Fumonisin Occurrence in Corn from High- and Low-Risk Areas for Human Esophageal Cancer in China

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Forty-seven corn samples were collected in 1989 from Linxian and Shangqiu Counties in Henan Province, the high- and low-risk areas, respectively, for human esophageal cancer in the People's Republic of China. The samples were analyzed for fumonisin (fumonisin B1 [FB1] and FB2) contamination. Of the fumonisin-positive samples, the mean levels in Linxian corn were found to be 872 ng/g for FB1 and 448 ng/g for FB2, while the Shangqiu corn had 890 ng/g of FB1 and 330 ng/g of FB2 per g. The incidence of fumonisin contamination of Linxian corn (48%) was about two times higher than that of Shangqiu corn (25%), and the former corn samples were frequently cocontaminated with trichothecenes. Fusarium species isolated from corn from Linxian County produced FB1 at levels ranging from 1,280 to 11,300 μg/g.

Different Fusarium species occur worldwide on a variety of plant hosts and cause a number of human and animal diseases after consumption of fungus-damaged plant products. Trichothecenes (TRIC) and zearalenone, produced principally by Fusarium graminearum and F. culmorum, are two of the well-known causative agents of several mycotoxicoses (24, 25).

F. moniliforme Sheldon also has been suspected of involvement in chronic toxicoses of animals, e.g., equine leukoencephalomalacia and porcine pulmonary edema syndrome (7, 11). Recently, a new mycotoxin, fumonisin B1 (FB1), was isolated from culture materials of F. moniliforme and was demonstrated to be an etiologic agent associated with equine leukoencephalomalacia and porcine pulmonary edema (4, 10). FB1 was shown to be both hepatotoxic and a promoter of carcinogenicity in rats (2). In addition, structurally related derivatives of FB1, namely, FB2 and FB3, as minor toxins of the fungus, were also demonstrated to possess toxic properties similar to those of FB1 (3). Moreover, FB1 and FB2 were detected in commercially available corn products intended for human consumption (13, 18, 20); hence, they are regarded as potential risks to human health.

On the other hand, the occurrence of F. moniliforme in corn appears to be correlated with the high rate of human esophageal cancer (HEC) in Transkei, South Africa, and in China (9, 26). FB1 and FB2 have been found in home-grown corn samples consumed by inhabitants of the rural areas in Transkei (22). Furthermore, a statistically significant correlation was demonstrated between the high incidence of HEC in this area and the fumonisin concentration in the staple (14, 21).

The major high-risk areas for HEC and esophagitis in the People's Republic of China are located in Henan, Hebei, and Shanxi Provinces, with Linxian County in Henan Province being the highest-risk area (5, 6). However, only limited surveys concerning the natural occurrence of mycotoxins in corn samples from HEC-prevalent areas have been made (8), and no study on the incidence of HEC in relation to levels of fumonisin has been reported. In this paper, we describe the natural occurrence of the Fusarium mycotoxins, including fumonisin, in corn samples from Linxian County and compare them with that in samples from Shangqiu County, an area at low risk for HEC. In addition, the ability of Fusarium isolates from Linxian corn to produce fumonisin is described.

MATERIALS AND METHODS

Corn samples. Corn samples analyzed in this study were those used in a previous study (8). A total of 47 corn kernel samples (at least 250 g each) were collected from Linxian and Shangqiu Counties, Henan Province, in 1989. Among these, 27 samples were obtained from 27 different families of HEC-affected patients (randomly selected) in Linxian. The remaining samples came from 20 different peasant families with no cases of esophageal cancer chosen at random in Shangqiu County. Originally, these samples were from corn stored on the cob both inside and outside. The corn was shelled, stored for a short time, and cooked without any selection for moldy ears or kernels. Individual corn samples were placed in zip-lock plastic bags and stored for approximately 18 months at −20°C prior to analysis.

Extraction and analysis. After 50-g corn samples were ground to a fine meal in a laboratory ultracentrifugal mill (Trio Blender 848-36MW; Trio Science Co. Ltd., Tokyo, Japan), a subsample (10 g) was extracted with acetonitrile-water (1:1; 50 ml) and filtered. The filtrate was partitioned with n-hexane, and the aqueous layer was evaporated to dryness. The residue was dissolved in acetonitrile-water (1:1; 5 ml) and applied to a preconditioned strong anion-exchange cartridge (Sep-Pak Accell Plus QMA; Millipore Corp., Milford, Mass.). The cartridge was eluted successively with methanol-water (3:1; 10 ml), methanol (5 ml), and 0.5% acetic acid in methanol (10 ml). The last eluate was evaporated to dryness and redissolved in acetonitrile-water (1:1; 2 ml) to provide a sample solution. This solution was analyzed for FB1 and FB2 according to the method of Shephard et al. (17), with a slight modification. Briefly, an aliquot of the sample solution was derivatized with o-phthalaldehyde and analyzed on a Shimadzu LC-6A high-performance liquid chromatograph (HPLC) equipped with a spectrofluorometer under the following conditions: column, Shim-Pack CLC-ODS, 250 by 4.6 mm (inside diameter) (Shimadzu Scientific Inc., Kyoto, Japan); emission wavelength, 440 nm; excitation wavelength, 335 nm. The mobile phase was methanol-sodium dihydrogen phosphate buffer (pH 3.3; 76:24) at a flow rate of 0.5 ml/min. Quantification of fumonisin was

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TABLE 1. Natural occurrence of fumonisin and TRIC in corn samples from Linxian and Shangqiu Counties

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples</th>
<th>No. (%) positive</th>
<th>Mean level (range) of positive samples (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linxian</td>
<td>27</td>
<td>13 (48)</td>
<td>872 (186-2,964) ± 448 (298-550) ± 781 (17-4,213)</td>
</tr>
<tr>
<td>Shangqiu</td>
<td>20</td>
<td>5 (25)</td>
<td>890 (197-1,732) ± 330 (213-447) ± 102 (14-612)</td>
</tr>
</tbody>
</table>

* Linxian samples were randomly collected from the families of patients with esophageal cancer; Shangqiu samples were randomly collected from peasant families with no esophageal cancer.
* Corn samples cocontaminated with fumonisin (FB1 and FB2) and TRIC at levels above 100 ng/g.
* The major TRIC in corn samples were deoxynivalenol and its 15-acetate. Data are total TRIC concentrations determined from a previous study (8). Mean TRIC levels in Linxian corn samples were significantly (P < 0.01) higher than those in Shangqiu corn samples.
* No significant difference was found between mean concentrations of fumonisins in Linxian and Shangqiu County samples (P > 0.05).

RESULTS AND DISCUSSION

The range and mean levels of FB1 and FB2 concentrations together with the number of samples positive for each fumonisin are given in Table 1.

FB1 was found in concentrations ranging from 186 to 2,964 ng/g (mean of positive samples, 872 ng/g) in 48.1% (13 of 27) of corn samples from Linxian, among which three samples were coincidentally contaminated with FB2 (298, 495, and 550 ng/g). On the other hand, 5 of the 20 Shangqiu corn samples (25%) contained FB1 in concentrations ranging from 197 to 1,732 ng/g (mean of positive corn samples, 890 ng/g). FB2 was also detected together with FB1 in two of the samples (213 and 447 ng/g). Thus, the incidence of fumonisin in corn samples from Linxian, the high-risk area, was about two times higher than in samples from Shangqiu, the low-risk area, although no significant (P > 0.05) difference in FB1 and FB2 levels between the two areas was found.

The incidence and mean levels of fumonisin in Linxian corns were close to those reported in corn-based human food products in the United States (18, 20) and Switzerland (13). In the United States, 94% of corn meal for human consumption analyzed in 1991 was contaminated, with mean levels of 1,048 and 298 ng/g for FB1 and FB2, respectively. However, fumonisin levels obtained from Linxian corns were apparently much lower than those found in corn samples from Transkei, the high-risk area for HEC in South Africa (21), where fumonisin levels were comparable to those determined in feed samples associated with field outbreaks of equine leukoencephalomalacia in several countries (16, 19, 22). Despite these facts, appreciably high levels of fumonisin were found in two corn samples (7.4%; 3,395 and 3,459 ng/g) from the high-risk area in China (Linxian), and similar levels of fumonisin have not been detected in commercially available corn-based human food products.

More remarkable was the co-occurrence of fumonisin and TRIC in the Chinese corn samples. Previously, we reported that the major TRIC found in these corn samples were deoxynivalenol and its 15-acetate and that the incidence and levels of these mycotoxins in Linxian corn samples were significantly (P < 0.01) higher than those in Shangqiu corns (8). Figure 1 illustrates the distribution of Linxian and Shangqiu corn samples (28 samples) contaminated with fumonisin (FB1 plus FB2; 18 samples) and/or TRIC (deoxynivalenol plus its 15-acetate; 24 samples) at concentrations of >100 ng/g; this level was the detection limit of the analytical method used. Among the 22 corn samples from Linxian County, only TRIC
was detected in 9 samples, while both fumonisins and TRIC were present in the remaining 13 samples. Only one of six corn samples from Shanggu was contaminated with both toxins. Thus, the incidence (48%) of Linxian corn samples cocontaminated with both fumonisins and TRIC was approximately 10 times higher than that of similarly contaminated Shanggu corn samples (Table 1).

The relative incidence of Fusarium species, including fumonisin-producing fungi, was not determined because of limited amounts of corn samples. From five corn samples from Linxian County, however, F. proliferatum and F. moniliforme were isolated and examined for fumonisin production. As shown in Table 2, all of the 12 isolates of F. proliferatum showed high levels of production of FB₁, ranging from 1,500 to 11,300 μg/g and produced FB₂ at levels ranging from 140 to 1,950 μg/g, while F. moniliforme (5 isolates) produced FB₁ at levels ranging from 1,280 to 10,200 μg/g and FB₂ at levels ranging from 210 to 1,130 μg/g. Although the toxin levels shown in Table 2 were determined on the basis of the wet weights of corn cultures, the isolates from Linxian corn samples had considerably higher toxin production levels than reported in previous papers (15). The FB₁/FB₂ ratios, varying from 4.0 to 17.4, for all isolates were similar to those reported previously (15, 23). The results suggest that corn samples from the high-risk area infected with both F. moniliforme and F. proliferatum contained high levels of fumonisins, which may be largely due to the latter organism.

This is the first report of a comparative study of the natural occurrence of fumonisins in staple foods in relation to the incidence of HEC in the People’s Republic of China. There are several differences in mean levels and incidence of this mycotoxin contamination when South Africa and China are compared. The levels of FB₁ and FB₂ in corn samples were significantly correlated with the incidence of HEC in South Africa (14, 21, 22), but the correlation was insignificant in China, as shown in this paper. On the other hand, the incidence and levels of TRIC in corn samples were clearly correlated with the incidence of HEC in China (8) but not in South Africa (21). Hence, continuous studies are needed to clarify the occurrence and level of Fusarium toxins in corn intended for human consumption in both areas.

**TABLE 2. FB₁ and FB₂ concentrations in corn cultures of F. moniliforme and F. proliferatum isolates from corn as a staple food of families of patients with HEC in Linxian County**

<table>
<thead>
<tr>
<th>Corn sample code</th>
<th>Conc (ng/g) in corn</th>
<th>Species and isolate</th>
<th>Conc (μg/g) in culture</th>
<th>Ratio, FB₁/FB₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FB₁</td>
<td>FB₂</td>
<td></td>
<td>FB₁</td>
</tr>
<tr>
<td>27H</td>
<td>2,964</td>
<td>495</td>
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<td>2,845</td>
<td>550</td>
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<td>11,300</td>
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<td>F. moniliforme KU1548</td>
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<td>ND</td>
<td>F. moniliforme KU1547</td>
<td>1,960</td>
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</table>

* Identification number of authors’ laboratory.

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**REFERENCES**


