5.1 The fungal hypotheses

Camille Chasseur, Georges Lognay, Carl Suetens, Sheero Rapten, Philippe Gillet, Pascal Kanyandekwe, Lixing He, Pelma Drolkar, Rinzen Wangla, Françoise Begaux, Eric Haubruge, Lobsang Rinchen, Lakpa Wangdu, William Claus, Françoise Mathieu

5.1.1. Moulds and KBD

The possible role of mycotoxins in KBD was already suggested by Russian researchers. Cereal grain contamination by *Fusarium sporotrichiella* Bilay in endemic areas was mentioned by Nesterov in 1964. In China, microbiological examinations indicated that wheat crops in KBD areas were contaminated by *Alternaria* sp. (Bai et al., 1990) and *Fusarium* spp. (Luo et al., 1992), and particularly *Fusarium oxysporum* Schlecht ex Fr. and *Fusarium monoliforme* Sheldon (syn: *Fusarium verticillioides* (Sacc.) Nirenh.) were also isolated from corn (Peng et al., 1992). Moreover, it was mentioned that other fungi should be considered. Mycotoxin contamination of corn and wheat grains was also investigated in KBD areas. For instance, cereal samples in high incidence areas of KBD were reported to be more heavily contaminated with trichotecenes (T2-toxins) as compared to those in low-incidence areas. Although *Fusarium* species are known to produce trichotecenes, these toxins are also formed by other cereal fungi species like *Trichothecium roseum* (Pers) Link (Ishii et al., 1986; Girisham et al., 1985), *Stachybotrys chartarum* (Ehrenb.) Hughes and *Myrothecium roridum* Tode ex Stendel (Girisham et al., 1985). For 20 years, *Fusarium* has been the main etiological mould studied by researchers. Lee et al. (1985) and Haynes et al. (1986) noted that chicks fed with TDP-1, a toxic component extract from *Fusarium roseum* Link, presented moderate to severe gross lesions of tibial dyschondroplasia. Results led Lee to conclude that the major toxic principle in *F. graminearum* Schwabe was a water extractable compound and was neither a trichothecene nor zearalenone. Krogh et al. (1989) and Wu et al. (1993) showed that this pathology was also induced in experimental conditions in chickens by fusarochromanone, a metabolite produced by *Fusarium equiseti* (Corda) Sacc. For Yang Jianbo (1997) who carried out his researches in the North of China, *Fusarium* is the main etiological factor of the disease, whereas in our studies led in endemic areas in T.A.R. on barley or in Gansu on wheat, this mould was seldom observed.

In 1995, our first mycological studies were started within the framework of a project supported by MSF-Belgium. The first fungal analyses were made on barley samplings at the beginning of storage and on barley flour or tsampa. During the MSF period, 2 main studies were carried out. A first detailed environmental and clinical study, concerning 40 families from endemic villages, in the north of Brahmaputra and from villages controls in non endemic area in the south of Brahmaputra (Rimpung) (Figure 5.1). Carried out in parallel, a second study was made with sampling in 575 families also selected in endemic area for a clinical study. After 2001, the projects supported by KBD Foundation were firstly focused on the reproduction of results during several successive years. Twenty villages were added to the 40 already studied, and the non endemic zone of Rimpung was extended to Gyantse and Nakartse (Figure 5.1). Second, the laboratory of Lhasa continued to analyse samples coming from the new clinical study (851 selected families). Specific and limited studies were also added, such as controls of the effectiveness of the disinfection of seeds in counties, and experiments on the effectiveness of keeping in bundles on fields or the impact of threshing machines. Thirdly, we began a comparative study in other endemic areas in China to compare the T.A.R. results with those obtained in very different environments. Surveys were still conducted in Gansu (2004), Heilongjang (2005) and Inner Mongolia (2006) (Figure 5.2). All these environmental studies were carried out with always the same objective: to find a relation between a specific fungal contamination and the KBD. Moreover, the first results also allowed to take already several actions, mainly concerning the storage conditions and disinfection of seeds.

5.1.2. Cereal samplings and storage

To sample cereal grains in sacks or containers in the storeroom, a grain trier is recommended. It is a probe with a long double tube normally made from brass. Both tubes have matching openings spaced along the tubes which permits to sample grain at different levels in bags or containers. It is important because fungal contamination may be different according to the depth (Plate 5.1).

In every storeroom, sample units taken in different sacks are gathered to constitute one homogeneous and representative sample. Until 2000, samples were brought in the laboratory in cool boxes, but later, osmofilm bags were used to carry grain to the laboratory. These bags were sealed immediately on site and were placed in a well ventilated (with bored holes) and dark box (not to pile up nor to compress the bags to facilitate a rapid drying until 12%). At the laboratory, samples are blended in a handle blender (15 to 20 seconds) before storage in a freezer (-20°C). Deep freezing is the best storage condition and also protects from insects.

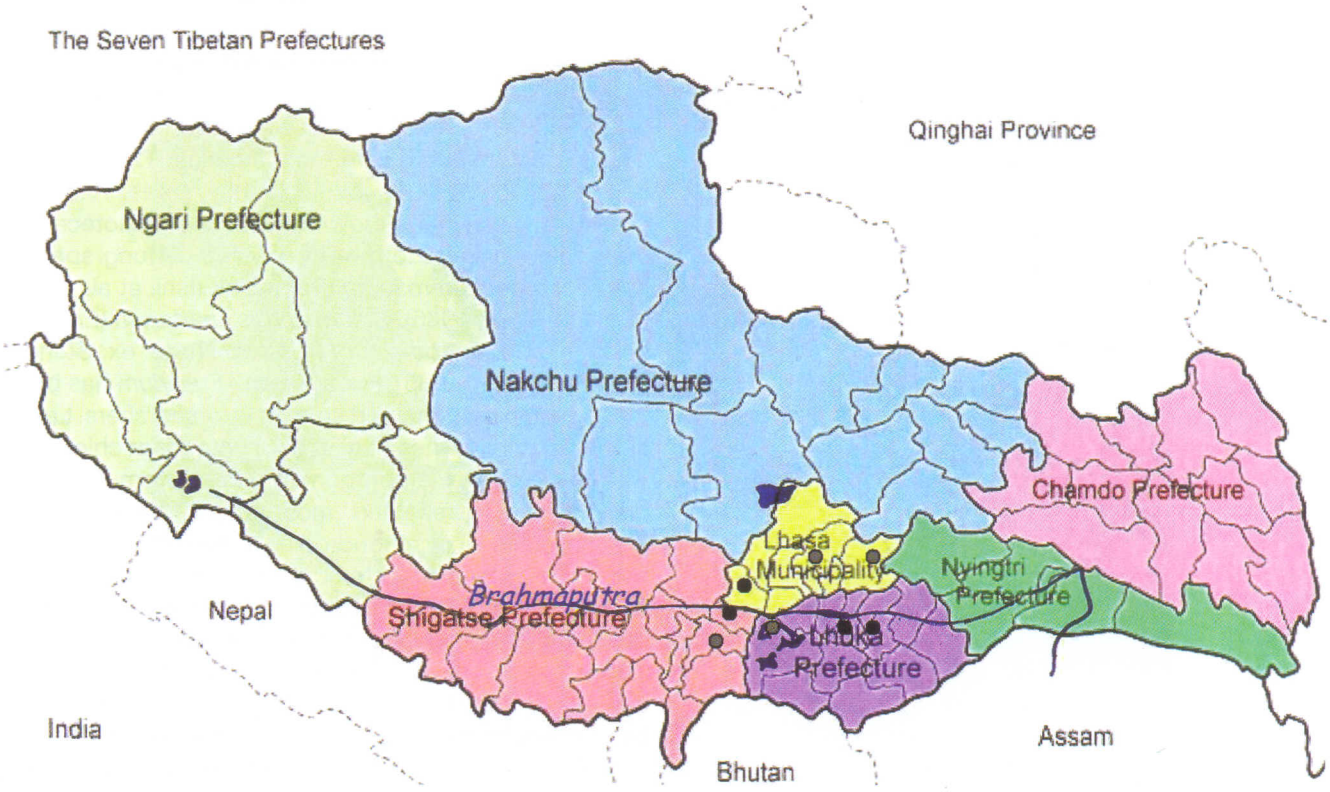


Figure 5.1. Tibetan Autonomous Region area with prefectures, counties and the Brahmaputra. A first mycological study was carried out in 2002 in 4 counties (black dots). A second study from 2004 to 2006, with 20 additional villages in 4 new counties (grey dots) (From Mapinfo geographic – version 4.1, modified).

Figure 5.2. China with T.A.R. (red) and other provinces of China where KBD missions were made from 2004 to 2006 (respectively Gansu in brown; Heilongjiang in blue; Inner Mongolia in green).

5.1.3. Fungal analyses: methods

Classical methods

Among the existing classical methods using nutritive agar media, two are the most used and are complementary: the direct plating method and the dilutions plating method.

To determine the percentage of contaminated grains, ***the direct plating method*** was used (Pitt et al., 1992). After a disinfection with a chlorine solution diluted 10 times, during 2 minutes, followed by 2 rinsings with sterile water, grains of each sample were planted in the agar medium with chloramphenicol: 50 grains in a malt agar, to isolate mesophilic species, and 50 grains in a MY50S, to isolate strongly xerophilic species. Plates were incubated at 25°C and enumeration and identification were made after 5-7 days. This method presents 2 advantages. First, disinfection and rinsing operations eliminate the moulds present in the sample dust which only preserves the moulds in cuticular or internal grain. Second, using living grain in culture allows to isolate and to identify semi parasitic moulds which are often difficult to grow and sporulate on synthetic media. But if this method permits to distinguish a contaminated grain from a not contaminated one, it is not appropriate to evaluate the contamination intensity in a grain and thus, in the sample (Plate 5.2).

The dilutions plating method is better adapted to flours (Pitt et al., 1992). If necessary, the sample is milled to a fine meal before being suspended in a physiological solution with tween. This primary suspension is then successively diluted 10 times, and plated on selected agar media.

After incubation at 25°C, first lectures and counting are made after 5-7 days, followed by a second examination after 15 days later. This method is more appropriate for a quantitative approach. Dilutions permit to analyse highly contaminated samples, but in favouring species with high sporulation. The choice of specific media is important to select some specific taxa (Malachite green Agar for *Fusarium*, for instance) or a group of species (mesophilic species growing on fields, xerophilic species growing in storage)

Chemical analyses

Generally, the classical microbiological methods are very useful to detect specific moulds and to evaluate a potential risk for the consumers. But the disadvantage of these methods is to evaluate the revivifiable germs without taking into account the dead germs, or germs which are not able to grow on the selected synthetic agar medium. Moreover, the mycotoxin production by a specific mould may vary according to a lot of parameters of cultures. So, the presence of a specific mould in a sample does not systematically mean the occurence

Plate 5.2. The fungal analyses: laboratory of mycology in Lhasa (CDC). A. laboratory of mycology installed in the CDC building in Lhasa in 1998, where specific trainings in mycology are regularly given – B. The team analyses every year several hundreds of grain samplings – C. Grains are analysed by direct plating method – D. Disinfected cereal grains are planted on agar medium in Petri dishes in order to force fungal contaminant inside the grain to go out and to grow in the synthetic media. Contaminated grains are counted, and interesting moulds are isolated on specific media. Most interesting moulds are identified, and stored after purification – Ea,b & c. *Alternaria alternata* (Fr.) Keissl. (Ea. stereomicroscopy 40x – Eb & c. microscopy 1000x) – Fa,b,c & d. *Alternaria tenuissima* (Kunze ex Pers) Wiltz isolated on barley grains in endemic area in T.A.R., but not or in low quantities in non-endemic area. (Fa. stereomicroscopy 80x – Fb & c. microscopy 400x – Fd. microscopy 1000x).

of its toxins, therefore when toxicological evaluation of grains is of concern, the quantification of mycotoxins is requested.

In this context, the objective of a «chemical» integrated and complementary project was to determine ergosterol as a fungal biomarker and to search some major toxic metabolites (mainly *Alternaria* mycotoxins) in barley from KBD non endemic (NEA) and endemic areas (EA). The study was also designed to compare grains collected in storeroom from KBD affected and non affected families in T.A.R.

Ergosterol is the predominant and specific fungal sterol that plays an essential role as a constituent of cell membrane. Its determination is a useful measure of mould contamination because it occurs in all fungi, it is not a natural metabolite of cereal grains and the molecule can be routinely analyzed by chromatographic methods such as high performance liquid chromatography (HPLC). In general, ergosterol analysis has been widely used as a screening test in fungal ecology to estimate mould biomass. Moreover, the quantitative determination of this molecule can support microbiological methods like the measurement of infection rate (percentage of seeds that yield fungal biomass after surface disinfection) which gives qualitative information. Thus specific chemical analyses were found useful and complementary within the KBD Foundation research programme. For that reason a performant analytical protocol was established and validated for ergosterol analysis. The method involved three complementary steps: the alkaline saponification of the sample to liberate ergosterol, the extraction of the unsaponifiable matter (lipidic fraction containing the molecule of interest) with a suitable solvent (i.e n-hexane) and finally the HPLC determination. Careful examination of the analytical protocol led to the conclusion that the developed method was performant and that reliable results could be recorded. Fourteen barley samples from NEA (taken as «references»), 21 from unaffected families and 25 from KBD affected families in EA were submitted to microbiological investigations (among which total moulds and *Alternaria* contamination expressed in CFU/g[4]) and to ergosterol analysis.

Beside ergosterol measurements, the determination of selected *Alternaria* sp. mycotoxins was carried out in order to compare grains from endemic and non endemic areas. Indeed microbiological assays demonstrated that *Alternaria* Nees ex Fr. was one of the three most common fungi prevalent in KBD endemic area from T.A.R. (Chasseur et al., 2001). The following programmed mycological surveys, undertaken after 2001, also confirmed a higher barley grain contamination with *Alternaria* in endemic KBD. It was therefore considered interesting to search some of the most common mycotoxins naturally produced by this genus and belonging to different structural chemical families:

a) the dibenzopyrone derivatives with altenuene, alternariol, alternariol monomethyl ether and
b) the perylene group with altertoxin I.

The validated HPLC method used for the study, adapted from Feng-qin and Takumi (2000) revealed similar analytical good performances. Raw mycotoxin extracts were also submitted to thin layer chromatographic determinations.

For the mycotoxin evaluation, 28 barley samples from different villages throughout EA and NEA were collected and carried to the laboratory in osmofilm bags. The grains were carefully sampled (Plate 5.1) during the harvesting period in 2004.

5.1.4. Fungal contamination of stored cereal

From May 15 to June 7, 1995, a preliminary study (Chasseur et al., 1996) was conducted on storage conditions in 296 families in 12 villages of Lhasa prefecture. In October 1995, the same villages were visited again during the harvest period. Structured observations included aspects of the house, ventilation and type of the storage place, type of containers used to store the grains. On the spot measurements included humidity of grains, of flours and of the storage room walls. This survey showed that barley grains or flour were stored in 97% of the families. Mean barley humidity was 17.5% (± 3.3) in October for recently harvested grains, and 12.7% (± 0.8), 6 months after storage. In May, barley humidity was more correlated with storage parameters than with the presence of KBD in the family, as reported in some regions of China. On the other hand, in October, statistical analyses suggested that barley humidity was significantly higher in affected than in unaffected families.

Preliminary mycological results showed an important barley grain and flour (tsampa) contamination. Fifty percent of grain samples (n = 21) presented more than 80% of contaminated grains on MEA medium and 17% on DG18 medium. Examined flours (n = 11) revealed 2,000 to 72,000 CFU/g on PDA medium and 2,000 to 32,000 on M40Y medium. In conclusion, storage conditions of home-produced grain and flour products in T.A.R. were far from optimal. So, on the basis of these results, we suggested that in T.A.R., there were at least 2 crucial periods for microbiological barley contamination related to KBD: first, when barley is kept in bundles on the field before the storage, and second, just after harvest during the beginning of the storage.

[4] Colony forming unit per gram of barley flour

The results of another mycological study were published in 1997 (Chasseur et al., 1997). This study concerned analysis of post-harvest grain samples obtained from 60 dwellings with 54 affected and 76 unaffected children aged of 5 to 15. Three elements were revealed: first, mesophilic fungal contaminations were significantly higher on barley grains stored in families with KBD (median = 66% of infected grains) than in healthy families (median = 43% of infected grains) (Kruskal-Wallis $P < 0,01$). Second, three common fungal taxa in grains were significantly associated with KBD: *Trichothecium roseum* (OR = 16.37, $P < 0.001$), *Drechslera* Ito (OR = 8.75, $P < 0.001$) and *Alternaria* (OR = 2.96, $P < 0.001$).

Third, a cumulative effect of different fungal associations was observed, as presented on the figure 5.3. «For instance, *Alternaria* alone presented an OR of 2.96 whereas the OR for the association of *Cladosporium-Alternaria* was 7.85, and for the association of *Cladosporium-Alternaria-Trichothecium* was 96.86. *Cladosporium-Drechslera* (OR = 26.58) and *Cladosporium-Alternaria-Drechslera* (OR = 83.43) were also highly correlated associations with illness. The presence of these four taxa in grains had an OR of 1027.72.»

These findings suggested that, in T.A.R., 4 crucial periods of fungal barley contamination could be related to KBD. First, during germination, by contaminated seeds (*Drechslera*). Second, on field, especially in summer, when some parasitic moulds (*Alternaria*, *Cladosporium*, *Drechslera*) may invade grains on ears. Third, just after harvest when barley is kept in bundles on the field before storage which should also require more attention in the future. And fourth, during the beginning of the storage, especially when the drying operation is not sufficient (*Trichothecium*, *Alternaria*, *Cladosporium*).

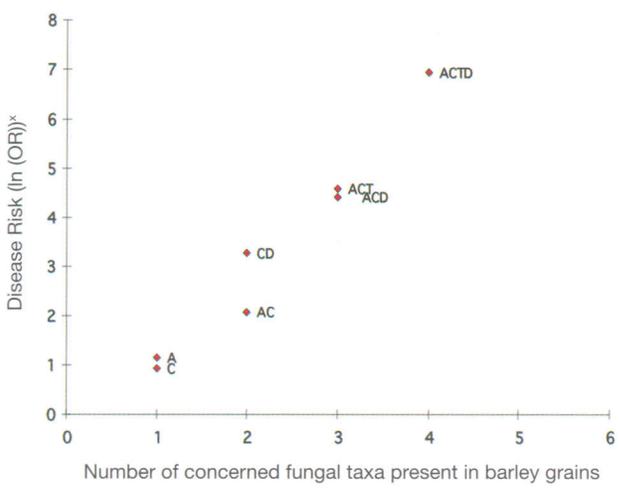


Figure 5.3. Evolution of KBD risk in Tibetan children as function of fungal taxa detected in barley grains (Chasseur et al., 1997) (C: *Cladosporium*; A: *Alternaria*; D: *Drechslera*; T: *Trichothecium*).

On the basis of these first environmental studies, several specific researches were also conducted during this period.

Barley grain sampled in KBD affected families and in non affected families were analysed in thin layer chromatography by the FUSAGx and these results were also presented in 2001 (Haubruge et al., 2001). A still unknown metabolite of *Alternaria* sp. was found, especially common on the barley grains of KBD affected families.

From the beginning of our studies, we also observed grains of different colours in our samples. Apart from the described barley varieties, we defined 6 groups easily identifiable in laboratory when planting in Petri dishes: blue, brown, black, and green grains, unclassifiable and ill-formed. Direct plating method allowed to determine the percentage of contamination in grains. Samples were collected in KBD endemic area of prefectures of Lhasa, Lhoca, and Shigatse, in both KBD affected and non affected families, and compared with those sampled in a control valley without KBD cases.

Results showed a positive correlation between *Alternaria* and *Trichothecium*, more often found on brown grains. This observation is all the more interesting as the brown grains are the most abundant in endemic area and rather weakly represented in control area. Moreover, we also noted a negative correlation, highly significant, with the blue grains which are more abundant in samples coming from control area. Contrary to our expectations, the black grains were less contaminated by *Trichothecium roseum*. Nevertheless, if the black grains were proportionally well represented in families with KBD, quantities were weaker than brown grains. So, these observations showed that barley varieties may be one parameter improving the development of the specific fungal contaminants precedently correlated with KBD.

Studies after 2001 were firstly focused on the reproducibility of the mycological results obtained on both sides of Brahmaputra. The significant difference ($p < 0.01$) between the two areas was confirmed during 3 years (2004-2006), and we noticed a difference ($p < 0.05$) between affected (92%) and healthy families (79%) for total mould contamination, but only one year out of three, in 2006. This last result recalls that the impact of fungal contamination on health could be different according to the years and that mycological studies should sometimes be conducted on a period of several years. More specifically, this study also confirmed a higher barley grain contamination with *Alternaria* in endemic KBD, although not in the same degree in all counties nor throughout the years.

As stated above, in 2005 (samples from 2004 harvesting period, ground grain) chemical investigations were undertaken in parallel with microbiological observations.

Plate 5.3. KBD action: educational programme. A. A general educational programme was established to inform the population of the risks of eating mouldy grains – B. Poster explaining the cycle of food chain with the different crucial levels: seed dressing in the county or in the village, the importance of preparing the soil, with organic manure and fertilizers before tillage, the precautions to take when preparing the biocides and spraying on field, the importance of drying grain in the sun before storage and how to improve the storeroom.

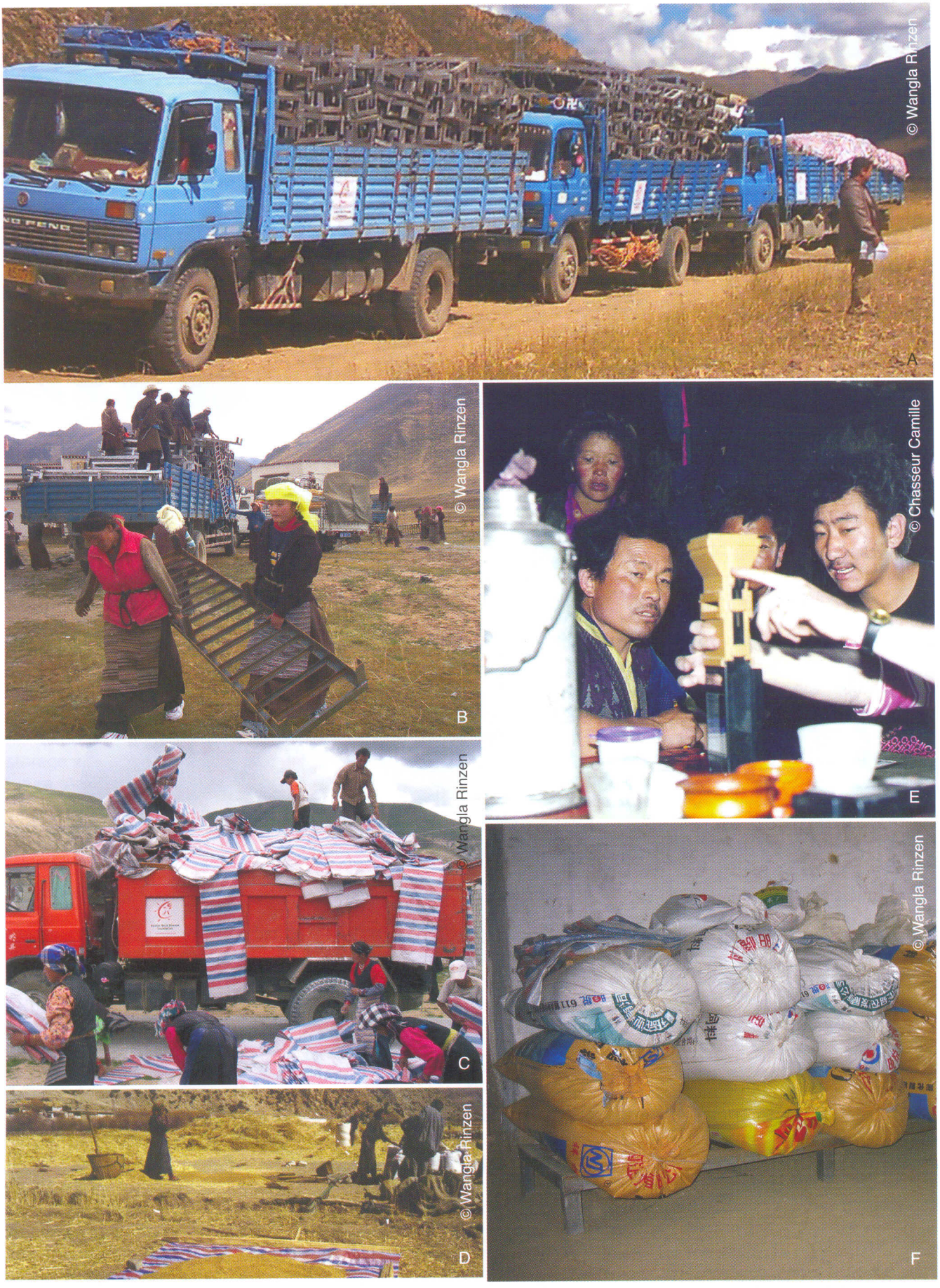


Plate 5.4. KBD action: improvement of the storage conditions. A, B & F. Metal racks to avoid humidity from soil in the bottom of the bags — C & D. Plastic covers were distributed to facilitate the grain drying in the sun – E. Distribution of one Samap device per village to measure grain humidity allows to control grain humidity before storage.

Plate 5.5. Threshing machines distribution. Threshing machines are an important help for peasants. Forty one machines were distributed by MSF in the prefectures of Lhasa, Lhoca and Shigatse between 2000 and 2002.

The results gathered from the study led to the following observations:

• Although significantly different ($p < 0.05$) the ergosterol concentrations in barleys from NEA (1.2 ± 1.1 µg/g) and EA (2.8 ± 2.2 µg/g) did not indicate acute mould proliferation. These results were in line with the mycological counting approach (341 ± 408 CFU/g and 1253 ± 1874 CFU/g in NEA and EA respectively) revealing also low to very low contamination levels. The additional ergosterol measurements performed on 482 samples (wheat and barley) from T.A.R. and Ganzu confirmed our observations (95.8% of them showed ergosterol level (< 5 µg/g). While taking as reference the indicative values proposed for cereal storage in western countries (http://www.fao.org/Wairdocs/X5160F/X5160f01.htm) doubtful quality of grains could be suspected for ergosterol concentrations > 10 µg/g !

• No difference ($P > 0.05$) could be made between KBD affected (2.8 ± 2.4 µg/g) and non affected (2.8 ± 2.0 µg/g) families in EA. This observation was also supported by CFU counting ($p > 0.05$).

• Among the 28 tested barley samples, none of them revealed detectable amounts of the four searched mycotoxins (p. 90) (the limit of detection was estimated at 30 µg/kg). Even so, it was demonstrated that strains of *Alternaria* isolated from Tibetan barley seeds collected in 2004 and cultured in the laboratory on non infected grains in high relative humidity were able to biosynthetize the four mycotoxins of interest but in different proportions. This observation can be considered as a proof of their ability to synthetize mycotoxins but the aforementioned results regarding mycotoxin analysis indicate that climatic, harvesting and storage conditions did not favour significant production of these toxic metabolites.

• Complementary T-2 toxin analyses were also all below to the limit of quantification (25 µg/kg) and until today, the investigations have not yet allowed the detection of the secondary metabolite reported previously by Haubruge et al. (2001).

Taking these observations into account, the results of the chemical study did not lead to a clear difference between barley from NEA and EA. Nevertheless it cannot be denied that other non investigated mycotoxins could be present but not detected during the survey. It is also noteworthy that the integrated «chemical» project was limited to only one harvesting year and that climatic and storage conditions could influence the development of «toxic» mycoflora.

In 2004, we also extended the mycological investigations in other endemic areas in China: several villages were examined in Gansu (November 2004), in Heilongjang (November 2005), and in Inner Mongolia (November 2006) (Figure 5.2). It was interesting to compare the results obtained in other endemic areas presenting very different environmental conditions, different cultivated cereal and food habits. This diversity could explain the difficulties to approach the KBD etiology. In Gansu, we sampled corn and wheat in 3 villages in plain and barley in two villages in altitude on the border of Tibetan plateau. In Heilongjang, barley is replaced by wheat, corn and rice, while in Inner Mongolia wheat is the mainly consumed cereal, with rice (but which is imported). First results (not yet published) showed in Gansu rather similar results as those obtained in T.A.R., with a dominant presence of *Alternaria* in barley and wheat while *Fusarium* species were seldom observed. In the North, the situation was different. In Heilongjang province, *Alternaria* was well represented, but we also noticed high percentage of grain contaminated by *Fusarium* spp. In inner Mongolia, similar results were recorded for sampled corn.

5.1.5. Other specific mycological studies

Period duration of keeping barley in bundles on field and fungal contamination

In our preliminary studies, we suspected that keeping barley in bundles on field after harvest for drying grain could be a crucial point to fungal contamination, especially the duration period on field. On the basis of information obtained in 530 families (from Lhasa, Lhoca and Shigatse prefectures) during a 5 years period (1997 to 2001) linked to the results of fungal contamination of stored grain, we noticed that this drying operation is effective and necessary, and that 10 days minimum on field was the optimum duration to record a significative reduction of the total fungal contamination, but results differed according to the 3 prefectures.

Study of seed disinfection effectiveness

Among the punctual studies, one was conducted to assess the disinfection effectiveness of seeds performed mechanically in the counties (Plate 5.6). Grain were sampled before and after the treatment, in 5 villages (Sheulba, Lhundurpkang, Chakton, Narme, Patsik), and analyzed in the Lhasa laboratory. Results (not published) are expressed in the figure 5.4, which shows that disinfection is only effective in 2 villages out of 5.

More specifically, concerning the 3 villages for which disinfection was ineffective (Sheulba, Lhundurpkang, Chakton), some taxa as *Cladosporium*, *Penicillium* and yeasts had a better resistance to the fungicide. But these taxa were also present in samples taken in Narme and Patsik villages for which disinfection was effective. The inefficiency of the treatment should rather be sought on the procedure level. For instance, we noticed that the preparation of the fungicide solution was not always correct.

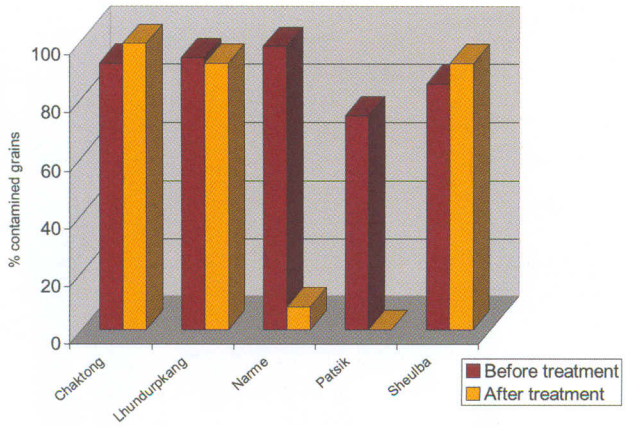


Figure 5.4. Total hygrophilic fungal contamination of seed before and after disinfection.

We also inquired in the villages far away from counties where the villagers are obliged to carry out this operation manually (Plate 5.7).

For instance, the villagers in Medrokonggar county received the biocide via their xiang leaders. But there were neither technicians nor explanations regarding the use of the biocide in terms of dosage and health. We noted that villagers mixed the biocide with water in cooking pots and other daily utensils. They disinfected the seeds on plastic sheets or cement concrete floor, and they did not wear gloves, nor masks; and they were not aware that the biocide was harmful. After disinfection process, people use normal soap to wash their hands but the color of the biocides remains for a few days on the hands. Some said the biocide smelled terrible and burnt eyes during the treatment. Regarding the harvest, the villagers considered the treatment according to the efficiency.

Threshing machines and fungal contamination

Threshing machines are an important help for peasants, and are gradually introduced by the Chinese farmers near built-up areas. We wanted to verify if using these machines would improve the fungal quality of grain and if the traditional drying of grains on field just after the harvest could be suppressed.

In 2001, two areas with barley were selected at Lume and Lundukang (Nyemo county, Lhasa prefecture). In these two areas, half of harvested barley was immediately threshed and stored, while half was maintained traditionally in bundles on field during 12 days for drying. We sampled grains for analyses just after the harvest, and at different moments of the drying in the storage room to follow the evolution of mould contamination. Evolution of fungal contamination was measured by direct plating method and by ergosterol measurements.

Results in the table 5.1 show that the total fungal contamination of grain was higher when there was no drying period on field. Results of ergosterol content in grain confirmed this conclusion. Ergosterol content never exceeded the critical threshold of 11 µg/g DM but a significant correlation between the fungal analyses and the chemical analyses was observed.

Threshing machines are an important help for peasants, but we concluded that concerning mould contaminations, the traditional drying period in bundles on field must be maintained.

Table 5.1. Comparison of humidity and fungal contamination between barley grains directly threshed after the harvest, and grains which were dried in bundles on field before being threshed.

Village of Lundukang - 2001	Dates	n°	Humidity %	Total fungal content* %	Ergosterol µg/g
				Directly threshed	
Storeroom	2001.08.31	1	25.0	66	4.0
	2001.09.12	2	14.0	96	5.3
	2001.09.24	3	14.0	98	6.7
	2001.10.05	4	14.0	100	5.6
			Dried in bundles on field before being threshed		
On field (12 days)	2001.08.31	1	25.0	66	4.0
Storeroom	2001.09.12	5	18.5	42	2.1
	2001.09.24	6	14.0	84	3.3
	2001.10.05	7	14.0	68	3.4

Plate 5.6. Seed dressing in the county. In the counties, the government proposes to treat seeds against moulds before planting. For families which are not too far from the county town, seeds must be brought to eliminate with mechanized sieves little stones and bad grains. This first operation, generally free, allows a selection of the best seeds. Families can also treat the seeds with chemicals. A pink dye was added to the treatment to avoid the consumption risks.

5.1.6. A sanitary programme

Following the previous results and research, we suggested measures to prevent fungal contamination at the crucial levels of the food chain, in proposing a sanitary programme. A large-scale curative action on field was started, including 44 villages in 3 prefectures (Lhasa, Lhoca and Shigatse prefectures).

To support these actions, in 1998, a laboratory of mycology (Plate 5.2) was installed in the CDC building of Lhasa. After a specific training in mycology, the team analysed every year several hundreds of grain samples. Training in mycology for the Tibetan staff, programs of validation with Ring-Test of fungal identifications, and checking of Lhasa laboratory fungal analyses in Belgium for validation of results are also included in this programme.

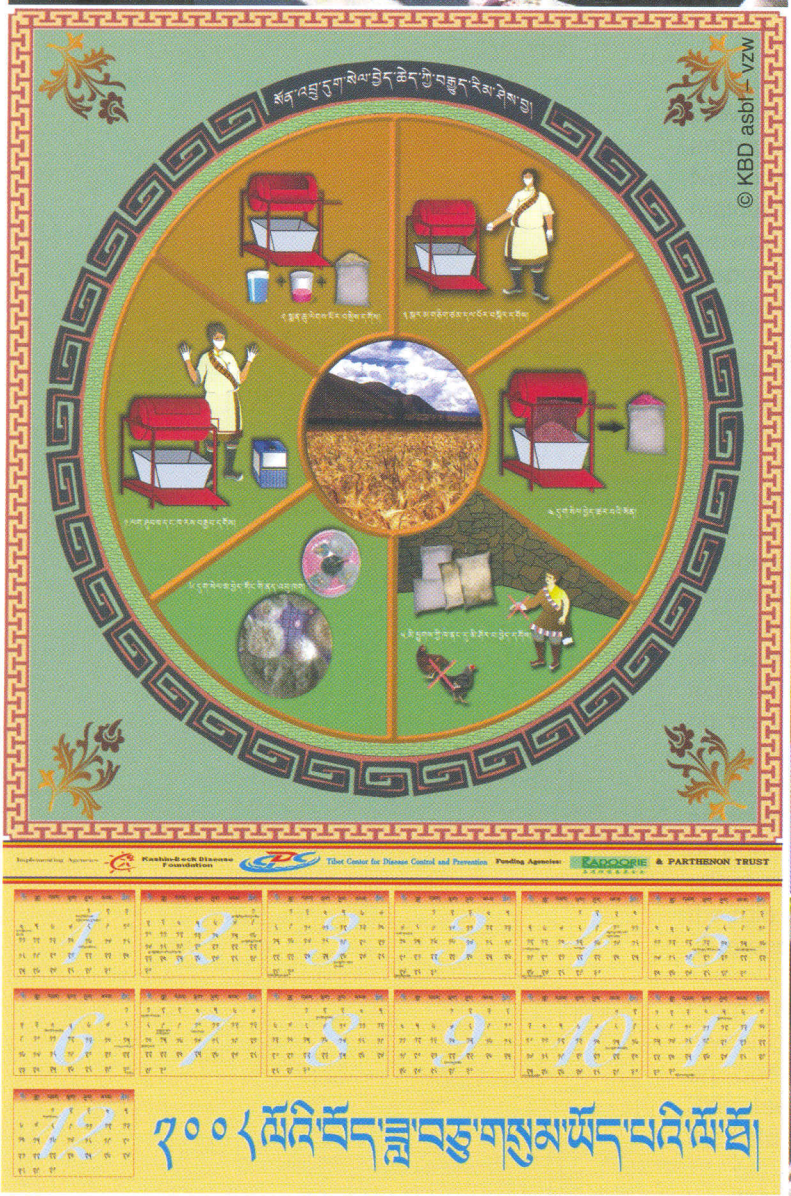
A general educational programme (Plate 5.3) was designed to inform the population of the risks of eating mouldy grains. A first poster was drawn. It allows to explain the cycle of food chain with the different crucial levels. It allows to approach the importance of seed dressing in the county or in the village, the importance of preparing the soil, with organic manure and fertilizers before tillage, the precautions to take when preparing the biocides and spraying on field, the importance of drying grain in the sun before storage and how to improve the storeroom.

Distribution of Samap devices to measure grain humidity was among the first curative actions. One person in each village – most of the time, the village leader – was in charge of controlling grain humidity before storage, which is accepted when the humidity level is below 14%. Plastic covers were distributed to facilitate the drying of grain in the sun. An important action also concerns the store-room arrangement to avoid humidity from soil in the bottom of the bags, and to improve ventilation. Metal racks and new plastic bags were distributed to each family. Soils and walls were cleaned, and training was given to explain the importance of the grain drying (Plate 5.4).

On the basis of results of the experiments, 41 threshing machines were distributed in the prefectures of Lhasa, Lhoca and Shigatse between 2000 and 2002 (Plate 5.5).

A similar programme was started in 2006 in regard to seed dressing in the villages which are too far from the counties to treat the grain mechanically. Five simple machines, easy to handle, were manufactured in Lhasa and tested in the villages in 2006. As results were satisfactory, 81 machines were distributed in 2007 (Plate 5.6) in order to equip the 86 villages covered by the programme of KBD Foundation (Plates 5.6, 5.7 and 5.8).

Plate 5.7. Seed dressing. In the villages far away from counties, the villagers are obliged to carry out seed dressing operation manually. There were neither technicians nor explanations regarding the use of the biocide in terms of dosage and health. We also noted that villagers mixed the biocide with water in cooking pot and other daily utensils, without being aware that the biocide was harmful.



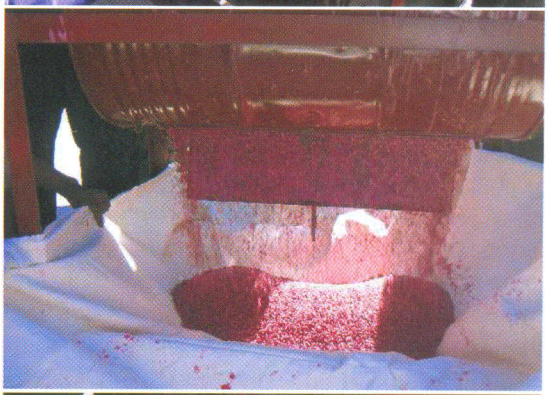


Plate 5.8. Seed dressing in the village – KBD F actions. In 2006 and 2007, 86 machines were planned, manufactured in Lhasa and distributed in the villages which are too far from the counties to treat the grain mechanically. A specific educational programme completed this action, insisting on the health's hazards to handle these chemicals.