FB_1 and 10.7 mg/kg FB_2 (*7*). In a further study, corn samples from various cultivars grown in the state of São Paulo showed mean levels of 5.6 mg/kg FB_1 and 1.9 mg/kg FB_2 (*12*). Corn samples collected at wholesale markets during the same season in central, southern, and southeastern Brazil had a mean FB_1 level of 2.2 mg/kg (*13*). *F. verticillioides* was the predominant *Fusarium* species detected in Brazilian corn in the southeastern state of São Paulo and the southern state of Paraná (*7*, *10*, *14*−*16*).

The current study was undertaken in the western, northern, and southern regions of the state of Santa Catarina, Brazil. Corn samples were collected from these regions during the year 2000 and analyzed for FB_1, FB_2, and FB_3 levels and fungal incidence. In Brazil the southern region has the highest incidence of esophageal cancer, and this is the first report of high fumonisin levels in corn from the state of Santa Catarina, southern Brazil.

MATERIALS AND METHODS

Corn Samples. Corn samples from the 1999−2000 harvest season were collected during the year 2000 from rural areas of the state of Santa Catarina, Brazil. These samples had been mechanically harvested and shelled, whereafter the grain was stored in silos. Corn intended for human consumption was collected from mountainous areas in the western (39 samples) and northern (17 samples) regions and from the prairies in the southern region (20 samples); corn intended for animal feed was collected in the southern region (14 samples). The corn samples were sent to the PROMEC Unit, South Africa, for fumonisin and mycological analyses.

Analytical Methods. *Fumonisin Analyses.* The standards were purified according to the method of Cawood et al. (*17*). FB_1, FB_2, and FB_3 levels were determined according to the method of Shephard et al. (*18*). Each sample was ground in a laboratory mill to a fine meal and extracted with methanol/water (3:1) by homogenization. An aliquot was applied to a strong anion exchange solid phase extraction cartridge (Varian, Harbor City, CA), and the fumonisins were eluted with 1% acetic acid in methanol. The purified extracts were evaporated to dryness, redissolved in methanol, and derivatized with *o*-phthaldialdehyde. The derivatized extracts were analyzed by reversed-phase high-performance liquid chromatography (HPLC) using an Ultracarb 5 ODS column (Phenomenex, Torrance, CA) and fluorescence detection.

Mycological Analyses. Samples were mycologically analyzed for fungal incidence (percent of kernels infected) using the method of Nelson et al. (*19*). Briefly, subsamples of kernels (80−100 g) were surface sterilized for 1 min in a 3.5% commercial sodium hypochlorite solution and rinsed twice in sterile water. One hundred kernels (5 kernels/ 90 mm Petri dish) were then transferred to malt extract agar (1.5%) containing novobiocin (150 mg/L), and the agar plates were incubated at 25 °C in the dark for 5−7 days. All of the isolated fungi were recorded and their frequencies determined using a stereomicroscope. *Fusarium* species were identified according to the system of Nelson et al. (*19*), and other fungi were identified on the basis of their cultural and morphological characteristics, that is, *Aspergillus* and *Diplodia* species.

Statistical Analysis. The results were statistically analyzed with the Systat 10 software package (SPSS Inc., Chicago, IL), and the correlation of the total fumonisin levels with the *F. verticillioides* incidence was analyzed with the STATA statistical software package (STATA Corp., College Station, TX).

RESULTS

The mean fumonisin (FB_1, FB_2, and FB_3) levels determined in the corn samples of the different regions are shown in **Table 1**. FB_1, FB_2, and FB_3 were present in all of the corn samples, except for two samples in which FB_3 was not detected. The mean level of the total fumonisins in the corn from all three regions in Santa Catarina combined was 2.89 mg/kg ($n = 90$, range = 0.02−18.74 mg/kg), indicating the occurrence of some very high levels (individual results are not shown). In this study 31 of 90 corn samples had FB_1 levels >2 mg/kg and 5 of 90 samples >4 mg/kg, whereas 46 of 90 corn samples had total fumonisin levels >2 mg/kg and 15 of 90 samples >4 mg/kg. In the western, northern, and southern regions 24 of 39, 14 of 17, and 8 of 27 samples, respectively, had total fumonisin levels >2 mg/kg. The 14 corn samples intended as animal feed, collected from the southern region, had a mean total fumonisin level of 1.53 mg/kg, whereas the 76 corn samples intended for human consumption had a mean total fumonisin level of 2.87 mg/kg (**Table 1**). There were no statistical differences ($p > 0.05$) in the fumonisin levels between the different regions and the corn samples intended for human and animal consumption, due in part to large individual variation in the samples as indicated by the standard deviations (**Table 1**).

Whereas the fumonisin contamination of the corn samples was 100%, *Fusarium* species were isolated from 93% of the samples. Even though *F. verticillioides* was the predominant fungus isolated from all three regions (**Table 2**), the incidence did not correlate with the fumonisin levels in the corn samples ($r = -0.14$, $p > 0.05$). There were no statistical differences ($p > 0.05$) in the mean incidence of *F. verticillioides* in the corn intended for human consumption between the western (14%, range = 0−62%), northern (11%, range = 0−66%), and southern regions (18%, range = 1−42%), respectively. There was no statistical difference ($p > 0.05$) in the mean incidence of *F. verticillioides* between the corn intended for human

Table 2. Incidence of Fungi in Corn Samples from the State of Santa Catarina, Southern Brazil

region	*n*	incidence of fungi[a] (% of kernels infected)				
		F. verticillioides	other *Fusarium* species[b]	*A. flavus*	other species[c]	total fungi
west	38[d]	13.5 ± 15.6	1.55 ± 2.85	3.37 ± 4.91	33.2 ± 18.5	51.6 ± 27.2
north	17	11.4 ± 18.7	0.88 ± 2.47	10.2 ± 21.9	20.5 ± 15.1	43.0 ± 34.3
south (intended for human consumption)	20	17.6 ± 12.5	1.25 ± 2.59	4.05 ± 7.74	36.1 ± 13.7	59.0 ± 23.9
combined (intended for human consumption)	75	14.1 ± 15.6	1.32 ± 2.68	5.11 ± 11.8	31.1 ± 17.4	51.6 ± 28.3
south (intended for animal feed)	14	10.4 ± 12.0	1.71 ± 2.64	8.64 ± 8.34	37.3 ± 13.2	58.1 ± 27.3

[a] Values are means ± standard deviation. Differences between the means in different regions are not statistically significant ($p > 0.05$). [b] Other *Fusarium* species includes *F. subglutinans* and *F. graminearum*. [c] Other species includes *Diplodia maydis* and *D. macrospora*. [d] One sample received as ground meal. Mycological analysis was performed by dilution plating. *F. verticillioides*, 0.9×10^6 colony-forming units (cfu)/g; other *Fusarium* species, 1.0×10^6 cfu/g; other species, 1.0×10^6 cfu/g; total fungi, 1.4×10^6 cfu/g.

consumption (14%, range = 0−66%) and the corn intended as animal feed (10%, range = 0−36%). There was also no statistical difference ($p > 0.05$) in the other mycological data obtained from the three regions. *F. subglutinans* was isolated in 16 of 38, 2 of 17, and 11 of 34 samples in the western, northern, and southern regions, respectively, with a mean incidence (percent of kernels infected) of 0.9% (range = 0−12%) for the combined regions. *F. graminearum* was present in only 9 of 90 samples, and other *Fusarium* species were present in 6 of 90 samples. *Aspergillus flavus* was isolated from 26 of 38, 12 of 17, and 23 of 34 samples in the western, northern, and southern regions, respectively, with a mean incidence of 5.7% (range = 0−90%) for the combined regions. *Diplodia* (= *Stenocarpella*) *maydis* and *Diplodia macrospora* were recorded in small numbers of samples (10 of 90 and 12 of 90, respectively) with a range of 0−2% of kernels infected.

DISCUSSION

High esophageal cancer incidence areas in South Africa, China, and Iran have been associated with populations consuming high levels of corn heavily contaminated with fumonisin (*2*−*5*, *20*). In Brazil, the southern region has the highest incidence of esophageal cancer (*8*). High production and consumption of corn and corn-based products occur in southern Brazil (*7*, *9*). Previous studies that investigated the levels of fumonisin in Brazilian corn were confined almost exclusively to Paraná, southern Brazil, and São Paulo, southeastern Brazil (*7*, *10*−*12*, *16*, *21*−*23*). This study is the first to report fumonisin levels in corn in the state of Santa Catarina, southern Brazil.

The mean FB_1 level in corn samples from Santa Catarina intended for human consumption was 1.89 mg/kg, which is similar to FB_1 levels in other high esophageal cancer incidence regions, for example, 1.84 mg/kg in Centane, South Africa, in 1989, 2.27 mg/kg in Mazandaran, Iran, in 1998, and 2.73 mg/kg in Linxian County, China, in 1994 (*2*, *3*, *20*). Similar mean FB_1 levels have been reported in other southern Brazil states. During the 1997/1998 season corn from central, southern, and southeastern Brazil had a mean FB_1 level of 2.2 mg/kg in 214 samples, and in the 1995/1996 season from central-western Paraná a mean level of 2.4 mg/kg in 86 samples was reported (*13*, *22*). The first investigation on fumonisin contamination in corn-based food products conducted in São Paulo revealed a mean FB_1 level of 0.4 mg/kg, whereas cornmeal samples collected from the same markets had a mean FB_1 level of 2.3 mg/kg (*11*).

The mean total fumonisin level in corn intended for human consumption was 2.87 mg/kg, almost double the 1.53 mg/kg level in the corn intended as animal feed. However, the difference was not statistically significant, due in part to individual variation in the samples as indicated by the standard deviation. On the basis of this mean total fumonisin contamination of 2.87 mg/kg and the assumption that a 70 kg person from the rural area in Brazil consumes 11−39 g of dry corn per day (*11*), the probable daily intake (PDI) of fumonisins by this population was up to 1.6 μg/kg of body weight. This is double the tolerable daily intake (TDI) of 0.8 μg/kg of body weight, which was based on a NOEL (no observed effect level) of 25 mg of FB_1/kg of diet in rats and a safety factor of 1000 for carcinogenicity (*24*). However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a provisional maximum tolerable daily intake (PMTDI) for total fumonisins of 2 μg/kg of body weight based on a NOEL of 0.2 mg/kg of weight/day and a safety factor of 100 (*25*). Considering this recommendation the exposure of the rural population of Santa Catarina would be below the level proposed by JECFA.

Similar to previous Brazilian investigations on the mycoflora of corn, *F. verticillioides* was the predominant fungus isolated in this study (*7*, *10*, *14*−*16*). However, the incidence of *F. verticillioides* was not significantly correlated with the fumonisin levels in the corn as reported in other South African corn studies (*2*, *26*). In contrast to these studies, a correlation was observed in investigations on corn conducted in Argentina, South Africa, and the United States (*27*, *28*). The presence or absence of significant positive correlations might be attributed to various factors that influence the production of fumonisins by *F. verticillioides*, for example, the corn hybrid, the abiotic stress on the host plant, and symptomless systemic infection (*2*, *7*, *29*). Even though *F. subglutinans* was isolated in 29 of 89 samples, the mean incidence was only 0.9% and that for *F. graminearum* only 0.2%, and neither of these two *Fusarium* species produce fumonisins (*30*). Therefore, compared to *F. verticillioides* the incidence of the other *Fusarium* species in this study was insignificant. The second most abundant genus of fungi isolated from the corn was *Aspergillus* (5.7%). Co-contamination with aflatoxin and fumonisin has previously been reported in Brazilian corn from central, southern, and southeastern Brazil, specifically in the states of Paraná and São Paulo (*13*, *14*, *23*). Further investigation is required into the seasonal variation of the chemical and mycological data in the corn of Santa Catarina, Brazil, as differences may be expected due to changing environmental conditions such as annual rainfall, temperature, and insect infestation. Additional epidemiological data on ASIR of esophageal cancer in Santa Catarina are also urgently required. The high level of fumonisin contamination in corn intended for human consumption in yet another region where the incidence of esophageal cancer is high is a further indicator for an association between the consumption of corn contaminated with fumonisins and esophageal cancer.

ABBREVIATIONS USED

FB_1, fumonisin B_1; FB_2, fumonisin B_2; FB_3, fumonisin B_3; ASIR, age-standardized incidence rate; PDI, probable daily intake; TDI, tolerable daily intake; NOEL, no observed effect level; JECFA, Joint FAO/WHO Expert Committee on Food Additives; PMTDI, provisional maximum tolerable daily intake.

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