Natural occurrence of mycotoxins in corn, samples from high and low risk areas for human esophageal cancer in China

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章 紅^{*1,2}, 長嶋 等^{*2}, 後藤 哲久^{*2,3}: 中国の人食道ガン高発生率地域と低発生率地域におけるトウモロコシのマイコトキシン汚染

Summary

A total of 246 apparently healthy corn samples were collected from nine villages in the counties of Cixian, Linxian, Anyang, Fanxian and Yanqing in the People's Republic of China. All of the samples were harvested in the autumn of 1995 and were intended for human consumption. The samples were analyzed for fumonisin and aflatoxin B₁ contamination by ELISA. Of 164 corn samples collected from areas in which the risk of human esophageal cancer (HEC) is high, fumonisin was detected in 106 samples (0.5-16.0 ppm, average ; 0.70 ppm), but of 82 samples collected from HEC low risk areas, fumonisin was found in 23 samples (0.5-1.5 ppm, average ; 0.20 ppm). The frequency of fumonisin contamination in the high risk areas was approximately twice that of low risk areas, and the average content of fumonisin in samples from HEC high risk areas was about three times higher than that from HEC low risk areas. For aflatoxin B₁, the concentration and frequency of aflatoxin B₁ positive samples varied greatly from village to village but in general, aflatoxin contamination was low in HEC low risk areas. Although a clear relationship between fumonisin contamination and HEC incidence could not be distinguished, it is evident that people living in HEC high risk area sustained more exposure to fumonisin and aflatoxin than people living in HEC low risk area.

Key words: Fumonisin, フモニシン ; Aflatoxin, アフラトキシン ; Human Esophageal Cancer, 人食道ガン

Aflatoxin B₁ (AFB₁) a metabolite, produced by some members of the *Aspergillus flavus* group fungi, is well known for its hepatocarcinogenicity¹⁻³). This mycotoxin is a frequent contaminant of various agricultural commodities including corn and corn products⁴). Fumonisin was first reported in 1988 by Gelderblom *et al.*⁵) and the carcinogenicity of this toxin was also reported⁶). Fumonisin is produced by *Fusarium moniliforme* and are naturally occurring contaminations of corn in many parts of the world⁷). Fumonisin B₁ (FB₁) is the major fumonisin found both in *F. moniliforme* cultures and in naturally contaminated samples. The toxicity of this toxin has been well-documented in several species and exposure may result in leukoencephalomalacia in the horse⁸), porcine pulmonary edema in swine⁹) and liver tumors in rats^{6,10}). As evidence of the

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carcinogenic activities of fumonisins mounted⁵⁾, epidemiological studies were done and a relationship between exposure to fumonisins and risk of human esophageal cancer (HEC) was indicated¹¹⁻¹⁴⁾.

In some areas of South Africa and the People's Republic of China, epidemiological correlations between HEC incidence and *F. moniliforme* and fumonisin contamination of corn and corn-based products for those populations in which corn products constituted a major portion of the daily diet were reported^{11,15-18)}. However, there are limited surveys relating the natural occurrence of mycotoxins in home grown corn samples in the People's Republic of China have been done¹⁹⁻²¹⁾. In China, the annual incidence of HEC is second only to stomach cancer, and is therefore a health risk of major concern. In some areas, the annual mortality rate due to HEC exceeds 100 per 100,000¹⁷⁾. One of the main high risk areas is located in Henan, Hebei and Shanxi Provinces, which are located along the border of the Taihang Mountains. Before 1985, the people in these areas consumed corn-based foods as a major part of their diet; even now corn products still make up about 15-20% of total food consumption. In Linxian County in Henan Province, some epidemiological studies were done due to the high mortality rate for HEC²²⁾. In Linxian county, analysis of corn samples collected from family members of HEC patients' revealed *Fusarium* mycotoxin contamination^{19,23)}. The incidence of fumonisin contamination in Linxian corn was about two times higher than that of the corn found in the low risk area of Shangqiu. In addition, the corn samples from Linxian were frequently co-contaminated with trichothecenes²¹⁾. Chu and Li²⁰⁾ reported that even healthy appearing corn samples collected from households in Cixian County, another HEC high risk area, contained FB₁ at a mean level of 39.5 ppm (range 30.3 to 47.9 ppm), providing additional evidence that FB₁ and other mycotoxins may be causative agents of HEC in the People's Republic of China.

In this report, analysis for fumonisin and AFB₁ in corn samples collected from the HEC high risk areas of Cixian, Linxian and Anyang Counties and the HEC low risk areas of Fanxian and Yanqing Counties are compared.

Materials and Methods

Samples A total of 246 corn samples harvested in the autumn of 1995 were collected directly from farmer in six villages (Ducun, Lintian, Xiazhuangdian and Xiguanglu in Cixian County, Tingtoucun in Linxian County and Shuiye in Anyang County) situated in HEC high risk areas and from farmers in three villages (Chengguanzheng and Baiyi in Fanxian County and a village in Yanqing County in Beijing suburb) situated in HEC low risk areas¹⁷⁾. The samples were collected between October of 1995 and March of 1996. Cixian County is belong to Hebei Province. Lixian County and Anyang County are belong to Henan Province. Yanqing County is located in Beijing suburb (Fig. 1). The HEC high risk areas are located in hilly or mountainous area and, with the exception of Yanqing County which is mountainous, the low risk areas are located in plain area. The distances from the HEC high risk areas to Fanxian County is about 100-150 km, and to Yanqing County is about 400 km. The incidences of HEC were more than 80/100,000 in high risk areas and less than 20/100,000 in low risk areas. All of the samples were intended for human consumption and did not contain visible signs of mold contamination. Each sample was

Fig. 1 Map of the area sample collected.

milled by an Ultra Centrifuge Mill ZM-1 (Retsch, Germany) equipped with a 1 mm mesh screen and stored at -20°C until analysis.

Analysis of Fumonisin Fumonisin in corn samples was detected by ELISA using a kit developed by Kikkoman Co. Ltd (Noda, Japan) with some minor modifications²⁴. The minimum detection limit of the kit was 0.5 ppm for FB_1 in corn samples. Ten grams of milled samples were shaken with 50 ml of 70% methanol for 15 min and filtered through No. 2 filter paper (Advantec Toyo, Tokyo, Japan). The $350\ \mu\text{l}$ of filtrate was transferred to the well of a 48-wells concentrating plate and dried at 30°C overnight. The residue was dissolved in the well in $70\ \mu\text{l}$ of 70% methanol to which $70\ \mu\text{l}$ of enzyme conjugate was added. After mixing, $100\ \mu\text{l}$ of the mixture was transferred to an antibody coated well and incubated for one hour at 30°C . After the plate was washed $100\ \mu\text{l}$ TMB substrate solution (Sigma, MO, USA) was added to each well and incubated for 10 min at room temperature in the dark, at which time $100\ \mu\text{l}$ stop reagent ($0.5\ \text{N}\ \text{H}_2\text{SO}_4$) was added. The color was measured by a microplate reader (Model 450, BIO-RAD, CA, USA) at 450 nm and the amount of FB_1 in the sample was calculated from the absorbance of FB_1 standards.

Analysis of Aflatoxin Aflatoxin B_1 in corn samples were also analyzed by ELISA²⁵. The 70% methanol extract was diluted 20 times by phosphate buffered saline. A polyclonal antibody for AFB_1 and AFB_1 -oxime-horseradish peroxidase conjugate were used to detect and quantitate AFB_1 . The minimum detection limit of this method for AFB_1 is approximately 2 ppb and quantitatively more than 5 ppb may be measured in corn sample.

Results

Contamination of Fumonisin The level of fumonisin contamination in corn samples from HEC high and low risk areas are shown in Fig. 2 and Table 1. There were differences in the frequency and concentration of fumonisin contamination in corn samples from HEC high risk areas relative to low risk areas. Of 164 corn samples from HEC high risk areas, 65% of the samples were

Fig. 2 Fumonisin contamination of corn samples.
N.D. : not detected (less than 0.5 ppm).

Table 1 Frequency and amounts of fumonisin contamination.

Village	No. of Samples	No. of FB ₁ Positive	Ratio of FB ₁ Positive	Average* (range of positive) FB ₁ contents (ppm)
Ducun	42	24	57.1	0.58 (0.5 ¹⁾ -4.1)
Lintian	29	20	69.0	0.57 (0.5-4.2)
Xiazhuangdian	29	19	65.5	1.30 (0.5-16.0)
Xiguanglu	30	23	76.7	0.63 (0.5-2.2)
Jingtoucun	8	5	62.5	0.35 (0.5-0.6)
Shuiye	26	15	57.7	0.56 (0.5-6.0)
Chengguanzheng	26	8	30.8	0.22 (0.6-1.5)
Baiyi	25	6	24.0	0.19 (0.5-1.4)
Yanqing	31	9	29.0	0.19 (0.6-0.8)

* : The average data comes from average of all samples analyzed.
¹⁾ Minimum ditecton limit and minimum quantitation limit of this analytical method.

contaminated with fumonisin, but of 82 corn samples from HEC low risk areas, only 28% of the samples were contaminated. The average level of fumonisin contamination was 0.7 ppm in the HEC high risk area samples versus 0.2 ppm in the HEC low risk area samples. So not only was fumonisin contamination twice as likely to occur in the HEC high risk areas, but the level of contamination was more than three times higher than that the level of contamination in HEC low risk areas. The frequency of fumonisin contaminated samples varied from 57.1% to 76.7% in the six villages in the HEC high risk area and from 24.0% to 30.8% in the three villages in the HEC low risk areas. No samples from low risk areas were contaminated with more than 1.5 ppm of FB₁. In contrast, 13.4% of samples from high risk areas contained more than 1.0 ppm of FB₁ and one sample in Xiazhuangdian was contaminated with 16 ppm FB₁.

Contamination of Aflatoxin B₁ The levels of contamination of AFB₁ in corn samples from HEC high and low risk areas are shown in Fig. 3. In general, the AFB₁ contamination levels were low

Fig. 3 Aflatoxin B₁ contamination of corn samples.
N.D. ; not detected (less than 2 ppb).
trace : between 2 to 5 ppb.

Table 2 Frequency and amounts of aflatoxin B₁ contamination.

Village	No. of Samples	No. of AFB ₁ Positive	Ratio of AFB ₁ Positive (%)	Average* (range of positive) of AFB 1 Contents (ppb)
Ducun	42	26	61.9	4.70 (trace ¹⁾ -13.5)
Lintian	29	25	86.2	2.90 (trace-9.7)
Xiazhuangdian	29	15	51.7	3.58 (trace-8.7)
Xiguanglu	30	5	16.7	0.70 (trace-8.4)
Jingtoucun	8	8	100.0	9.38 (8.0-10.7)
Shuiye	26	7	26.9	1.35 (trace-12.0)
Chengguanzheng	26	9	34.6	trace (trace)
Baiyi	25	12	48.0	1.14 (trace-6.1)
Yanqing	31	0	0.0	N.D. ²⁾

* : When calculating the incidence and average of AFB₁ contamination, the trace level contamination was considered as positive sample and zero level. The average data comes from the average of all samples.

¹⁾ between 2 to 5 ppb, ²⁾ not detected (less than 2 ppb).

in the HEC low risk areas. AFB₁ was detected in only 25.6% of the samples, some of which was only trace level contamination. In contrast, 52.4% of the corn samples from HEC high risk areas were contaminated with AFB₁ and 6.7% of them contained more than 10 ppb. However, as shown is Table 2, the levels of AFB₁ content and the frequency of AFB₁ positive samples varied greatly from village to village. No AFB₁ was detected in samples from Yanqing County which is considered an HEC low risk areas, but all of the samples from Jingtoucun, which is one of the HEC high risk areas, contained more than 5 ppb of AFB₁ and nearly 40% of them contained more than 10 ppb. However, 83.3% samples from Xiguanglu did not contain detectable amount of AFB₁ even though this village is situated in a HEC high risk area. The highest concentration of AFB₁ was found at Ducun (13.5 ppb) and 7 of 26 positive samples were contaminated with more

than 10 ppb of AFB₁.

Discussion

This study revealed that the frequency of fumonisin contamination in household corn samples collected from six villages in HEC high risk areas was about two times higher than that from three villages in HEC low risk areas. A relatively high concentration of fumonisin, (average level of 0.70 ppm) was found in corn samples from HEC high risk areas compared to the average level (0.2 ppm) found in samples from HEC low risk areas. In addition, 16 ppm of FB₁ was detected in a sample collected at Xiazhuangdian of Cixian County, whereas the highest level of FB₁ contamination detected in a HEC low risk area was 1.5 ppm. The frequency and level of FB₁ contamination was consistent with the HEC incidence.

Our observations regarding the frequency of fumonisin contamination were comparable to those reported in a previous study reported by Yoshizawa *et al.*²¹⁾ in which the frequency of fumonisin contamination of Linxian (HEC high risk area) corn was also about two times higher than that of Shangqiu (HEC low risk area) corn. However, the amount of detected in high versus low HEC risk areas was less than the values reported by Sydenham *et al.*¹¹⁾. In the latter study, in which the corn samples were from South Africa, the mean level of FB₁ contamination associated with HEC high risk areas was at 2.1 ppm and was 20 times greater than the level associated with low risk areas. Also the FB₁ contents of our samples from HEC high risk areas were much lower than the levels previously reported by Chu and Li²⁰⁾.

Unlike fumonisin contamination, the frequency and concentration of AFB₁ contamination in corn samples were varied greatly among the nine villages. Also the concentrations of AFB₁ contamination obtained in this study were very low and were similar to the results reported by Chu & Li²⁰⁾.

There have been several hypotheses put forth in efforts to explain the high incidence of HEC in China, in which nutritional deficiencies or irregularities in eating habits, nitrosamine exposure, alcohol consumption, tobacco use, and fungal metabolites have been proposed as the causative agent or agents¹⁷⁾. This is the first report indicating the difference in the natural occurrence of fumonisin in a staple food source between high and low risk areas for HEC in Cixian and Anyang Counties (HEC high risk areas) and Fanxian, Yanqing Counties (HEC low risk areas) in People's Republic of China. It has been shown that the people residing in an area with a high incidence of HEC received more exposure to fumonisins, although the exposure varied greatly. The unequivocal positive relationship between fumonisin and AFB₁ concentrations in foodstuffs and incidence of HEC could not be drawn from this study. Since fumonisins have been shown to be potent carcinogens, the simultaneous presence of fumonisins and other carcinogenic compounds may play an important role in human carcinogenesis. More extensive study are needed to clarify the relationship between fumonisins contamination and the incidence of HEC in China.

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References

- 1) Schroeder, H. W., Boller, R. A.: *Appl. Environ. Microbiol.*, **25**, 885 (1973).
- 2) Sargeant, K., Sheridan, A., O'Kelly, J., Carnaghan, R. B. A.: *Nature*, **192**, 1096 (1961).
- 3) Halver, J. E.: "Aflatoxins", (ed. Goldblatt, L. A.), p. 265 (1969), Academic Press, New York.
- 4) Goto, T.: *Food Reviews International*, **6**, 265 (1990).
- 5) Gelderblom, W. C. A., Jaskiewicz, K., Marasas, W. F. O., Thiel, P. G., Horak, R. M., Vleggaar, R., Kriek, N. P. J.: *Appl. Environ. Microbiol.*, **54**, 1806 (1988).
- 6) Gelderblom, W. C. A., Kriek, N. P. J., Marasas, W. F. O., Thiel, P. L.: *Carcinogenesis*, **12**, 1247 (1991).
- 7) Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., Shephard, G. S., Gelderblom, W. C. A., Nieuwenhuis, J. J.: *Appl. Environ. Microbiol.*, **57**, 1089 (1991).
- 8) Marasas, W. F. O., Kellerman, T. S., Gelderblom, W. C. A., Coetzer, J. A. W., Thiel, P. G., van der Lugt, J. J.: *Onderstepoort J. Vet. Res.*, **55**, 197 (1988).
- 9) Harrison, L. R., Colvin, B. M., Greene, J. T., Newman, L. E., Cole, J. R.: *J. Vet. Diagn. Invest.*, **2**, 217 (1990).
- 10) Voss, K. A., Plattner, R. D., Bacon, C. W.: *Food Chem. Toxicol.*, **27**, 89 (1989).
- 11) Sydenham, E. W., Thiel, P. G., Marasas, W. F. O., Shephard, G. S., Van Schalkwyk, D. J., Koch, K. R.: *J. Agric. Food Chem.*, **38**, 1900 (1990).
- 12) Sydenham, E. W., Gelderblom, W. C. A., Thiel, P. G., Marasas, W. F. O.: *J. Agric. Food Chem.*, **38**, 285 (1990).
- 13) Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S., Van Schalkwyk, D. J.: *Phytopathology*, **82**, 353 (1992).
- 14) Norred, W. P.: *J. Toxicol. Environ. Health*, **38**, 309 (1993).
- 15) Marasas, W. F. O., Van Rensburg, S. J., Mirocha, C. J.: *J. Agric. Food Chem.*, **27**, 1108 (1979).
- 16) Marasas, W. F. O., Wehner, F. C., Van Rensburg, S. J., and Van Schalkwyk, D. J.: *Phytopathology*, **71**, 792 (1981).
- 17) Yang, C. S.: *Cancer Res.*, **40**, 2633 (1980).
- 18) Cheng, S. J., Jiang, Y. Z., Li, M. H., Lo, H. Z.: *Carcinogenesis*, **6**, 903 (1985).
- 19) Luo, Y., Yoshizawa, T., Katayama, T.: *Appl. Environ. Microbiol.*, **56**, 3723 (1990).
- 20) Chu, F. S., Li, G. Y.: *Appl. Environ. Microbiol.*, **60**, 847 (1994).
- 21) Yoshizawa, T., Yamashita, A., Luo, Y.: *Appl. Environ. Microbiol.*, **60**, 1626 (1994).
- 22) Zhen, S. F., Wei, H. J., Luo, X. M., Hu, G. G., Shang, A. L.: *J. China Tumor*, **4**, 174 (1982) (in Chinese).
- 23) Chu, C. H., Jian, L. W., Xiao, Q. L., Yun, S. L.: *Cancer Detection and Prevention*, **13**, 79 (1988).
- 24) Fukuda, S., Nagahara, A., Kikuchi, M., Kumagai, S.: *Biosci. Biotech. Biochem.*, **58**, 765 (1994).
- 25) Chu, F. S., Ueno, I.: *Appl. Environ. Microbiol.*, **33**, 1125 (1977).