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# Mycotoxins in Stored Barley (*Hordeum vulgare*) in Tibet Autonomous Region (People's Republic of China)





*Mycotoxins are naturally occurring toxic chemical compounds produced by fungi infesting agricultural crops both during crop growth and storage. Such secondary metabolites, when ingested, can produce toxic syndromes in humans. This study is the first survey that documents the occurrence of mycotoxins in stored barley in Tibet Autonomous Region [P.R. China]. Twenty-five samples of barley collected from Tibet were analyzed for the presence of aflatoxins, fumonisins, ochratoxins, zearalenone, deoxynivalenol, and T-2 toxin using an easy, sensitive, competitive direct enzyme-linked immunosorbent assay. Ninety-six percent of the samples were contaminated with zearalenone at concentrations ranging from 25 to 270 µg/kg. Seventy-six percent of the samples were contaminated with T-2 toxin at concentrations ranging from 1 to 163 µg/kg. In contrast, deoxynivalenol was observed in only 12% of the samples, with toxin concentrations ranging from 25 to 270 µg/kg. Aflatoxin was observed in only 4% of the contaminated samples.*

***Keywords:*** *Mycotoxins; stored barley; Tibet Autonomous Region; Kashin–Beck disease.*

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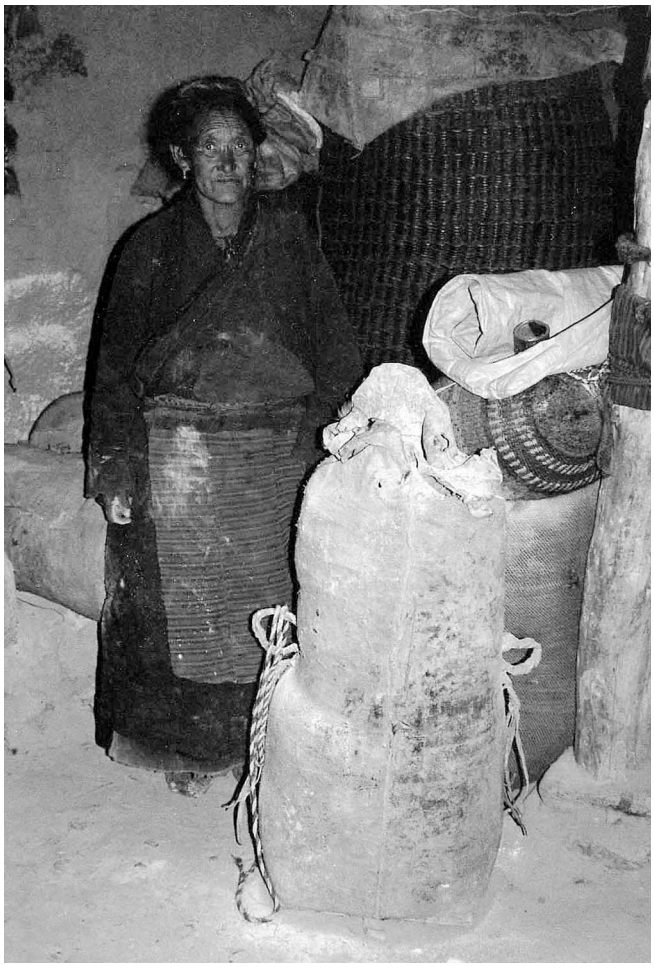

**FIGURE 1** Yak skin bags for storage of *tsampa* (roast barley flour) in Tibet Autonomous Region, P. R. China. (Photo courtesy of Eric Haubruge)

## Introduction

In Tibet Autonomous Region, both high altitude and extremely severe climatic conditions affect the availability of natural resources and land use (Haubruge et al 2000). Nevertheless, some Tibetan barley species and cultivars are tolerant of these severe environmental conditions and have constituted the staple diet of farmers for more than 4000 years BC, as revealed by archaeological research in the Sera temple near Lhasa (Vavilov 1926; Daniggelis 1995). Barley grains stored in 97% of Tibetan homes are usually roasted and ground into *tsampa,* which is eaten with butter tea, and are also used to make *tchang,* the traditional local alcohol (Haubruge et al 2000). The barley crop is grown in the short, humid summer season from June to August and is harvested early in August or later in September when the differences between the daily minimum and maximum temperatures are high. Because of their high moisture content ranging from 15.7% to 17.5%, the grains are particularly susceptible to mold (Chasseur et al 1996). After a field-drying period, ears are beaten with flails, and every year, naked grains are poorly stored in the same baskets made of yak skin or bags made of yak hair (Figure 1). Under these climatic conditions, the contamination of barley grains with fungi that are capable of producing mycotoxins is very important (Chasseur et al 1997). Damage from fungal plant pathogens often affects the quality of Tibetan grains, in which saprophytic fungi (*Alternaria* sp, *Cladosporium* sp) and *Drechslera* sp with parasitic properties cause grain discoloration and black point on kernels (Chelkowski 1989).

The objective of this work was to monitor the quality of stored barley by evaluating the presence of mycotoxins in several samples of barley grains collected in Tibet. We used a competitive direct enzyme-linked immunosorbent assay (CD-ELISA), which is a practical

**TABLE 1** Mycotoxins detected in 25 samples of barley from farms in Shigatze Prefecture of Tibet Autonomous Region.

| Mycotoxins | Positive samples (%) | Dose (mg/kg) | |
|---|---|---|---|
| | | Range[a] | Mean |
| **Aflatoxins** | 4 | — | 0.04 |
| **Fumonisins** | 56 | 100–1100 | 450 |
| **Ochratoxins** | 52 | 1–46 | 14 |
| **Deoxynivalenol** | 12 | 100–200 | 161 |
| **Zearalenone** | 96 | 25–270 | 161 |
| **T-2 toxin** | 76 | 1–163 | 39 |

[a]In positive samples.

and sensitive method to quantify the presence of mycotoxins in cereals (Scott 1995; Tanaka et al 1995; Barnavetro et al 1997). Barley samples were collected soon after the harvest because preharvest contamination is of main interest for agricultural practices.

## Materials and methods

### Samples

Barley samples were collected in October 1997 within 1–4 weeks after harvest. The samples were randomly collected from 25 farms located in 2 villages (Rinpung and Lundrupste) of Shigatze Prefecture of Tibet Autonomous Region. The moisture content in the grain samples was determined directly with an electronic moisture meter (SAMAP®, Cereal Tester, Belgium). The samples were refrigerated upon arrival in the laboratory and kept at -20˚C until analysis.

### Competitive direct enzyme-linked immunosorbent assay

For detection of aflatoxins, fumonisins, ochratoxins, deoxynivalenol, zearalenone, and T-2 toxin, CD-ELISA test kits (Veratox®, Neogen Corporation, Lansing, MI, USA) were used in accordance with the manufacturer's instructions.

Mycotoxins were extracted by shaking 10 g of grains with 5 volumes (wt/vol, ie, 150 ml) of a 70:30 (vol/vol) solution of methanol and water for 2 minutes. The extract was filtered through a Whatman no. 1 filter paper. A 100-mL portion of the filtered sample was diluted (1:80) with a 10% aqueous methanol solution. Mycotoxin standards (Veratox®, Neogen Corporation, Lansing, MI, USA) or diluted sample extracts (100 mL) were added to mixing wells containing 100 mL of mycotoxin (fumonisin B1, aflatoxin, T-2 toxin, zearalenone, ochratoxin A, or deoxynivalenol)–horseradish peroxidase conjugate. The sample and conjugate solutions were mixed with a multichannel pipettor, and 100 mL of this mixture was transferred to antibody-coated wells and incubated for 15 minutes at room temperature. Reagents were washed from the antibody wells with distilled water. Then 100 mL of an enzyme substrate was added and incubated for 15 minutes. The development of color was stopped by the addition of a stopping reagent. Mycotoxin concentrations were assessed by recording optical density readings at 650 nm using a Bio-Tek EL301 microwell strip reader.

## Results and discussion

The moisture content of all samples ranged from 9.2% to 15.2%. The frequencies and levels of mycotoxins in the 25 barley samples are summarized in Table 1.

Zearalenone was the most dominant mycotoxin, occurring in 96% of the samples; the mean and maximum contents were 161 and 270 mg/kg, respectively. T-2 toxin, fumonisins, and ochratoxins occurred in 76%, 56%, and 52% of the barley samples, respectively. Analyses of barley grains by CD-ELISA showed that only 12% of the samples were positive for deoxynivalenol with levels ranging from 100 to 200 mg/kg. Aflatoxins were detected in less than 4% of the barley samples at a low level (<0.05 mg/kg).

Although surveys of barley grains collected from European countries revealed that deoxynivalenol was the major toxin (Gareis et al 1989; Müller and Schwadorf 1993), the dominance of zearalenone was observed in grains collected in Tibet. Among barley samples collected in the Netherlands, 83% and 100% were contaminated with deoxynivalenol and zearalenone, respectively (Tanaka et al 1990).

In Finnish cereal samples collected after a cool and very rainy growing season in 1998, deoxynivalenol was detected in 71% of the samples in the concentration range 5–111 µg/kg (Eskola et al 2001). Nivalenol and HT-2 toxin were detected, respectively, in 3 and 2 samples in the concentration range 10–20 µg/kg.

In China, mycotoxins in cereals were associated with gastrointestinal disorders in 1991. Deoxynivalenol was detected by gas chromatography–mass spectroscopy and by high-performance liquid chromatography in all samples as a major trichothecene (16–51,450 mg/kg); zearalenone was also found in all corn and barley samples (46–3079 mg/kg) (Li et al 1999). In 1993, a survey on the occurrence of mycotoxins in Korean barley was conducted; deoxynivalenol, nivalenol, and zearalenone were the major contaminants, with mean levels of 170, 1001, and 287 mg/kg, respectively (Kim et al 1993). In Argentina, zearalenone has been reported in corn at levels ranging from 30 to 750 mg/kg (Chulze et al 1989). About 25% of European wheat is found to be contaminated with zearalenone at concentrations ranging from 1 to 2000 mg/kg (Gareis et al 1989).

It is interesting to note that in barley of Tibetan origin, T-2 toxin and zearalenone occur at low frequencies. In Norway, Sundheim et al (1988) also detected a lower incidence of T-2 toxin in barley compared with

deoxynivalenol and zearalenone. Hietaniemi and Kumpulainen (1991) analyzed 45 samples of Finnish barley meant for food and feed use, and determined the incidence of deoxynivalenol, T-2 toxin, and zearalenone to be 93%, 4%, and 4%, respectively. Our survey demonstrated that the Tibetan barley contained T-2 toxin at levels ranging from 1 to 163 mg/kg. In 1992 and 1993, samples of diets and feeds that included barley grain were examined in Poland mycotoxicologically; T-2 toxin occurred at 100–3750 mg/kg (Bocarov-Stancic et al 1995).

Mycotoxins such as aflatoxins, ochratoxin A, zearalenone, and deoxynivalenol have been the subject of guidelines in various countries. Although approximately 60 countries in the world at present have legal limits for mycotoxins in food, these limits vary widely (Table 2).

Although the sample size was small, there is unequivocal evidence of global contamination of barley grains with mycotoxins. The present study indicates that barley grain-based foodstuffs for human consumption in Tibet Autonomous Region are contaminated principally with trichothecenes and zearalenone. Mycotoxins are a large group of naturally occurring fungal secondary metabolites with very diverse toxic effects in humans and animals. Although the "mycotoxin problem" is old and intoxication from moldy foods and feeds in humans and animals has been known for centuries, the discovery of aflatoxin as one of the most potent carcinogens in the early 1960s has generated considerable research in this area (Goldbatt 1969). In Tibet Autonomous Region, an endemic osteoarthritic disease, the Kashin–Beck disease (KBD), has been suggested to be associated with exposure to mycotoxins (Chasseur et al 1997; Haubruge et al 2000). The theory of the involvement of mycotoxin in KBD etiology held sway in the Soviet Union at the time of Nesterov's (1964) review, in which the view was proposed that moldy grains act selectively on the joints. In laboratory rats fed with grain infected with *Fusarium sporotrichiella* from KBD-endemic areas, Nesterov (1964) observed an atrophy of the growth plate and osteopenic skeletons but not the characteristic articular lesions of KBD. Therefore, the "mycotoxins hypothesis" was not considered the cause of the disease. However, since 1982, the possibility that fungi are an etiological factor has claimed attention again. Indeed, KBD has been observed in areas of China where cereals infected with *Alternaria* sp and *Fusarium* sp have been observed frequently (Yang et al 1983). More recently, in Shaanxi and Shanxi prefectures, Luo et al (1992) have shown that *Fusarium* sp producing known trichothecenes are involved in the fungal contamination of staple foods in KBD endemic areas where 80–100% of cereal samples (maize and wheat) were found to be contaminated with nivalenol and deoxynivalenol, respectively. To clarify the association of mycotoxins with the prevalence of KBD, further studies need to be conducted on the incidence and levels of mycotoxins in barley samples in Tibet, where high-risk KBD area is absent.

**TABLE 2** Maximum tolerance levels of mycotoxins in cereals and derived cereal products used for direct human consumption. (Source: Anonymous 1997)

| Countries | Mycotoxins | Tolerance (mg/kg) |
|---|---|---|
| **Australia** | Aflatoxins | 5 |
| **Austria** | Zearalenone | 60 |
| | Deoxynivalenol | 500 |
| **Brazil** | Ochratoxin A | 50 |
| **Canada** | Deoxynivalenol | 2000 |
| **Finland** | Aflatoxins | 10 |
| **The Netherlands** | Aflatoxins | 5 |
| | Ochratoxin A | 5 |
| | Deoxynivalenol | 500 |
| **Russia** | Deoxynivalenol | 1000 |
| | Zearalenone | 1000 |
| | T-2 toxin | 100 |
| **United Kingdom** | Aflatoxins | 10 |
| **United States** | Aflatoxins | 20 |
| | Deoxynivalenol | 1000 |


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