

Big Bone Disease

A multidisciplinary approach
of Kashin-Beck disease
in Tibet Autonomous Region (P.R. China)



François Malaisse and Françoise Mathieu

Big Bone Disease

In the same collection

- Atelier Sorcier. *Ordre et désordre*. 2005, 48 p. couleur.
- Atelier Sorcier. *L'arbre et l'eau*. 2007, 60 p. couleur.
- Atelier Sorcier. *Dans l'air*. 2008, 62 p. couleur.
- Boudru M. *Forêt et sylviculture. Boisements et reboisements artificiels*. 1992, 348 p., 6 fig., 36 tab., 5 photos.
- Bousson E. *Gestion forestière intégrée. Approche structurée basée sur l'analyse multicritère*. 2003, 303 p., 79 fig., 52 tab., 7 photos.
- Buldgen A. et collaborateurs. *Aviculture semi-industrielle en climat subtropical. Guide pratique*. 1996, 128 p., 10 fig., 15 tab.
- Buldgen A., Dieng A. *Andropogon gayanus var. bisquamulatus. Une culture fourragère pour les régions tropicales*. 1997, 171 p., 18 fig., 28 tab., 15 photos.
- Dagnelie P. *Principes d'expérimentation. Planification des expériences et analyse de leurs résultats*. 2003, 397 p., 73 fig., 83 tab.
- Dagnelie P. *Analyse statistique à plusieurs variables*. 1975, 362 p., 20 fig., 28 tab.
- Dagnelie P., Palm R., Rondeux J., Thill A. *Tables de production relatives à l'épicéa commun*. 1988, 124 p., 44 tab.
- Dagnelie P., Palm R., Rondeux J., Thill A. *Tables de cubage des arbres et des peuplements forestiers*. 1999 (2^e édition), 128 p., 88 tab.
- Delacharlerie S., de Biourge S., Chéné C., Sindic M., Deroanne C. *HACCP organoleptique. Guide pratique*. 2008. 176 p., photos couleur.
- Delvingt W. *La forêt des hommes. Terroirs villageois en forêt tropicale africaine*. 2001, 288 p., 39 fig., 51 tab., 22 photos en couleur.
- Delvingt W., Vermeulen C. *Nazinga*. 2007, 312 p., 38 fig., 54 tab., 282 photos couleur.
- Demol J. *Amélioration des plantes. Application aux principales espèces cultivées en régions tropicales*. 2002, 584 p., 77 fig., 20 tab., 72 photos en couleur.
- Diépart J.C., Dogot T., Viboth Ly, Chanthy Loeung, Bora Kathy. *Le Monde Rural dans la Plaine Centrale du Cambodge. Analyse comparative à partir de cinq communes*. 2005, 154 p., 66 fig., 47 tab.
- Hoyoux J.M. *Le vocabulaire de l'apiculteur, illustré d'extraits littéraires*. 2002, 279 p., 99 ill.
- Lebailly P., Dogot T., Pham Van Bien, Tran Tien Khai. *La filière rizicole au Sud Viêt-nam. Un modèle méthodologique*. 2000, 168 p., 48 fig., 109 tab., 38 photos couleur.
- Leclercq A., Seutin E. *Les ennemis naturels du bois d'œuvre*. 1989, 140 p., 4 fig., 14 tab., 34 photos dont 15 en couleur.
- Ledent A., Burny P. *La politique agricole commune des origines au 3^e millénaire*. 2002, 600 p., 4 fig., 41 tab.
- Lepoivre Ph. *Phytopathologie*. 2003, 427 p., 39 fig., 15 tab.
- Malaisse F. *Se nourrir en forêt claire africaine. Approche écologique et nutritionnelle*. 1997, 384 p., 63 fig., 73 tab., 307 photos dont 260 en couleur.
- Mohimont A. *La Faculté de Gembloux dans l'ancienne abbaye de Gembloux*. 2005, 72 p., 42 ill.
- Nanson A. *Génétique et amélioration des arbres forestiers*. 2004, 712 p., 103 ill., 25 tab.
- Nederlandt P. *La coutellerie à Gembloux*. 2007, 136 p.
- Neuray G. *Des paysages. Pour qui ? Pourquoi ? Comment ?* 1982, réimpression 2004, 590 p., 300 ill.
- Nyabyenda P. *Les plantes cultivées en régions tropicales d'altitude d'Afrique. Tome 1. Légumineuses alimentaires - Plantes à tubercules et racines - Céréales*. 2005, 225 p., 7 fig., 57 tab., 32 photos.
- Nyabyenda P. *Les plantes cultivées en régions tropicales d'altitude d'Afrique. Tome 2. Cultures industrielles et d'exportation - Cultures fruitières - Cultures maraîchères*. 2006, 250 p., 10 fig., 39 tab., 40 photos.
- Oestges O. *La mécanisation des travaux agricoles*. 1994, 248 p., 207 fig.
- Oestges O. *La mécanisation des travaux à la ferme*. 1986 (2^e édition), 236 p., 222 fig.
- Paterson P. D. *L'apiculture*. 2008, 158 p., 61 fig., 6 tab.
- Planète femmes. *Des racines pour retrouver des ailes*. 2008, 56 p., photos noir et blanc, poésies.
- Rondeux J. *La mesure des arbres et des peuplements forestiers*. 1999, 544 p., 70 fig., 67 tab., 20 photos.
- Rondeux J. *La forêt et les hommes. Arrêt sur images 1900-1930*. 1997, 94 p., 82 photos.
- Rondeux J., Thibaut A. *Tables de production relatives au douglas*. 1996, 152 p., 5 fig., 61 tab.



Big Bone Disease

François Malaisse and Françoise Mathieu, editors

Les Presses agronomiques de Gembloux

Contributors

- Bally Pascale**, Kashin-Beck Disease asbl-vzw, Belgium
- Begaux Françoise**, Médecins Sans Frontières - Belgium (until 2002)
- Bock Laurent**, Laboratory of Geopedology, Gembloux Agricultural University, Belgium
- Chasseur Camille**, Section of Mycology, Scientific Institute of Public Health, Belgium
- Chasseur Stéphanie**, Faculty of Psychology, University of Mons-Hainaut, Belgium (until January 2008)
- Claus William**, Kashin-Beck Disease asbl-vzw, Belgium
- Delcarte Emile**, Bureau of Environment and Analysis, Gembloux Agricultural University, Belgium
- De Kesel André**, Department of Cryptogamy (Bryophyta & Thallophyta), National Botanic Garden of Belgium
- De Maertelaer Viviane**, IRIBHM & Department of Biostatistics and Medical Computing, Faculty of Medicine, Université Libre de Bruxelles (ULB), Belgium
- Drolkar Pelma**, Kashin-Beck Disease asbl-vzw, Tibet Autonomous Region, P.R.China
- Durand Marie-Claire**, Médecins Sans Frontières – Belgium (until 2000)
- Eddyani Miriam**, Department of Microbiology, Institute of Tropical Medicine, Belgium
- Gillet Philippe**, Department of Clinical Sciences, Institute of Tropical Medicine, Belgium
- Goyens Philippe**, Nutrition and Metabolism Unit, Department of Pediatrics, University Children's Hospital Queen Fabiola (HUDERF), Université Libre de Bruxelles (ULB), Belgium
- Haubrue Eric**, Unit of Functional and Evolutive Entomology, Gembloux Agricultural University, Belgium
- He Lixing**, Centre for Disease Control and Prevention, Tibet Autonomous Region, P.R.China
- Hinsenkamp Maurice**, Department of Orthopaedic Surgery and Traumatology, University Hospital Erasme, Université Libre de Bruxelles (ULB), Belgium
- Kanyandekwe Pascal**, Section of Mycology, Scientific Institute of Public Health, Belgium
- Lacroix Daniel**, Laboratory of Geopedology, Gembloux Agricultural University, Belgium
- Lagrange Marjorie**, Médecins Sans Frontières – Belgium (until 2001)
- Leteinturier Béatrice**, Laboratory of Ecology, Gembloux Agricultural University, Belgium (until 2002)
- Lognay Georges**, Unit of Analytical Chemistry, Gembloux Agricultural University, Belgium
- Malaisse François**, Laboratory of Ecology, Gembloux Agricultural University, Belgium
- Mathieu Françoise**, Kashin-Beck Disease asbl-vzw, Belgium
- Rapten Sheero**, Centre for Disease Control and Prevention, Tibet Autonomous Region, P.R.China
- Rinchen Lopsang**, Kashin-Beck Disease asbl-vzw, Tibet Autonomous Region, P.R.China
- Samdrup Tashi**, Centre for Disease Control and Prevention, Tibet Autonomous Region, P.R.China
- Stallenberg Bernard**, Department of Radiology and Medical Imaging, University Hospital Erasme, Université Libre de Bruxelles (ULB), Belgium.
- Suetens Carl**, Epidemiology Unit, Scientific Institute of Public Health, Belgium
- Tserin Dawa**, Center for Disease Control and Prevention, Tibet Autonomous Region, P.R.China
- Van Damme Patrick**, Laboratory of Tropical and Subtropical Agronomy and Ethnobotany, Ghent University, Belgium
- Van Marsenille Christiane**, Artiste peintre - illustratrice
- Wangdu Lakpa**, Kashin-Beck Disease asbl-vzw, Tibet Autonomous Region, P.R.China
- Wangla Rinzen**, Kashin-Beck Disease asbl-vzw, Tibet Autonomous Region, P.R.China
- Wathelet Bernard**, Unit of Industrial Biological Chemistry, Gembloux Agricultural University, Belgium
- Yangzom Dikki**, Kashin-Beck Disease asbl-vzw, Tibet Autonomous Region, P.R.China
-

Contents

Preface	7
Acknowledgements	9
Chapter 1 Kashin-Beck disease	
1.1. Historical background	11
1.2. Clinical description	13
1.3. X-ray description	14
1.4. Anatomopathology	15
1.5. Epidemiological studies	15
1.6. Ethiology	16
1.7. Treatment and prevention	16
1.8. Conclusion	17
Chapter 2 Framework	
Introduction	19
2.1. Geographical framework	20
2.2. General geological framework	20
2.3. Ecoclimatology	23
2.4. About soil and Tibetan soils	24
2.5. Plant and vegetation diversity	25
2.6. Animal husbandry	32
Chapter 3 The land-ecosystem	
Introduction	37
3.1. Barley cultural practices	39
3.2. Other cultivations	41
3.3. Breeding	45
3.4. Food habits in the villages	45
Chapter 4 Kashin-Beck disease in T.A.R.: 15 years of programmes	
Introduction	50
Chapter 4.1 Clinical study	
4.1.1. Objectives of the study	52
4.1.2. Population and methodology	52
4.1.3. Some results	53
Chapter 4.2 Radiological study	
Introduction	57
4.2.1. Objectives	57
4.2.2. Population and methodology	57
4.2.3. Results	59
Chapter 4.3 Physical therapy study	
4.3.1. Objectives	62
4.3.2. Population, materials and methods ...	62
4.3.3. Some results	62
Chapter 4.4 Prevalence study	
.....	65

Chapter 4.5

First epidemiological study

Chapter 4.6

Second epidemiological study: prevention trial

Chapter 4.7

Nutritional issues in KBD endemic rural areas

Chapter 4.8

Third epidemiological study: clinical trial based on nutritional issues

Introduction	67
4.5.1. Population and methodology	67
4.5.2. Some results	67
4.5.3. Conclusion	68

Introduction	69
4.6.1. Population and methodology	69
4.6.2. Some results	70
4.6.3. Conclusion	73

Introduction	75
4.7.1. Exploratory nutritional survey	75
4.7.2. Food consumption	76
4.7.3. Anthropometric surveys	76
4.7.4. Specific nutritional deficiencies	78
4.7.5. Intervention studies	80
4.7.6. Conclusion	80

.....	81
-------	----

Chapter 5

Studies and actions concerning several hypotheses

Chapter 5.1

The fungal hypotheses

5.1.1. Moulds and KBD	85
5.1.2. Cereal samplings and storage	86
5.1.3. Fungal analyses: methods	87
5.1.4. Fungal contamination of stored cereal	89
5.1.5. Other specific mycological studies	94
5.1.6. A sanitary program	97
.....	101

Chapter 5.2

The mineral deficiency hypothesis

Chapter 5.3

The alternative food path or the very little diversified diet hypothesis

5.3.1. Background	105
5.3.2. Alternative foods	106
5.3.3. Edible mushrooms	109
5.3.4. Edible potherbs	117
5.3.5. Spices, aromatic herbs and condiments	122
5.3.6. Other plant organ's foods	128

Chapter 5.4

Protinet

Introduction	131
5.4.1. Nettle as diet in T.A.R.	131
5.4.2. Nettles diversity in T.A.R.	133
5.4.3. Phytotechny and cultivation of nettles in South Central Tibet	133

References	137
------------------	-----

Appendix 1	145
------------------	-----

Appendix 2	149
------------------	-----

Preface

Kashin-Beck disease is probably one of the most neglected diseases and known only by a little number of practitioners and scientists.

This disease occurs mainly in remote parts of China but nowadays the largest and most serious cases are located in Tibet Autonomous Region.

Since more than 15 years, the Centre for Disease Control and Prevention of Tibet Autonomous Region, together with the help and constant support of Kashin-Beck Disease Foundation are studying this disease and are implementing some measures for the benefit of these patients. The programmes are targeting mainly the children and aimed to improve their health and nutritional status.

This joined initiative started in 1992 when Dr. Françoise Mathieu who was working with the international non governmental organization Médecins Sans Frontières (MSF) came for the first time in Tibet Autonomous Region (T.A.R.) and checked these patients. Later on, she presented to MSF Brussels office a physical therapy programme which aimed to reduce the handicap of these patients by releasing their joint pain and improving their mobility.

This triggered the start of various programmes and studies focusing on the research and better knowledge of this etiology.

Ten years later, when MSF withdrew from T.A.R., a new non profit organization was created and named the Kashin-Beck Disease foundation. Since that time, the collaboration was even reinforced.

Along all these years, a network of scientists coming with different backgrounds was built. At present, scientists from the *Université Libre de Bruxelles*, the Gembloux Agricultural University, the Scientific Institute of Public Health, the Department of Pediatrics of the University Children's Hospital Queen Fabiola, the Institute of Tropical Medicine,... among others are still involved in the KBD issues. They pay regular field visits to share their expertise with us.

The present book to which several authors have contributed is a synthesis of the different programmes and studies which were carrying on during more than 15 years in T.A.R. Some of the results are also presented. They are exposed in a chronological order.

The different chapters are reviewing the multidisciplinary approach of the disease, prioritizing always a better knowledge of the environment and the population. The authors are endeavoured to find a long-term solution for these patients, integrated with their natural resources.

This long commitment, that all of us decided to take, is a rich and unique experience. The constant mutual exchange that we have is irreplaceable and very valuable. Hopefully this book is reflecting this spirit and will share with the reader our enthusiasm.



Dr. Sheero Rapten

Director of Centre for Disease Control and Prevention,
Tibet Autonomous Region

2018



2008, LES PRESSES AGRONOMIQUES DE GEMBOUX, A.S.B.L.
Passage des Déportés 2 — B-5030 Gembloux (Belgique)
Tél. : +32 (0) 81 62 22 42
E-mail : pressesagro.gembloux@uliege.be URL : www.pressesagro.be
D/2008/1665/93
ISBN 978-2-87016-093-0

Cette œuvre est sous licence Creative Commons. Vous êtes libre de reproduire, de modifier, de distribuer et de communiquer cette création au public selon les conditions suivantes:

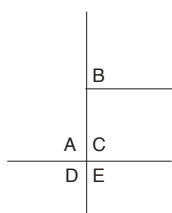
- paternité (BY): vous devez citer le nom de l'auteur original de la manière indiquée par l'auteur de l'œuvre ou le titulaire des droits qui vous confère cette autorisation (mais pas d'une manière qui suggérerait qu'ils vous soutiennent ou approuvent votre utilisation de l'œuvre);
- pas d'utilisation commerciale (NC): vous n'avez pas le droit d'utiliser cette création à des fins commerciales;
- partage des conditions initiales à l'identique (SA): si vous modifiez, transformez ou adaptez cette création, vous n'avez le droit de distribuer la création qui en résulte que sous un contrat identique à celui-ci.

À chaque réutilisation ou distribution de cette création, vous devez faire apparaître clairement au public les conditions contractuelles de sa mise à disposition. Chacune de ces conditions peut être levée si vous obtenez l'autorisation du titulaire des droits sur cette œuvre. Rien dans ce contrat ne diminue ou ne restreint le droit moral de l'auteur.

<http://creativecommons.org/licenses/by-nc-sa/4.0/deed.fr>

Publié avec l'aide du Service public de Wallonie
(Aides à la promotion de l'emploi)

Photos of the cover:



- A. A boy suffering from KBD (Lejeune Vincent)
- B. Lesions of the tarsus (Hinsenkamp Maurice)
- C. *Alternaria tenuissima* (Chasseur Camille)
- D. Barley harvest (Malaisse François)
- E. Nettle soup (Chiu Lorna)

Cover background: Namtso lake (Malaisse François)

Acknowledgements

The implementation of the different programmes and studies on the field as well as the editing of this book is the work of a whole team during more than 15 years. We would like to take the opportunity to deeply thank everybody who participates closely or from afar to this building.

First of all our greatest acknowledgements go to our partners and direct collaborators in Tibet Autonomous Region:

- from 2002 until now, our partner is the Centre for Disease Control and Prevention;
- from 1996 to 2002, as the programmes extended to other prefectures, the *Médecins Sans Frontières* counterpart was the Regional Health Bureau of Tibet Autonomous Region;
- from 1993 to 1996, the *Médecins Sans Frontières* counterpart for Kashin-Beck disease programmes in Tibet Autonomous Region was the Lhasa City Health Bureau.

Of course, nothing would be possible without the incredible work done on a daily basis by our whole team in Lhasa. Let us express our deepest and warmest gratitude to all of them: Dikki Yangzom, Lakpa Wangdu, Lixing He, Lopsang Rinchen, Nyima Ganden, Pelma Drolkar (the Small one and the Big one), Rinzin Wangla, Samdrup, Tsewang Lhakdrun, Chongtak.

We are also in debt for the constant help we received from the Institute of Nutrition and Food Safety, Centre of Disease Control and Prevention, P.R.China.

Various institutions and universities in Europe co-operated with Kashin-Beck Disease Foundation and *Médecins Sans Frontières* for all the studies, taking part very punctually or with a longer commitment in some stages of the research and carrying out some lab analyses.

The main partners are:

- The *Université Libre de Bruxelles* (ULB), Belgium;
- The Institute of Public Health (IPH), Belgium;

- The Gembloux Agricultural University (FUSAGx), Belgium;
- The University Children's Hospital Queen Fabiola (HUDERF), Belgium;
- The Laboratory of Water Chemistry and Environment (LCCE) – University of Poitiers, France;
- The Institute of Tropical Medicine, Belgium.

For the editing of this work, we are in debt to:

- Mrs. Christel Binard for the reviewing of part of the manuscript;
- Mrs. Alison Marschner for the English translation of parts of the thesis of F. Mathieu;
- Mrs. Nathalie da Costa for drawing the figure 2.2, Mrs. Lorna Chiu and Mr. Vincent Lejeune for placing some tremendous photos at our disposal;
- The team of the *Presses Agronomiques de Gembloux* and notably Mrs. Claire Parmentier and Mrs. Dominique Verniers for their valuable contribution and their outstanding availability.

Last but not least, for the last 4 years we received a substantial financial support and permanent valuable advices from the Kadoorie Charitable Foundation and the Parthenon Trust. We like to deeply thank them. They have to receive the expression of our deepest appreciation.

For the editing of this book, we are also in debt with the National Lottery of Belgium, Dr. Stanley Ho from Hong Kong and Mr. Ph. Hammer from France who gave us the financial opportunity for the realization.

François Malaisse and Françoise Mathieu
June 2008

1.1. Historical background

1.1.1. Russian period

According to Sokoloff (1989), the first traces of this disease go back to the Bronze Age. However, it was not until 1849 that a surveyor, I. M. Yurenskiy, described it for the first time as a clinical condition (Figure 1.1). The patients he observed lived exclusively along the banks of the Urov River. At that time, the disease occurred among the populations living in Eastern Russia, near Baikal Lake, between the Argun and Shilka rivers. From 1856, the staff headquarters of a military detachment of Cosacks established in the region observed that an abnormally high percentage of the population was suffering from physical deformities that made them unable to serve in the army. Dr. N.I. Kashin, the Cosack brigade doctor conducting a health survey found, in addition to bony deformities, the presence of cases of goitre and malaria (Figure 1.2). In the same year, he wrote a report entitled «A description of endemic goitre and other diseases existing along the Urov River» (Sokoloff, 1987). In 1861, N. I. Kashin enlightened that a distinction had to be made between people of small stature presenting a limp encountered east of Baikal Lake and those suffering from hypothyroidism, a pathology also endemic in the region (Malinovskii, 1957).



Figure 1.1. Ivan M. Yurenskiy (Sokoloff, 1989; reproduced with permission of the Medical Society of the State of New York).



Figure 1.2. Left: Nikolai I. Kashin. Right: Cosack Vassily Ivanov aged 50 years (drawing made in 1856 by N.I. Kashin) (Sokoloff, 1989; reproduced with permission of the Medical Society of the State of New York).

For a long time, the disease was known as Urov's disease, from the name of the Urov River, flowing into the Argun River along which the affected populations were living. The authorities decided to move the populations of some villages towards the Argun River. Unfortunately, there was no medical follow-up of these villagers and no conclusions can be drawn from this experiment (Nesterov, 1964).

From 1899 to 1902, E. V. Beck – also a military doctor with the Cosack army in the Transbaikal region – together with his wife, A.N. Beck, made a detailed study of the clinics and the prevalence of the disease, which they called «*Osteoarthritis Deformans Endemica*» (Beck, 1906) (Figure 1.3). Beck devoted himself to the study of osteoarthritic lesions. He systematically studied 3,153 inhabitants from 11 villages.

In 1903, he transferred 6 patients, aged from 16 to 23 years, to the military hospital in St Petersburg in order to investigate the disease more deeply, as much from the anthropological point of view as from that of neuropsychiatry. E.B. Beck presented a paper, first published in the Russian journal *Russky Vrach* (Beck, 1906) and, two years later, in the German review *Archiv für Klinische Surgery* (Beck, 1908). From then on, the condition was known by the name of «Kashin-Beck disease» (KBD).

In 1925, the first medical conference in Eastern Siberia, in collaboration with the University of Irkutsk, created a commission to study the disease. In 1931, a research centre was opened in Chita, located between Baikal Lake and the Amur River. For thirty years, several studies



Figure 1.3. Eugene V. Beck, and the title page of Beck's thesis, 1906 (reproduced with permission of the Medical Society of the State of New York).

were carried out in this centre, in particular concerning the etiology of the disease.

1.1.2. Japanese period

The Japanese invasion of 1905 opened a new period of research into Kashin-Beck disease. In 1919, a military surgeon, T. Okano, observed that a large number of people living in the most northerly part of the Korean peninsula suffered from a progressive chronic deforming polyarthritis (Yamamuro, 2001). The clinical signs corresponded to those of Kashin-Beck disease. A local practitioner, Pak, told him that this disease, known locally as *Tojiru*, had been known in this region for over 300 years. Other Japanese doctors (Dr Nakamura in 1927, Dr Kato in 1928 and Dr Kim in 1929) confirmed the presence of cases in the same region. For the first time, Kashin-Beck disease was described outside Siberia.

During the same period, in 1931, N.I. Damperov observed that the clinical signs of the disease could also be found among the Chinese and Koreans who came to work in the regions of Baikal Lake (Voshchenko et al., 1990).

In 1935, Professor T. Takamori (Figure 1.4) revealed for the first time the presence of patients suffering from KBD in the north-east of China. He published a detailed radiological study and conducted systematic research into the disease up until the Japanese left the Chinese territory in 1945. Part of the results were confiscated. Years later, Takamori's work was published in English (Takamori, 1968). He named the disease *Dysostosis enchondralis endemica* (Egashira, 1990; Yamamuro, 2001).

1.1.3. Chinese period

Before the second world war, very few Chinese studies were published on Kashin-Beck disease.



Figure 1.4. Professor Tokio Takamori (reproduced with permission of K. Shimizu, department of Orthopaedic Surgery, Gifu University, School of Medicine, Japan).

A disease very close to Kashin-Beck was described in Geography of Anze County, Shaanxi Province in 1664 (Tan, 1989). According to Yang (1990), KBD was mentioned for the first time in 1908, by a local administrator named Liu Jianfeng, in the mountainous region of Chang Bai, close to Korea. According to other authors (Tan, 1989), it was Zhang Fengshu, in 1934, who made the first detailed description in the province of Jilin (North-Eastern China). In 1956, Hypabres published the first scientific study on *Ta Gu Ji Ping*, which translates literally into «big joint disease».

After the second world war, the USSR developed health programmes with its allies. Research into KBD was included in these. The last document co-published by the USSR and the People's Republic of China dates from 1956. It included ten chapters covering the publications that had already appeared in each of the respective countries (Sokoloff, 1989).

1.1.4. Present situation

Today in China, there are three schools of medicine with research units into Kashin-Beck disease: Harbin, Xi'an and Changchun. Institutes at provincial level are also working on the subject. Some of these researchers have been studying the disease for over 40 years. It was a national health priority for several years. In Russia, the principal research centre is still in Chita.

Interest in KBD has extended to the international scientific community. The World Health Organisation and the Chinese Ministry of Health organised a conference in Beijing in 1985. Since 1986, UNICEF has been encouraging and participating in a research programme in China.

The scientific knowledge of Kashin-Beck disease is not very old. The main elements are summarised below.

1.2. Clinical description

1.2.1. Symptoms

Clinically, Kashin-Beck disease is characterized by articular stiffness accompanied by deformities and enlargements of one or several joints. These signs are, most of the time, bilateral and become progressively installed during childhood and adolescence. According to most of the studies, it is the interphalangeal and metacarpophalangeal joints and the joints of the wrists that are the first to be affected (Nesterov, 1964; Sokoloff, 1988; Yin, 1990). Later on, the elbows, the knees and the ankles are also affected.

These joints are painful and muscular atrophy can develop, associated with a limitation in the articular range. Patients feel more easily tired by effort and progressively weaker. Cases of dwarfism are observed, with a marked shortening of the limbs (Kolsteren, 1992).

The later stages of the disease look very similar to the clinical symptoms of a pluriarticular osteoarthritis. The loss of articular mobility becomes greater with age. No inflammatory episode is reported at the level of the joints and the periarticular tissues (Kravchenko, 1959).

The Chinese doctors defined three conditions for establishing the clinical diagnosis. These were approved during the National Conference on KBD surveillance in 1989:

- the patient must live or have lived in an endemic area⁽¹⁾ for longer than six months;
- the principal complaints and symptoms must be those of Kashin-Beck disease;
- the other osteoarthropathies must be excluded.

⁽¹⁾ The authors (Anonymous, 1989) defined an area as being endemic when at least two of the following criteria are met:

- among patients, at least one presents a grade II;
- the prevalence rate is greater than 5% for grade I;
- several radiological changes can be found among children at the level of the distal extremity of the phalanges of the two hands.

1.2.2. Clinical classification

Beck and most of the Russian doctors distinguished three stages in the evolution of the disease (Nesterov, 1964; Voshchenko et al., 1990; Kolsteren, 1992). These are defined by clinical criteria with which radiological signs are associated.

The Chinese authors have a clinical classification in four stages (Anonymous, 1989; Kolsteren, 1992):

Early stage

The extremity of the fingers present a flexum. The fingers are «arched». There is crepitus at this level. Pain may be present in the knees and ankles.

Stage I

In addition to the previous lesions, there is also an enlargement of the joints of the fingers and of the other small joints, which present crepitus.

Stage II

The fingers and toes are shortened. Enlargement and loss of mobility are noted at the level of the medium-sized joints. There are lesions indicating muscular atrophy.

Stage III

Enlargements and loss of mobility are observed in the large joints, and a shortening of the extremities, as well as a reduction in the height of patients, sometimes to the extent of dwarfism.

According to Yin (1990), the early stage can sometimes be reversible. The joint deformities only appear at stage II. Finally, at stages II and III, the patient loses weight.

The Russian classification integrates clinical and radiological observations, while the Chinese have two independent classifications, one clinical and one radiological, both of them in four stages. The two clinical classifications are based on observation of the patient, without any objective measure.

The Russian classification apparently associates, in an arbitrary manner, certain clinical observations at the level of the different joints (first stage: deformity at the level of the interphalangeal joints, of the wrists and the ankles; second stage: restriction of the articular mobility at the level of the interphalangeal joints, the knees and the elbows). The deformity at the level of the elbows and the heels is not taken into account, nor is the reduction in the articular mobility of the wrists and the ankles. The shoulders and the hips are never considered.

The Chinese classification focuses, at the beginning of the disease, on an examination of the hands and the small joints, omitting a systematic analysis of the joints of the limbs. In our experience, many patients will escape diagnosis. This classification has the disadvantage that

it leads to many false negatives. In addition, it takes no account of the general state of the patient.

On the basis of these classifications, the present clinical examination for Kashin-Beck disease is mainly limited to a general observation of the patient and a detailed examination of the hands. The disease is revealed essentially at the level of the appendicular skeleton, a clinical examination of all the peripheral joints and an anthropometric examination of the limbs should provide complementary information.

There is a paradox within the criteria proposed by the Chinese colleagues for establishing the clinical diagnosis: one of the conditions is to have lived in an endemic region for more than six months. But the definition of an endemic region depends on previous radiological diagnosis of individual cases.

1.3. X-ray description

Professor T. Takamori (1968) analyzed the X-ray of 61 patients suffering from the disease at different stages. All the different radiological alterations were classified in 3 stages.

For the past twenty years, Chinese doctors have described the radiological signs on the basis of which they will establish the criteria for diagnosing and classifying the disease.

These different signs were summarized in 1985 and approved at the 1989 National Conference on KBD surveillance (Anonymous, 1989; Ying et al., 1990; 1994).

They are based on images of the hand and the foot:

- at the level of the epiphyso-metaphyseal plate of the metacarpus and metatarsus, the line of calcification becomes less visible, veiled, thinner, breaks up or disappears in places, corresponding to chondronecrosis. This necrosis is most often produced at the centre of the metaphyseal plate, thus forming a depression;
- alterations on the edges of the epiphyso-metaphyseal plate of the distal extremity of the phalanges, of the metacarpus, of the metatarsus, alterations of the bones of the carpus and of the tarsus, occurring after the chondronecrosis;
- deformity of the epiphysis with thickening of the articular cartilage, precocious closing of the metaphyseal plates following complete necrosis of the cartilage;
- enlargement of the interphalangeal joints corresponding to the effects of a secondary osteoarthritis.

According to the literature, radiology is the technique of choice for the diagnosis and evaluation of the disease. Most of the authors (Anonymous, 1989; Ying et al., 1990; Wang et al., 1996; Yu et al., 2002) agree that a standard X-ray of the hand (or of both hands) is amply sufficient.

However, we should note that chondronecrosis cannot be seen on an X-ray. It is only when the edges of the zone of necrosis begin to calcify that the first radiological lesions appear. In our opinion, radiology therefore does not allow detection of the first effects of KBD.

1.4. Anatomopathology

There have been few histological studies of Kashin-Beck disease. The small number of research teams who have studied the histopathology had only basic techniques available.

In the light of different observations (Nesterov, 1964; Mo, 1987; 1990; Pasteels et al., 2001), it would appear that the cartilage changes are produced in patients in a slow and progressive manner. The main characteristic is the osteochondral necrosis. After the death of the chondrocytes, secondary changes intervene. Healing accompanied by the appearance of cicatricial tissue can be observed. Osteophytes and joint dysplasias that could be associated with diminished growth of the diaphysis of the long bones are also visible. Different stages can be found simultaneously on different sites in the same patient. The pathogenesis of this process has not yet been clearly demonstrated.

1.5. Epidemiological studies

1.5.1. Geographic distribution of KBD

Kashin-Beck disease only occurs in Asia, in a crescent-shaped region stretching from the South-East of Siberia to Tibet, affecting the northern and central provinces of China (the North-East of Heilongjiang province, Jilin province, Inner Mongolia, Hebei, Shandong, Shanxi and Shaanxi up to the south-west with Gansu, Qinghai, Sichuan and T.A.R.) and the north of North Korea.

At the present time, in China, KBD affects 303 countries in over 13 provinces and 2 autonomous regions. The geographic distribution of the disease was studied for several years by Tan J. and collaborators (Tan, 1989). The regions with the largest number of cases are the mountainous regions of Changbai, Xingan and Xiao Xingan, as well as the plateau of Loess and the mountains in the west of Sichuan.

Most of the regions of China affected by KBD are grouped along a wide belt going from the North-East to the South-West of the country and forming a transition zone between the tropical and sub-tropical, hot and humid South-East, and the arid and semi-arid North-West (Figure 1.5). The disease is not uniformly distributed within this crescent. There are some small «islands» that are no doubt protected by environmental variations. For example, in the west of Sichuan and in

T.A.R., the disease is only found at a certain altitude (the author does not say which one) in zones where the earth has a drab brown colour (Tan et al., 1990).

Sokoloff (1987) states that most of the cases are found in rural communities living in a mountainous environment, more particularly in enclosed valleys where the quality of the soil is very poor and easily washed by the water runoff. The winters are long and severe, and the summers warm and humid.

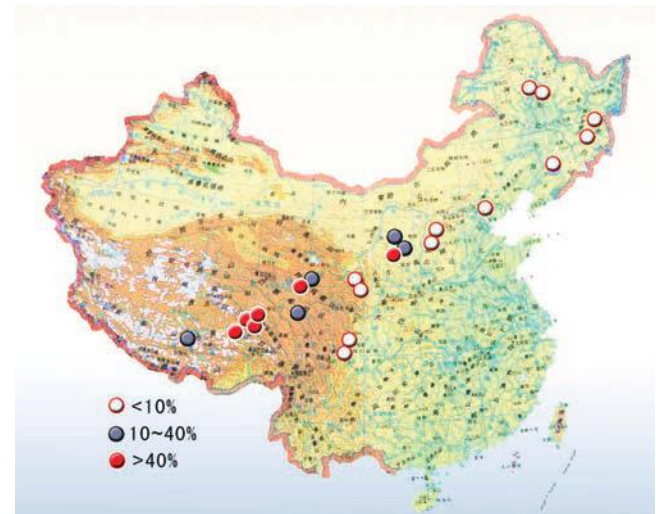


Figure 1.5. Prevalence map of Kashin-Beck disease in China (reproduced with permission from Centre for Endemic Disease Control, China CDC, 2003).

1.5.2. Demographic data

Many publications present epidemiological information, but few of them give precise details of the prevalence of the disease or its incidence. In addition, no article mentions the mortality rate (Allander, 1994). The life expectancy among these populations would perhaps be significantly reduced because of the selenium deficiency problem and the presence of Keshan disease, factors associated with Kashin-Beck disease (Foster et al., 1995).

In China, more than 30 million people are living in endemic regions and are thus exposed to the disease, while some 2 million are clinically affected. To these cases must be added the cases in Siberia and probably also in North Korea (Levander, 1987; Sokoloff, 1989).

The prevalence varies from one place to another (Table 1.1). It can go as high as 96% in some communities. According to a study carried out in 1950 in the North of China, the average prevalence in an endemic zone is from 30 to 40% (Kolsteren, 1992). It seems that only sedentary peasants and semi-nomads are affected by KBD. It is not described among urban and nomadic populations (Allander, 1987).

Table 1.1. Summary of the different geographic data.

Authors	Year	Region	Population	Prevalence	Incidence
Allander, 1987	1906	Eastern Siberia	Children	-	31.6%
Allander, 1987	1941	Eastern Siberia	Children	-	24.2%
Kolsteren, 1992	1950	North of China	Total	30%-40%	-
Allander, 1987	1959	Eastern Siberia	Children	-	12.5%
Allander, 1990	1966-1979	Heilongjiang	Children	70%	-
Allander, 1987	1979	Eastern Siberia	Children	-	8.6%
Wang, 1990	1981	Heilongjiang	Total	2.08%	-
Allander, 1990	1983	Heilongjiang	Children	30%	-
Allander, 1990	1984-1986	Heilongjiang	Children	10% –	-
Deng, 1990	1989	Sichuan villages	Total	20%	-
Tan, 1989	1989	Highly endemic regions in China	Children <10 years	7.8%-96% > 50%	-
Allander, 1991	1992	Province of Shaanxi	Total	0.7%	-

In China, in 1989, the National Conference for KBD Surveillance defined the concept of an endemic region. The smallest entity is the natural village, which is considered endemic if two of the following criteria are met:

- patients are present at clinical stage 2 or above;
- the prevalence of stage 1 patients is above 5%;
- the appearance of several radiological changes in the distal extremity of the fingers among children. These changes must be bilateral.

Criteria of severity have also been established for these regions:

- severely affected region: the clinical prevalence is equal to or greater than 30%;
- averagely affected region: the clinical prevalence is between 10 and 30%;
- lightly affected region: the clinical prevalence is lower than 10%.

The definition of disease «activity» is based on a radiological diagnosis of the metaphyses of the right hand of children aged between 7 and 13:

- «active» region: the rate of radiological detection of metaphyseal anomalies is greater than 50%;
- relatively «active» region: the rate of detection is between 25 and 50%;
- «non-active» region: the rate of detection is lower than 25%.

1.6. Etiology

The research into the pathogenic factors of Kashin-Beck disease has led to the formulation of a number of hypotheses.

At this time, the etiology remains unknown, but a consensus has been established in favour of a multifactorial origin.

The principal risk factors are held to be:

- the fungal contamination of cereals accompanied by a production of mycotoxins;
- the selenium deficiency;
- the presence of certain organic matter, such as humic and fulvic acids in the drinking water.

We have attempted a very succinct summary of the different etiological theses (Table 1.2).

Up to now, no single etiological factor has successfully explained the epidemiological characteristics, the clinical table and the anatomopathology of the disease. The different teams of researchers agree in acknowledging the multifactorial aspect of the etiology of Kashin Beck disease.

1.7. Treatment and prevention

1.7.1. Treatment

Given the uncertain etiology of the disease and the lack of knowledge about the physiopathological mechanisms, few treatments have been proposed and still fewer results have been published. Most of the treatments are palliative.

In 1981, mandated by the World Health Organisation, Egashira evaluated the importance of the problem in China. He concluded that there is no treatment for the disease. He noted that acupuncture and moxibustion procure relief in the short term, while the ingestion of medicines such as sodium sulphate and magnesium sulphate and traditional Chinese medicines have no particular effect (Egashira, 1990).

As the etiology of KBD remains to be established, there is no specific treatment for Kashin Beck disease. The traditional treatments have a high rate of relapse and have been abandoned. Surgical treatment offers a good solution for sustained improvement in the handicaps

Table 1.2. Summary of the different etiological hypotheses.

Etiological hypotheses	Arguments in favour	Arguments against
Fungal and mycotoxin contamination	Relation between the presence of fungi in cereals and KBD (Yang, 1990; 1995; 1997; Bai et al., 1990; Chasseur et al., 1996; 1997; 2001).	No study isolates the one (or more than one) mycotoxin(s) capable of reproducing KBD lesions.
Lack of selenium	Presence of KBD in the zones deficient in selenium (Mo, 1987; Li et al., 1990; Ge et al., 1993). Therapeutic and preventive effect on precocious cases of a selenium supplement (Li, 1979; Mo, 1987; Li, 1987; Liang et al., 1987; Liang, 1990; Wang, 1990; Yin, 1990).	Insignificant difference between the level of selenium in a KBD population and a control population (Tan, 1989; Li et al., 1990; Ma et al., 1993; Ge et al., 1993; Moreno-Reyes et al., 1998). The experimental studies do not allow the reproduction of the lesions observed in KBD (Wei et al., 1986; Walser et al., 1988; Mo, 1990).
Lack of iodine and selenium	Low level of urine iodine, high TSH and TBG serum levels among KBD patients (Moreno-Reyes et al., 1998).	Only one reference, limited to a region. No therapeutic or preventive effect demonstrated.
Organic acids	The incidence of KBD stands in relation to the levels of humic acids (Zhai et al., 1980; Zhai, 1990; Ren et al., 1991).	Contradictory studies (Zhang et al., 1982; Levander, 1987). Properties of the humic acids not well known (Wang et al., 1991).
Deficiency or excess of certain minerals		Little data (Rosin et al., 1982; Tan, 1989; Voshchenko et al., 1990).
Mixed hypothesis	Integration of the different risk factors (Peng et al., 1987; 1990; 1991). Permits, in part, a pathological explanation of the disease (Wang et al., 1991; Zhang et al., 1991; Peng et al., 1992; 1999).	No therapeutic or preventive effect demonstrated

produced by the disease in its advanced phase (Zhang, 1988; Liu et al., 1998).

1.7.2. Prevention

Based on the etiological hypotheses of KBD, a number of prevention programmes have been set up, most often without a strict evaluation of their effects, despite the evaluation criteria defined by the National Conference for KBD surveillance (Anonymous, 1989).

Tan (1989) summarized the situation by observing that if no programme had by then succeeded in preventing Kashin-Beck disease, certain measures could, according to him, reduce the incidence. The target population must be the children. He proposed the following measures:

- selenium supplements either orally (in the form of sodium tablets) or by spraying selenium onto the crops, or by selecting a diet particularly rich in selenium;
- substituting crops, which was already taking place in some endemic regions in the north of China, by planting rice instead of cereals;
- improving the drinking water by using sanitation programmes: microbiological filtration of the water, chemical controls, deviation and protection of water sources, digging deep wells.

He also advised measures as a curative treatment: adding supplements of vitamins C and E, as well as medicinal herbs in order to improve the blood circulation and reduce the pain (the same as those used in cases of rheumatism).

All these studies, attempting to prove the efficacy of some prevention programmes, were based on observations of the radiological lesions of the disease and never on a clinical examination.

1.8. Conclusion

In his review of the literature, Allander (1994) observed that the majority of the articles on Kashin-Beck disease were written in Russian, Chinese and Japanese. The restricted diffusion of these publications, as well as the translation difficulties, limited their accessibility to the worldwide scientific community. Few articles have been translated and published in English. Very often, only summaries exist. These complications have represented a major problem to understanding and synthesizing the studies that have already been conducted into this disease.

More recently, Sudre et al. (2001) reported another observation. They were surprised by the extent of the disease presenting a high prevalence and a morbidity in well-defined zones affecting several million people compared to the small amount of well-conducted research into the etiology of the disease. According to them, there are several reasons for this situation. Kashin-Beck disease is a disease affecting very poor, rural populations. It is widespread in isolated regions that are difficult to access (geographically and politically) and have very little contact with urban centres. KBD is not described in any industrialised country, which is where most of the resources for medical research are to be found. Most of the scientific teams taking an interest in KBD have worked in an isolated manner. However, an etiological explanation, which is more than likely to be multifactorial, would require collaboration between specialised individuals or teams coming from very different domains and sharing their knowledge and experience.

Prospective randomised clinical trials and cohort studies are the most credible. For Kashin-Beck disease, these have only rarely been conducted and the results produced have been equivocal (Liang et al., 1987; Liang, 1990; Yin et al., 1990). Many other comparisons have been made: control groups, transversal, 'before and after' and ecological (Li, 1979; Li et al., 1987; Liang et al., 1987; Li et al., 1990; Wang et al., 1990; Yang, 1990; Zhai, 1990; Ge et al., 1993; Yang, 1995; Chasseur et al., 1997; Moreno-Reyes et al., 1998). The better-conducted studies rely on animal models, laboratory experiments and anatomopathological data (Wei et al., 1986; Mo, 1987; Wang et al., 1991; Zhang et al., 1991; Yang, 1995). Nevertheless all these studies have produced only indirect evidence (Sudre et al., 2001).

François Malaisse, Daniel Lacroix, Laurent Bock, Eric Haubruge, Camille Chasseur,
Christiane Van Marsenille, William Claus, Françoise Mathieu

Introduction

The concept of «ecosystem» was one of the most important steps in the evolution of ecological concepts during the XXth century. More recently human welfare was taken into account (di Castri, 1981). From an ethno-ecological approach, this led to the definition of a new unit: the «ethno-ecosystem», which may be defined as «the whole of elements regarding an environment where mankind lives and through which he establishes intuitive and cognitive relations with each of those elements» (Pujol, 1975). The ethno-ecosystem adds the cultural dimension to the spatial and temporal ecosystem preoccupations (Marchenay, 1975). The adoption of this concept, mainly regarding agriculture, into our understanding and environmental management approaches will be a source of development for the concerned societies (Malaisse, 1997). Consumption of little known wild edible products should not be avoid (Gaud, Semwal, 1983).

A first step towards a better agro-environmental knowledge consists in an ecosystemic diversity approach based upon a recognition, and further identification of the main vegetation types as well as the agricultural systems. The plant society or phytocenosis furnishes the reference regarding the ecosystemic units' denomination. Differentiation's criteria rely upon the evolutionary series concept, density of the plant cover layers, habitat characteristics as well as, last but not least, species composition.

Therefore, a vegetation survey was carried out in the action area of MSF–Belgium in South Central Tibet in July and August 1998. Our study was completed with the constitution of a plant reference collection of some 650 voucher specimens. This collection is conserved at the Belgium National Botanical Garden (BR according to Holmgren et al., 1990).

The aim of the present chapter is to develop first the abiotic factors of the area of concern. Later aspects of

the biocenosis are investigated, including a typology of the diverse vegetation units recognized during our field survey.

2.1. Geographical framework

2.1.1. Tibet Autonomous Region (T.A.R.)

Tibet Autonomous Region, as the main part of the Qinghai-Xizang Plateau, is located in the south-western border area of China between east longitude 78° 25' - 90° 06' and north latitude 26° 50' - 36° 53'. Its continental frontiers are about 4,000 km, separating T.A.R. from Uyggur Autonomous Region and Qinghai province to the North, Sichuan province to the East, Yunnan province at South-East and from Myanmar, India, Buthan, Sikkim and Nepal to the South (Figure 2.1). Its superfcy is about 1.228 million km², or 12.8% of P.R. China. T.A.R. is on the Tibetan Plateau, the world's highest region. Most of the Himalaya mountain range, one of the youngest mountain ranges in the world, is only 4 million year-old and lies within T.A.R. Its most famous peak, Mount Everest, is on Nepal's border with P.R. China.

Several major rivers have their source in the Tibetan Plateau. These include Yangtze, Yellow river, Indus, Mekong, Ganges, Salween and Bramhaputra or Yarlung Tsangpo river. The Bramhaputra river originates from a lake (Tso Mapham in Tibetan) in Western Tibet, near Mount Kailash (Khang Rinpoche in Tibetan), and Lhasa river is one of its tributaries. Tibet has numerous high-altitude lakes (*tso* or *co* in Tibetan). Two of them are included in the study area, the Namtso Yumco and the Yamdrok Tso (Plate 2.1).

Based on natural topography, T.A.R. can be roughly divided into four parts, valleys and drier regions in the South, plateau in the North, high mountains with river valleys in the South-West and wet forest regions in the East.

At the end of 2000, the T.A.R. total population was estimated on 2.62 million inhabitants, whilst over 1.3 billion people are quoted for China mid-2007.

2.1.2. Area of concern

The present survey was carried out in South Central Tibet. The area studied covers three prefectures (Shigatse, Lhasa and Lhoca). The network consists of 84 villages distributed in ten counties (Figure 2.2). People enrolled speak Tibetan (alternate name Central Tibetan). The Tibetan population in the area is estimated for 1,200,000 inhabitants. This ethno-linguistic group includes several dialects, of which Ü and Tsang subgroups are here concerned. Written Tibetan is reported to be based on a southern dialect, Ü-Chan. It is the common script used by all of them. In central and northern T.A.R., there are also around 500,000 Drokba, nomad herders; they are not included in the present study.

2.2. General geological framework

Tibetan Plateau is formed by collage of tectonic continental microplates, namely from north to south, the Kunlun, Songban-Ganzi, Qiangtang, and Lhasa terranes, the latter being the region investigated in this book.

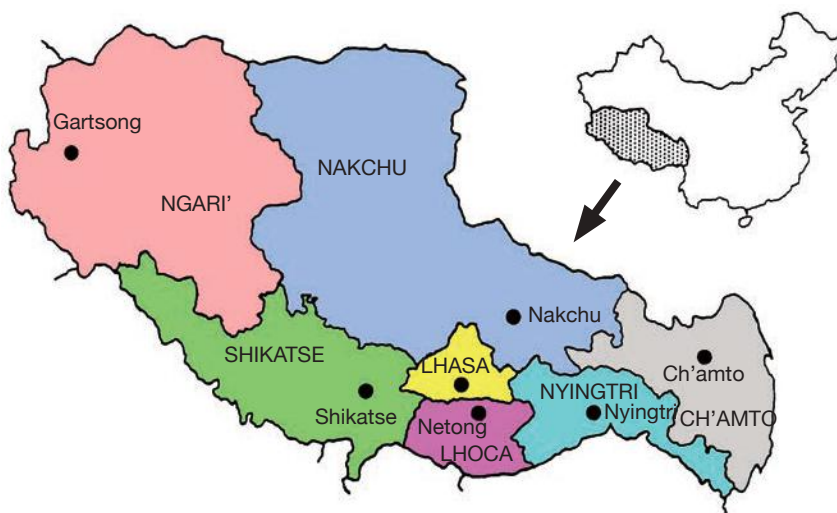


Figure 2.1. P.R. China, T.A.R. and the seven prefectures.



© Matisse François

Plate 2.1. Aspects of hydric environment in the area of concern (rivers, lakes, streams, ponds, springs). A. The Yarlung Tsangpo river or Bramhaputra in the vicinity of Gongkar – B. The Lhasa river in the vicinity of Meldrogungkar – C. Namtso Chukmo or Namtso lake (altitude 4,718 m) – D. Yamdrok Tso (altitude 4,439 m) – E. Reting Tsangpo river (vicinity of Tanggo) – F. Hot water spring (Derdron) – G. Low land pond – H. Low land small river – I. High mountain stream – J. Skin coracle on Tsangpo river in rainy summer season.

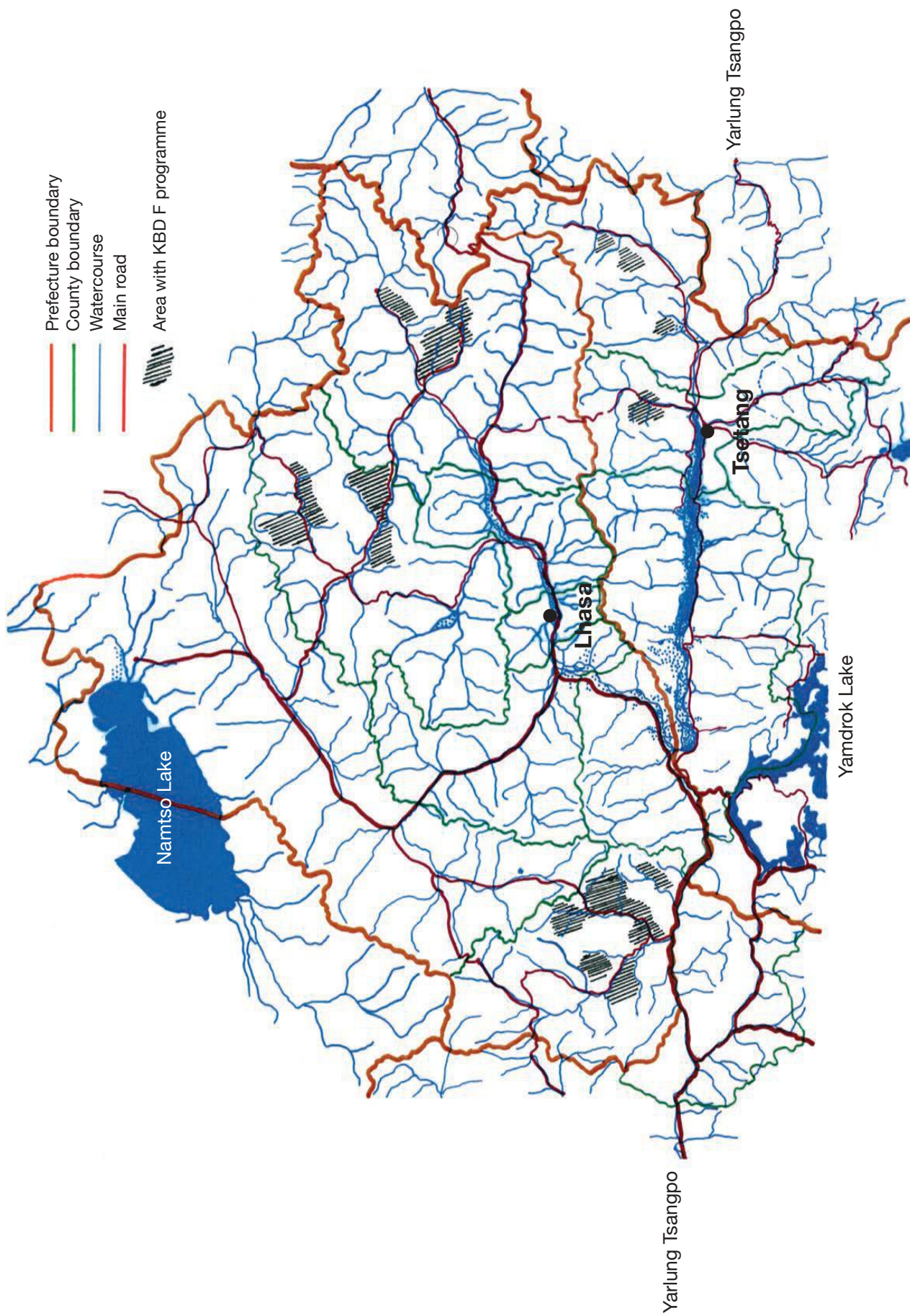


Figure 2.2. South Central Tibet with the implementation sites of KBD F programmes.

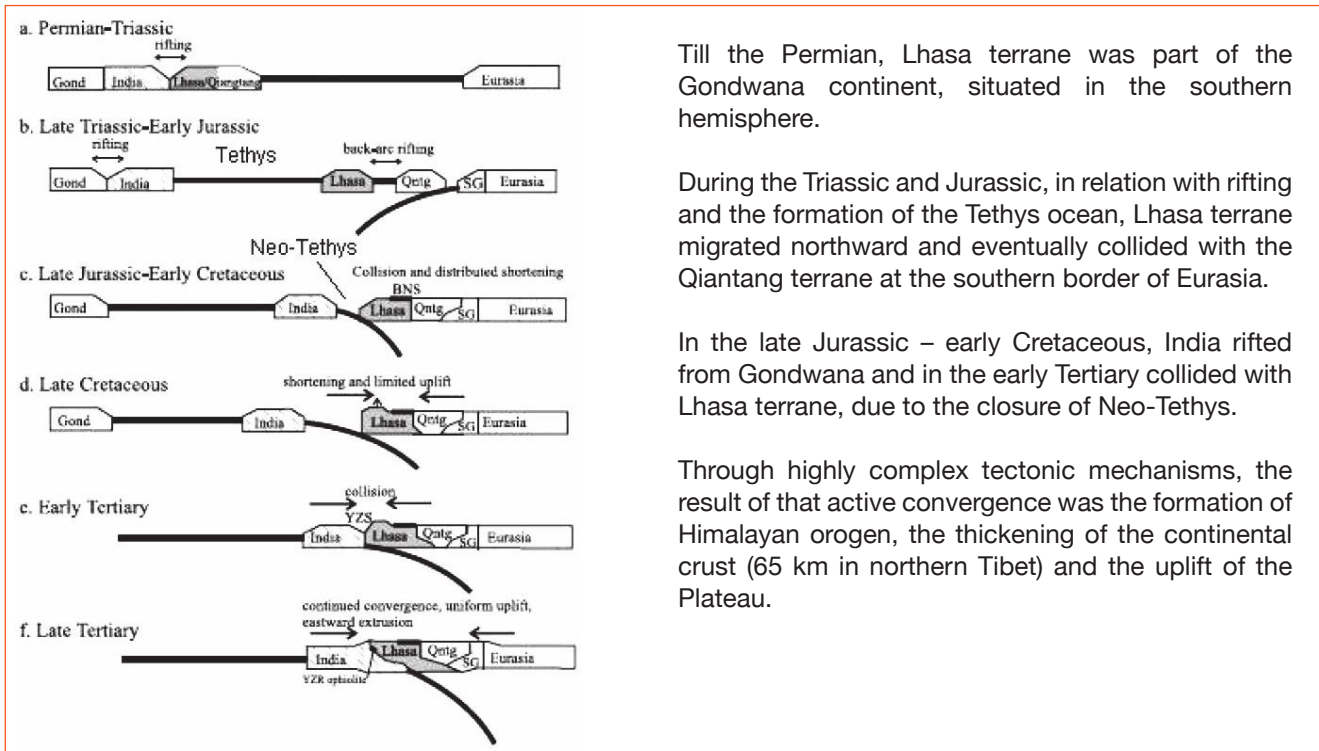


Figure 2.3. Lhasa block (Haines et al., 2003).

Till the Permian, Lhasa terrane was part of the Gondwana continent, situated in the southern hemisphere.

During the Triassic and Jurassic, in relation with rifting and the formation of the Tethys ocean, Lhasa terrane migrated northward and eventually collided with the Qiantang terrane at the southern border of Eurasia.

In the late Jurassic – early Cretaceous, India rifted from Gondwana and in the early Tertiary collided with Lhasa terrane, due to the closure of Neo-Tethys.

Through highly complex tectonic mechanisms, the result of that active convergence was the formation of Himalayan orogen, the thickening of the continental crust (65 km in northern Tibet) and the uplift of the Plateau.

2.2.1. Geology of Lhasa region

The following information comes from Harris 1988, Pearce 1988, Yin 1988.

Lhasa terrane is delimited by two dismembered ophiolitic sutures, remnants of the oceanic lithospheres caught between Qiantang block to the north (Bangong-Nujiang suture) and India to the south (Yarlung – Tsangpo suture).

Southern part of Lhasa block is characterized by widespread exposures of the Cretaceous calco-alkaline Gangdese batholith and Eocene volcanic rocks, both due to the subduction and closure of Neo-Thetys ocean.

Carboniferous glacio-marine and volcanic formations and Permian limestones and sandstones outcrop in Lhunzub area.

In Lhasa district, Triassic is represented by calcareous and clastic deposits with volcanic intercalations. Volcanic rocks are also important in the lower part of Jurassic whose upper part is constituted by argillaceous limestones and sandstones. Cretaceous continental and shallow marine sandstones, siltstones and limestones, intercalated with volcanic formations, are well exposed in Lhasa terrane.

Yarlung – Tsangpo ophiolite is widely exposed south of Lhasa, particularly in Xigaze area.

To the south of Yarlung – Tsangpo suture, flysch deposits belong to Himalayan domain.

2.3. Ecoclimatology

Ecoclimatological data have been presented and described in previous papers (Malaisse et al., 2001; 2003).

Two main factors control the climatic characteristics of Tibet: the topographic configuration and the atmospheric circulation. A South-East – North-West orientated cross-section indicates a clear line with regard to mean annual rainfall, which varies between more than 2,000 mm in the tropical monsoon area and less than 50 mm in North-West. Climatic zones varies from arid polar alpine ice-snow zone to humid low mountain tropical zone. During winter, western winds are dominant; in summer, the southern and south-eastern parts of the plateau are exposed to warm, wet monsoon winds. The climate is very dry during wintertime. Schweinfurth (1956; 1984) and Chang (1981) have described the dry valleys of the Tibetan Himalaya. The Tsangpo valley appears as a subzone at the margin of two continental plates. There, annual precipitation is usually between 300 and 500 mm (Figure 2.4). The mean annual temperature is between 4°C and 8°C. The mean temperature during the warmest month varies between 10°C and 16°C. Sunshine is abundant and the growing season is longer than in the higher colder meadows. Around Lhasa, rains

are frequent from late June to early September (Malaisse et al., 2001). The atmosphere is severely dry 8 months of the year, and average annual snowfall is only 35 cm, due to the rain shadow effect whereby mountain ranges prevent moisture from the ocean from reaching the plateau. Western passes, *la* in Tibetan, receive small amounts of fresh snow each year but remain traversable all year round (Figure 2.5). Low temperatures are prevalent throughout the western regions, where most vegetation is low, and where wind sweeps unchecked across vast expanses of plain. The Indian monsoon exerts some influence only on eastern Tibet, whilst northern Tibet is subject to higher temperatures in the summer and intense cold in the winter.

2.4. About soil and Tibetan soils

2.4.1. Soil definition

Soil is defined as the epidermis and life support of emerged land. It is a formation of a few meters thick at most whose mineral and organic compounds, generally loose, differ from the original products under the influence of different factors and processes.

Ideally, this formation is natural, structured, often differentiated in horizons and a medium of biological activities in the presence of water and air.

Soil is thus a bio-reactor at the interface between the lithosphere, the biosphere and the atmosphere whose characteristics can vary in space and time. These definition and concepts can be summarized by the following formula:

Soil = a function of (Cl, p, r, v, o) t_1 + mt_2

with Cl for climate, p for parent rock (or material), r for relief, v for vegetation, o for soil organisms, t_1 for varying time of natural processes, m for human management and t_2 for time of this human influence.

2.4.2. Tibet and the World Soil Resources Map

According to these factors and the map at small scale (1:30,000,000) of World Soil Resources, soils of Tibet could be mainly:

– **Leptosols** for very shallow soils over hard rock or in unconsolidated very gravely material. These soils are very young or continuously rejuvenated by soil removal (erosion) and their characteristics are clearly dominated by the rock factor.

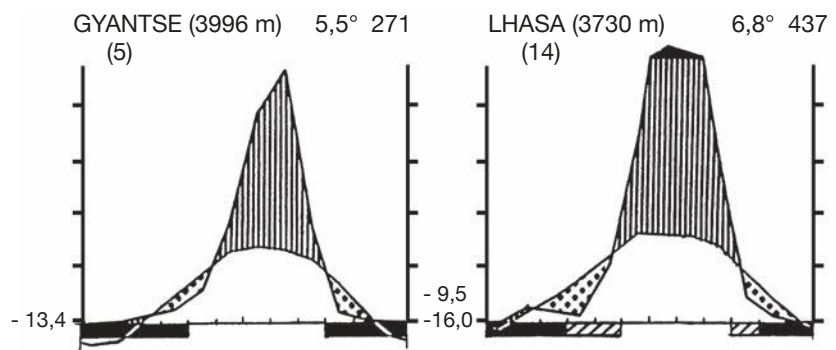


Figure 2.4. Omrothermic diagrammes for Lhasa and Shigatse (Malaisse et al., 2003, modified).

- **Regosols** for soils with very limited soil development. These soils are also very young or continuously rejuvenated but here, by soil deposits (colluviums) at foot slope. Their characteristics are influenced by the nature of the deposits and of course, the relief factor.
- **Cambisols** for weakly to moderately developed soils. These soils show generally a diagnostic Bw horizon, w for onset of weathering, identified by a change of structure (root influence) or of colour (iron individualization). They indicate that the position is relatively stable (safe from erosion) and that the biological activity is progressively increasing.
- **Calcisols** for soils with accumulation of secondary calcium carbonates. These soils result from a (re)precipitation of calcium carbonates (CaCO_3) and generally correspond to a climate with a dry season at the limit between sub-humid and semi-arid conditions. The original material is limestone or calcium-rich rocks.
- **Luvissols** for soils with subsurface accumulation of high activity clays and high base saturation. These soils show a diagnostic Bt horizon, t for clay (ton in German) or for textural, and, suggesting more stable weathering/leaching conditions within the soil, would be the most developed ones in T.A.R.

More locally, we can add to this list: histosols (soils of organic materials), anthrosols (soils with profound human influence, namely terraced soils), arenosols (sandy soils on rising dunes or granitic sand), fluvisols (soils in alluvial deposits) and gleysols (soils permanently or temporarily humid).

All these soils fit well this context of:

- high mountains and rock outcrops,
- slope, fluvio-glacial or aeolian (including soils developed on loessic material) deposits,
- a climate colder and dryer in the northern part and slightly warmer and humid in the south-eastern part.

2.4.3. A soil fertility assessment of five villages in Lhasa region

A soil fertility assessment was conducted in five villages around Lhasa by two FUSAGx students on behalf of the Kashin-Beck Disease Foundation.



Figure 2.5. The Chak pass in November 2007.

Tsingda and Wapuk are located in Pondo county, 60 km north-east of Lhasa along the Reting Tsangpo (river) as representative of a volcano-sedimentary geological context and semi-nomad tradition. Sheu and Lume are located in Nyemo county, 90 km south-west of Lhasa along a tributary of the Yarlung Tsangpo (Bramhaputra river) as representative of a granitic context and a more sedentary tradition. These 4 villages are located in endemic area's for Kashin-Beck disease.

Targye is located in Rimpung county, 130 km south-west of Lhasa and south of the Bramahpoutra river as representative of a sedimentary context, a semi-nomad tradition but also as a region unaffected by the Kashin-Beck disease.

In this survey, it is noticed that cultivated soils are brownish black to dull yellow orange, 15/20 to 60 cm thick and slightly stony to stony.

The main laboratory analyses for topsoil give the following results:

- a texture corresponding either to loamy sand, sandy loam or (silt) loam;
- pH_{water} values never below 6.1 and frequently between 6.9 and... 8.2;
- a total carbonate content of maximum 1.8%;
- total organic contents between 0.8 and 3.9% and C/N ratios between 8.0 and 13.5;
- cation exchange capacity (CEC) measurements between 8.5 and 20.8 cmolc/kg⁻¹, the highest values correspond to higher carbon contents and the lowest to loamy sand texture;
- base (Na, K, Mg, Ca) saturation ratios frequently higher than 80% or reaching 100%;
- and a quick estimation suggests clays with a relatively high CEC.

All these indicators are those of weakly developed soils (leptosols, regosols and fluvisols), very shallow and stony in some places, slightly acid to basic, with a relatively variable organic content but a satisfactory index of mineralization, which show a good CEC (potentiality to retain elements) and are quantitatively well base saturated (effectiveness of this potentiality or concept of fertility). However, soil depth and stoniness influence greatly the fine earth percentage and thus the real soil contents and moreover, fertility is not only a question of element quantity but also of nutrient availability in well balanced proportions.

Amongst land improvement, removal and piling up of stones, man-made terraces, (old) irrigation channels and levees along rivers are observed.

Secondary calcium carbonate accumulations mined out for wall liming, loamy soils mined out for bricks and charcoal remains in soil are other anthropic features.

Different vernacular expressions exist to qualify high, medium and low soil suitability like «*rap, ding and tha*» in Tsingda and Wapuk, «*saka shean, aui sagyus, karpel*» in Sheu and «*gya wang chepo, raku and ghashon*» in Lume. These farmers' appreciations correspond fairly well to the scientific criteria of diagnosis.

2.5. Plant and vegetation diversity

2.5.1. Flora diversity in South Central Tibet

China is among the world's richest countries in terms of plant biodiversity. Besides the abundant flora, containing some 33,000 vascular plants (30,000 angiosperms, 250 gymnosperms, and 2,600 pteridophytes), there is an extraordinary diversity, as well as a large pool of both wild and cultivated germplasms.

According to Wu and Feng (1992) on the Tibetan plateau there are over 12,000 species of 1,500 genera of vascular plants, over 5,000 species of 700 genera of fungi.

Our knowledge of the flora of Tibet results both from rare foreign expeditions (Ward, 1935) and from several expeditions of the Chinese Academy during the last decades (Zhang et al., 1982). The flora of Tibet (Plate 2.2) comprises 164 families, 1,145 genera and some 5,296 species of Spermatophyta, with about 955 endemics (Wu et al., 1981). According to the areal

types, this flora may be classified under five major geo-elements: the north temperate (7.4%), the central Asiatic (7.0%), the Tibetan (5.8%), the Sino-Himalayan (54.0%) and the tropical (12.9%) geo-element (Zheng, 1983). Five floral regions have been recognized, namely from south-east to north-west: the indo-malayan – belonging to the paleotropical empire –, the sino-himalayan, a transition zone, the Tibetan and the Central Asian floral regions belonging to the holarctic empire (Zheng, 1983).

The phytogeographical background of the studied area needs also to keep in mind the diversity of the various vegetation belts. It has been concluded that neither the traditional altitudinal zonation (Troll, 1939; 1972), nor low mountain latitudinal zonation can be applied to T.A.R. Because of its special vegetation characteristics, the Tibetan plateau as to be recognized as an independent region, for which a special high plateau zonation as been proposed (Chang, 1981; 1983). From south-east to north-west the following belts occur: tropical evergreen forest, tropical monsoon forest, mountain evergreen broadleaf forest (upper limit of 1,800 m), mountain mixed forest, mountain coniferous forest, subalpine thickets, high-cold meadow, high-cold steppe, semi-desert and high-cold desert.

The Tsangpo valley is generally recognized as a xeric shrubland-steppe plateau zone (Schweinfurth, 1957; Chang 1981). With increasing altitude other vegetation belts are observed which have been studied regarding their flora and vegetation (von Wissmann, 1961; Hartmann, 1972; 1987; 1990; 1995; 1997; Zheng et al., 1979; Li 1980; Billiet et al., 1986; Wu et al., 1995; Miehe, 1997; Dobremez et al., 1998). Moreover spatial heterogeneity of man activity provides a second way of differentiation. According to land use patterns four ethno-ecological zones may be recognized, namely: the urban zone, the peri-urban zone, the agricultural zone and the pastoral zone.



Figure 2.6. Tibetan children helping during botanical exploration.

2.5.2. Vegetation diversity in South Central Tibet

Approach of vegetation diversity has taken place both through surveys (Figure 2.6) and from interviews of the peasants. The diverse vegetation units recognized (Plates 2.3 to 2.6) were allocated into two main groups, namely terrestrial and half-aquatic or aquatic. The formers allow to distinguish closed versus open vegetation units. For the later, lakeside regarding lotic conditions are to be kept apart. The diverse habitats related to streaming water are recognized by local people under the name of *doutchou*.

Some examples are briefly described below.

- Closed woody unit: the *Sabina przewalskii* Kom. forest

Those coniferous forests presented formerly wider extension. Remnant forestry patches were noted in the Reting valley, namely in the vicinity of Tsangchung. Several local uses, mainly collecting of branches as incense (or *pama*) and clear cutting for firewood, maintain a heavy anthropic pressure on those forests, whose rarefaction increases continually.

- Open forest unit: *Hippophae salicifolia* D.Don open forest

The occurrence of those open forests is restricted to narrow ecological conditions, mainly on alluvial sites supporting contrasting hydrological conditions due to the large amplitude of fluctuations of the ground water according to seasons. The presence of such woody patches was noted on islands and stream banks in several valleys, namely along the Nyemo river, in the vicinity of Nyemo, as well as in the Reting valley.

- Woodland unit: Willow-birch woodlands (*Salix sclerophylla* Anders and *Betula* sp.)

This unit is linked with poor acidic soils in the lower part of the subalpine belt. Birch woodlands are not at all rare. The presence of a *Ribes* sp. was observed. For the area under consideration, such woodlands were quoted between Tsingda and Tsangchung.

- Shrubbery steppe unit

A regressive stage of several woody climax units is realized by several shrubby vegetation units. A preliminary set of shrubs has been listed, including spindle-tree, currant-bush, jasmine and honeysuckle (surroundings of Djamey).

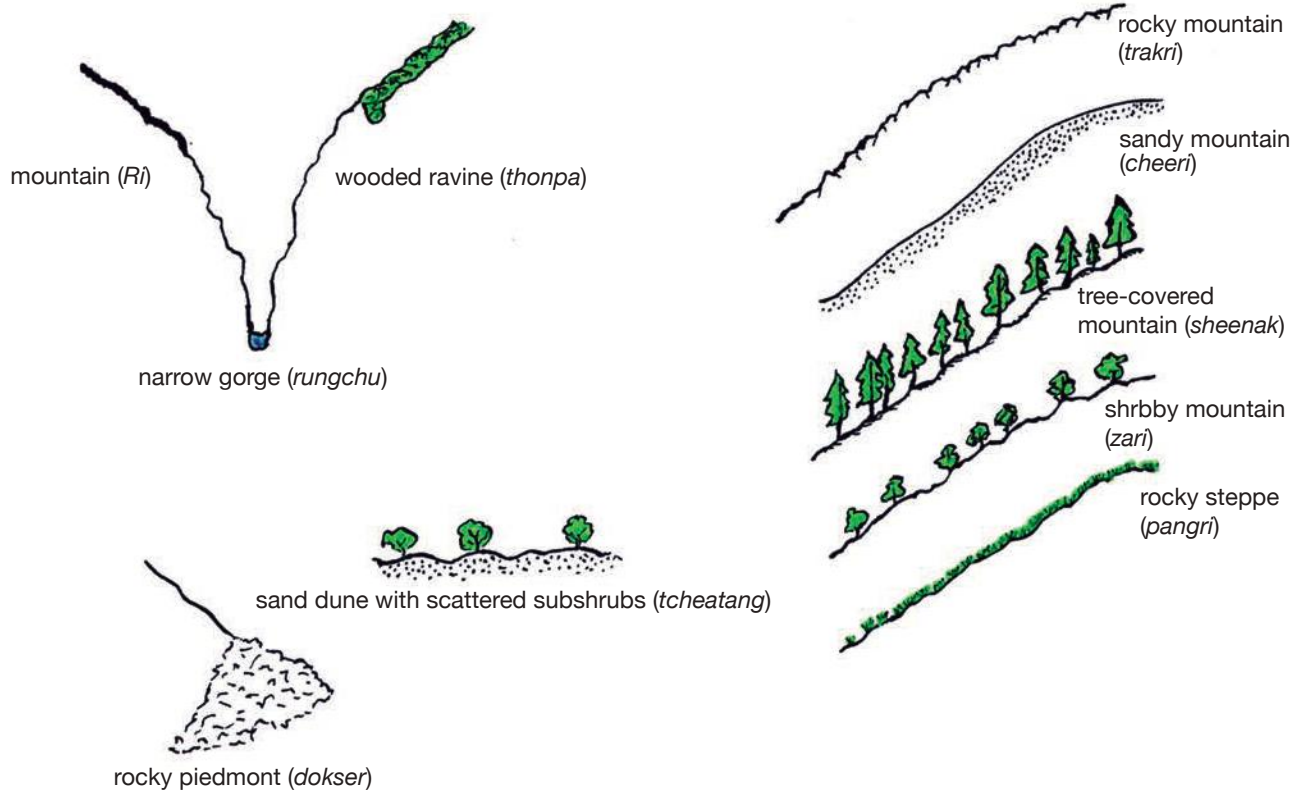
- Pioneer thicket unit: *Myricaria germanica* (L.) Desv. pioneer thicket
Those thickets, 1-2,5 m height, are linked to riversides, namely gravely



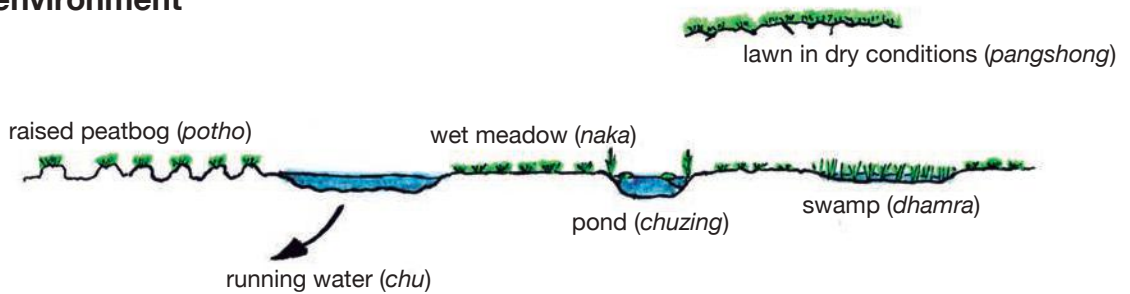
© Matisse François

Plate 2.2. The reference voucher collection of the flora of South Central Tibet. Some plants collected during the surveys. A. *Clematis tangutica* (Ranunculaceae): habit – B. *Clematis tangutica* (Ranunculaceae): flower – C. *Pedicularis longiflora* var. *tubiformis* (Scrophulariaceae) – D. *Gentiana lhakangensis* (Gentianaceae) – E. *Arisaema flavum* (Araceae) – F. *Viola biflora* (Violaceae) – G. *Myricaria germanica* (Tamaricaceae) – H. *Meconopsis horridula* (Papaveraceae).

Mountain (*Ri*) environment (*Kul*)



Valley (*Kil*) environment



Country (*Dongsep*) environment

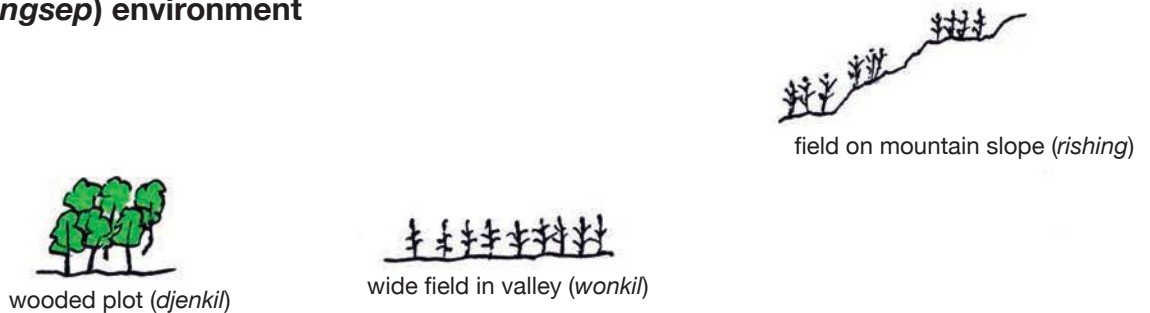


Plate 2.3. Main landscape units recognized by Ü peasants in South Central Tibet (Malaisse et al., 2003, modified).



Plate 2.4. Some vegetation units of South Central Tibet. A. *Sabina przewalskii* forest or *shennak* (Reting) – B. *Hippophae salicifolia* open forest (Tanggo) – C. Birch-willow woodland, winter aspect (Dumta) – D. Birch-willow woodland, summer aspect (Tsingda) – E. Raised peat bog or *poto*: summer aspect (Batchen) – F. Raised peat bog or *poto*: winter aspect.

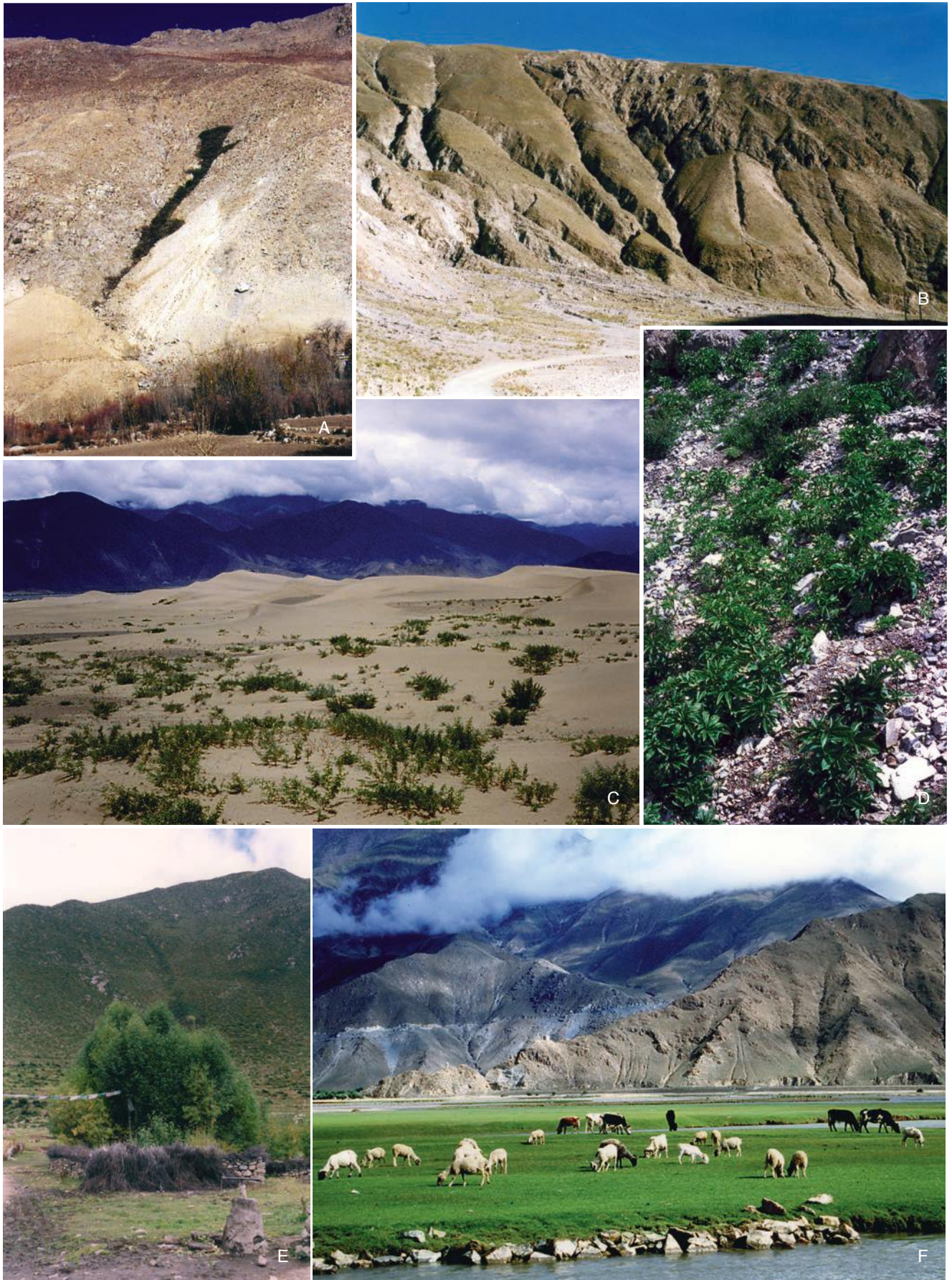


Plate 2.5. Some vegetation and landscape units of South Central Tibet. A. Wooded ravine or *thonpa* – B. Rocky steppe or *pangri* – C. Sand dune with scattered subshrubs or *tcheatang* – D. *Arisaema flavum* clumps on stony scree-covered slope or *dokser* – E. A wooded plot or *djenkil* – F. Wet meadow or *naka*.



Plate 2.6. Some vegetation and landscape units of South Central Tibet. A. Rocky mountain or *trakri* – B. Rocky mountain with dominance of red schists or *martrakri* – C. Sandy mountain or *cheeri* (Gongkar) – D. Himalayan-alpine meadow or *pangri* – E. *Berberis* sp. shrubby steppe or *zari* – F. Dense steppe on rocky slope – G. Narrow gorge or *rungshu* – H. Slope peatbog – I. Shore of pond with *Nymphaeoides* sp. or *chuzing* – J. *Hippuris vulgaris* swamp or *dhamra*.

riverbeds. The pinkish-red inflorescences developed in June-July easily pinpoint this typical vegetation unit.

- Meadow units

Fields (agro-ecosystems)

Reap fields versus pasture-lands are a classic approach of differentiation. The first are very rare, while the later are common. Grazed meadows allow to be split according to two main ecological factors, soil richness (rich, medium and poor) and soil hydration (dry, fresh, moist and very damp). For instance, *Caltha palustris* L. var. *himalensis* (D.Don) Mukerjee occurs on damp places on grazing grounds; *Potentilla anserina* L., locally well known as *toma* or *droma*, on fresh soils. Short overgrazed lawns, located near streams, are dominated by sedges and called *naka*.

- Steppe units: the biotite rocky steppe

Steppe vegetation of Tibet has been approached by Schweinfurth (1957) and reviewed by Wang (1980; 1988a; 1988b), who recognizes four types of community, namely the tussock-grass, the rhizome-grass, the rhizome-sedge and the semi-undershrub steppe. If the former is the widest distributed at high altitudes and the most typical, the last one occurs frequently in the area under consideration. In fact, several units occur according to lithology, altitude, exposition, etc. Steppes extend until 5,200, even 5,400 m elevation.

Biotite rocks exposed to the south-east carried open vegetation. In the crevices, a rare sandy substratum accumulates which permits the development of dense carpets of *Selaginella pulvinata* (Hook. & Grev.) Maxim. and *Corallodiscus kingianus* (Craib.). Less frequent are *Aleuritopteris argentea* (S.G.Gmel.) Fée and *Lepisorus*. Locally shrublets, such as *Ceratostigma griffithii* C.B.Clarke and *Lonicera tibetica* Bur. & Franch. are scattered (Plate 2.7).

- Sandy deserts

They occur in the Tsangpo valley, between Neudong and Djamey, around km 41. Their origin relies on large amounts of sand deposited along the Tsangpo during swellings, which are later reworked by wind in sand-dunes. Sandy slopes on mountain are also observed, their local name is chiri, whilst at piedmont with scattered plants, such as *Arisaema flavum* (Forsskål) Schott, locally recognised as *katang*.

- Aquatic and half-aquatic vegetation units

Those habitats are of primary interest, as they provide water for humans. Water of streams and rivers are primarily used for toilette and washing.

Chemical characteristics of the lakes of T.A.R. have been discussed by Fan (1980). On a general pattern the mineralization increases from south-east toward north-west, fresh water lakes becoming gradually salt water lakes and then saline lakes. Lakes' shores vegetation

relies of this pattern; nevertheless these ecosystems have not been approached during our survey.

Total area of marshes in Tibet is about 11,000 km² (Zhao et al., 1980). They have been classified into two major types (gley fens versus peat moors) and eleven subtypes according to geomorphology situation and the regime of water supply. Their upper limit is about 5,200 m above sea level. There study is beyond the scope of our work.

2.6. Animal husbandry

Animal diversity is also high in China. According to Wu and Feng (1992) on the Tibetan plateau there are 210 species belonging to 29 families of mammals, 532 species of birds, and 115 species of fishes. The Tibetan steppe hosts a tremendous wildlife; whose mammals are presented in details by Schaller (1998).

Although dogs, cats, ducks, chickens and geese are present in all Tibetan villages, yaks, sheep and horses comprise the main animals in the total agricultural zone. In the intensive agricultural zone, black pigs are often found in households; these pigs are not local-bred but originally brought from Russia (Daniggelis, 1995).

The yak is one of main participants of Tibetan economy; it is most favourably adapted to the high altitude and cold climate. Animal products from the yak are hair (tents, clothes), milk (children drink, butter, cheese), dried meat, skin (clothes, leather bags, shoes, leather ropes), bones (manufactured glue) and dung (fuel for heating and cooking).

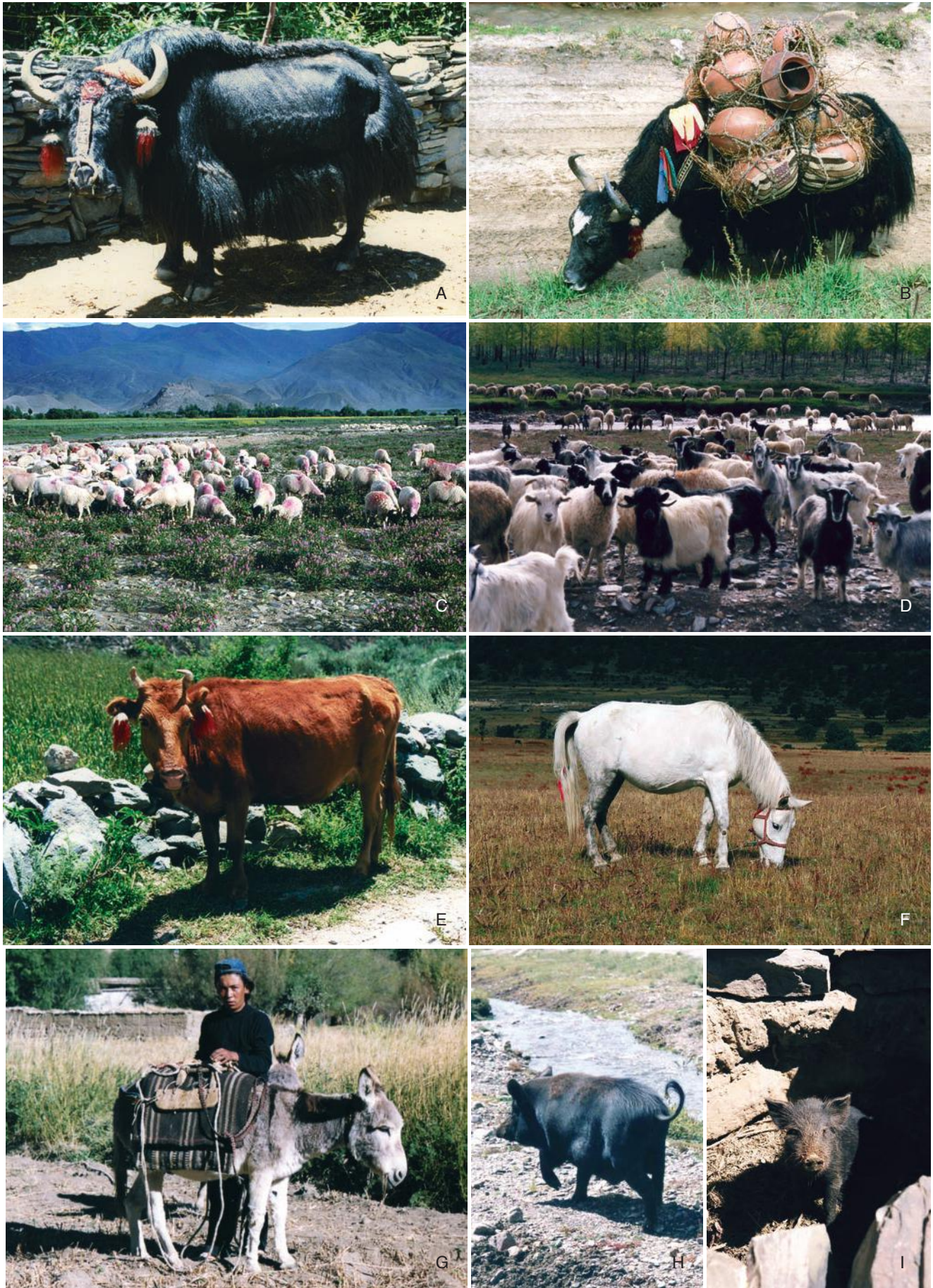
The Tibet Autonomous Region is one of the five pasturelands in China. Natural grasslands total 82,052 million hectares, or 23% of the national total. In 2001, the meat and dairy production of Tibet got close to 150,000 tons and 200,000 tons respectively.

The variety of livestock in T.A.R. includes yak, cattle, *dzo* (offspring of a bull and a female yak), horse, sheep, goat and to a lesser degree pig, donkey (Plate 2.8). Livestock products are major resources and the materials for the improved livelihood of the Tibetan people, and also the main raw materials of the textile industries. They play an important role in the Tibetan economy. Tibetan livestock husbandry enjoys a history of several thousand years and herdsmen moving about in search of pastureland are frequent. Animal husbandry remains of importance, primarily in the nomad zone. By 2000, various livestock numbered 23 million head, including 4 million head of yaks, 980,000 oxen, 270,000 *dzo*, 1.55 million milk cows, 140,000 horses, 11.4 million sheep, 5.77 million goats and 180,000 pigs. The output of meat products reached 149,300 tons, and the production of sheep wool came to 8,629 tons (Plates 2.8 and 2.9).



© Malaisse François

Plate 2.7. The biotite rocky steppe. A. General view: Lume – B. More detailed view: habit – C. *Ceratostigma griffithii* – D. *Aleuritopteris argentea* – E. *Lonicera tibetica* – F. *Corallodiscus kingianus* – G. *Selaginella pulvinata*, autumn aspect – H. *Selaginella pulvinata*, summer aspect – I. *Lepisorus waltonii*.



© Matisse François

Plate 2.8. Livestock in South Central Tibet. A. Yak – B. Dzo – C. Sheep – D. Goats – E. Cow – F. Horse – G. Donkey – H & I. Pigs.



Plate 2.9. Sheep wool technology. A. Heap of sheep fleece – B. Wool twist (nomad zone) – C. Distaff – D. Card – E. Wool, card and material – F. Spinning wheel – G. Beige dyed wool by the Himalayan walnut's husk or *tarbak*.

Chapter 3

The land-ecosystem

Camille Chasseur, François Malaisse, Eric Haubruge, Rinzen Wangla, Carl Suetens, Rinchen Lobsang, Lakpa Wangdu, Stéphanie Chasseur, William Claus, Françoise Mathieu

Introduction

Before 1960, the Tibetan society was built on a feudal system. The majority of the grounds belonged to the first leader of the country, the Dalai Lama. The fertile areas were divided into properties governed by a high-ranking official from the government, a monastery, a noble or by the State itself. The lands were lent to peasants who paid taxes generally in the form of goods or services. As long as they kept to their commitments and had a descendant, the peasants could not be expelled. The ground was subjected to the principle of joint possession and was transmitted by heredity. In the villages, each family had the same status except the servants. The heads of families constituted a council taking decisions for the whole village and made respect the obligations and right of everyone. One man was at the head of this council and played intermediary between the peasants and the owner. He was chosen by heredity, election or for his seniority. The agricultural work was carried out collectively but each family benefited from its own income (Stein, 1962; Goetghebeur, 1992).

After the creation of the People's Republic of China by Mao Tsé-Toung in 1949, the Popular Army of Liberation of China came to Tibet in 1950. Land reforms, carried out in every region and province of the New China, were extended to the Tibetan areas, except the Tibet Autonomous Region. In these first reforms, lands were directly confiscated from the previous owners and distributed to every family on the basis of the number of family members. The size of the agricultural fields distributed to people was based on the total size of the agricultural land of the village: there was administratively border-line from village to village. But in T.A.R., according to the 17 points Agreement between Beijing and Lhasa signed in 1951, Beijing planned not to interfere in Tibet's land and social reforms (Chasseur, 2008).

In 1959, after the departure of the Dalai Lama, T.A.R. had also to conform to the second step of the land reforms decided in China by Mao since 1957. This reform,

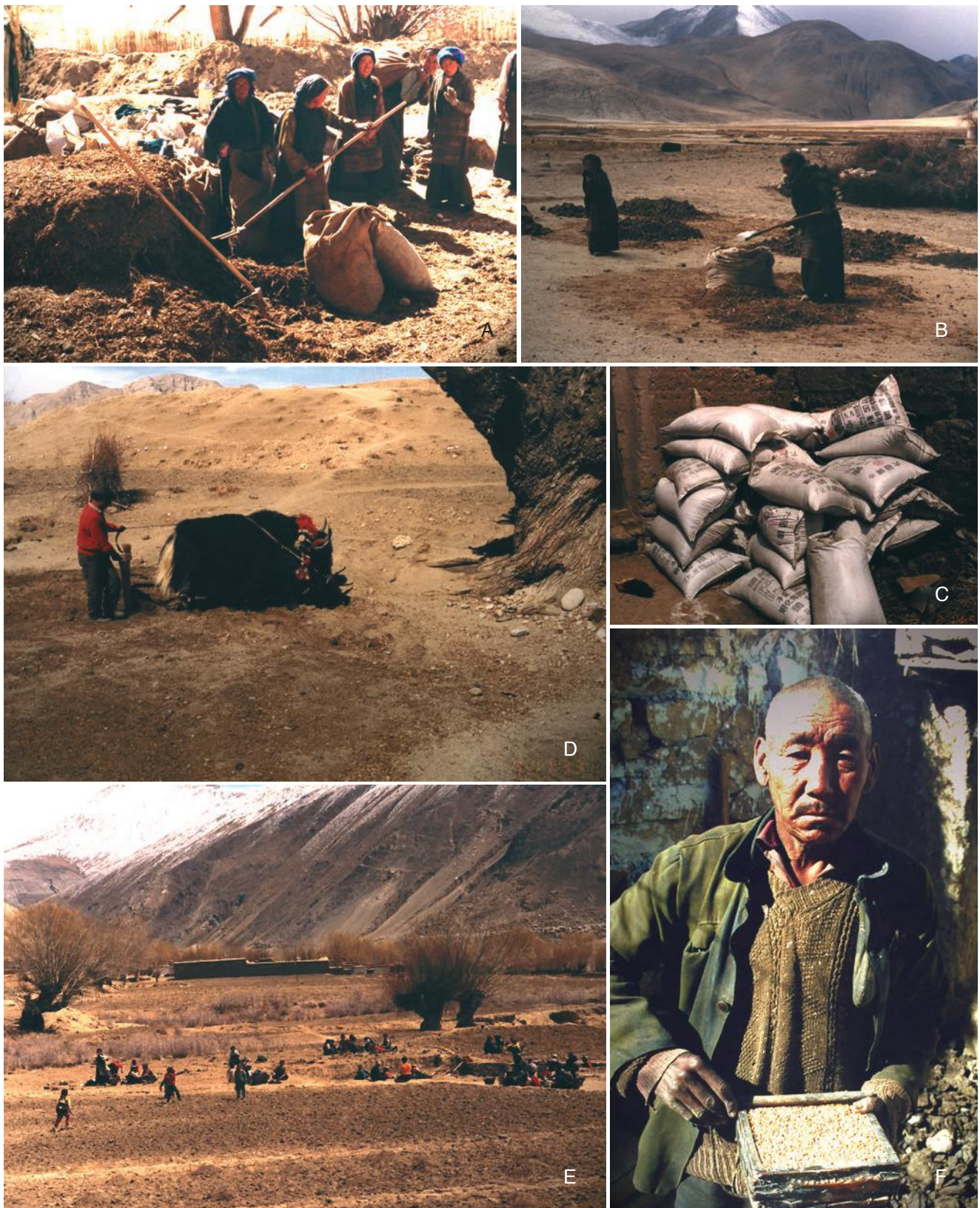


Plate 3.1. Traditional agriculture in Yarlung Tsampo basin: tillage and sowing. A. In the Yarlung valley, at the end of winter (March-April), the ground is prepared and enriched with organic manure. Mainly of human origin, manure is bagged – B. Manure manually spread on field – C. Mineral fertilizers more recently introduced – D. Traditional tillage with yaks or *dzô* (hybrid female yak with a bull) is a still used practice in T.A.R. – E. Spring barley is planted mid-April (to May) and all families are in field during spring works – F. Tibetans use a wooden box to measure 1 *bô* (1 *bô* = \pm 1 kg) of cereal to be planted, and plots of land are still measured in *mou* (649 m²). Another big wooden box is used to measure 1 *keru* or *ka* corresponding to \pm 14 kg, and generally, the farmer plants 1 *keru* of barley seeds in one *mou*. The measurements in hectares are only used for forests and collective vast lands.

called the «Great jump ahead» introduced the popular communes. All the arable lands, including the trees, were collectivized. Only some animals remained private property. The tools, the machines, were also gathered in collective goods. All the villagers worked together, in collective work units, under the upper communist party government leadership. The peasants were remunerated according to the work provided. They had to sell their surpluses of grains to the State who determined the prices. If harvests were bad, the State committed to sell grain to the peasants at the lowest prices. Executives were consequently necessary to manage the work, which generated the bureaucratization of the rural world (Bergère, 1989). This collective system did not work well and was very unpopular with the population.

In 1980, the policy of Deng Xiaoping favoured again the privatization and the abolition of the popular communes. All the fields were re-distributed to individuals. The size of agricultural land distribution was determined per person, and according to the total size of agricultural fields available in each village⁽²⁾. However, according to the Chinese constitution, the owner of agricultural land is the State and the farmers are users. Therefore, the farmers have no rights to sell their land, but are allowed to rent⁽³⁾.

Presently, the current agricultural land ownership in T.A.R. is still the same since 1980. Agriculture and breeding are managed by the State via governmental commissions, each sector having its own administration and its research centre. Each prefecture or municipality has offices working in these fields. Correspondents pass on the information between the prefecture and the counties. The administrative villages are managed by a council with a leader assisted by a deputy and a secretary who are elected every three years by the villagers (Osmaston, 1998).

The national policy aims at the intensification of the production of cereals in order to answer the need of an increasingly dense population. This policy brings a greater material prosperity but risks in the long run to involve serious environmental problems such as the impoverishment of the grounds becoming more vulnerable to erosion by the wind (Osmaston, 1998). Moreover, in spite of an agricultural policy that wants to be general in the whole T.A.R., in practice, in term of means, we still note a strong disparity between the villages located in the valleys far from the urban centres and the suburban areas. Rural areas remain one of the poorest regions in China and it is in these regions that the Kashin-Beck disease prevails particularly.

3.1. Barley cultural practices

In T.A.R., agriculture is strongly concentrated in the basin of the Yarlung Zangpo and its affluents. Altitude generally ranging between 2,500 and 4,500 m, weather conditions and poor soils prevent from cultivating a large diversity of species, and food is rather poor and little diversified (Plates 3.6, 3.7).

In 1995, in our first mycological studies we investigated more accurately the agricultural environment in order to better understand the living conditions in the villages, and to determine the potential crucial stages of fungal contamination in food chains. Several missions were made in different regions of T.A.R. (Lhasa, Shigatse, Lhoca prefectures), and were focused on food habits, traditional and still autarkical agricultural practices, cultivation chronology, and storage in a very constraining environment. The impact of the Chinese agricultural policy was also approached, especially what could be related to the fungal contamination of crops: selection and disinfection of seeds in the counties, use of fertilizers and biocides in fields, introduction of new cereals (wheat, corn), and mechanization near built-up areas.

We mainly focused on spring barley grain cultivation and its storage because barley is the staple food consumed by Tibetan villagers. It is also the oldest and traditional cultivation, which is important because KBD existed long before the new agricultural practices and the introduction of new cereals from 1960 on.

For Vavilov (1926), T.A.R. is an original genetic centre of barley (Figure 3.1). For the last 15 years, Chinese scientific expeditions have been set up to further define these taxa in this region. For Zhang et al. (1994), three varieties of barley are well represented in T.A.R., either in valleys or high table-land: *Hordeum vulgare* ssp. *vulgare* (HV), *Hordeum vulgare* ssp. *agriocrithion* (HA) and *Hordeum vulgare* ssp. *spontaneum* (HS). Many landraces were collected in T.A.R. (Ma Dequan, 2002)



Figure 3.1. Diversity of barley in T.A.R.

⁽²⁾ The size of agricultural land by person in the villages is really depending on the geographical conditions. In general, in some villages situated in vast valleys, each farmer can have 10 Mou (one Mou = ± 625 m²) but in some other villages situated in smaller valleys, a farmer will have only 2 Mou.

⁽³⁾ If farmers lost their land for public constructions, such as roads... They would get compensations in cash, but the amount of money is decided by the politicians. Farmers have no rights to refuse if the state asks to use the land for public purposes.



© Chasseur Camille

Plate 3.2. Growth and harvest of barley. A & B. Spring barley, well adapted to the mountain climate is the oldest and main cereal cultivated by Tibetan villagers – C. In the Yarlung Tsampo valley, after harvest, barley is generally kept in bundles up to maximum 20 days on field – D. Ears are traditionally beaten with animal help – E. Ears beaten with flails.

especially hullless ones. Among them, the «six-rows» (three spikelets on each axis of the stem) strongly dominate compared to the «two-rows» (ear made up of two lines of spikelets). Most of them have spring growth-habit, and a large diversity in the kernels color (black, purple, grey, green and carneus) is noted.

Despite some differences in the chronology cultivation, mainly due to the altitude and climatical difference, works in field begin in spring, with the tillage. At the end of the winter, the ground is prepared and enriched with organic manure. At this period, we can observe the ceaseless comings and goings of people, sometimes helped by donkeys, with organic manure in bags to deposit on field just before the tillage (Plate 3.1). Compost is mainly of human origin. Wood is rare and expensive, and animal excrements are rather used for heating and food cooking, like yacks dung which are collected and dried in the sun (Plate 3.5.F). This practice has important consequences on the contents of organic matter in cultivated soils.

We also noted, like Osmaston (1998), that «no fallows are used, and in view of the preponderance of grain crop (around 80-90%), it is not clear whether any crop rotation is being followed».

More recently, mineral fertilizers have been introduced (Plate 3.1.C). In 1998, in 22 villages in 3 prefectures (Lhasa, Shigatse and Lhoca), we noticed the use of two main chemical fertilizers. The «white» one (urea 40%), used just before planting seeds and the «black» one (P_2O_5 , N, $MgSO_4$) less used because more expensive. A third chemical fertilizer (Fenshiebo), a mixing of minor elements, was sometimes sprayed once on cultures but presently, it is no longer used.

In T.A.R., spring barley is planted from mid-April till May. The government proposes to the county to treat seeds against moulds before planting. For families which are not too far from the county town seeds must be brought to eliminate little stones and bad grains with mechanized sieves. This first operation allows a selection of the best seeds. Wealthy families can also treat the seeds with chemicals. A fungicide with carboxin and thiram is mainly used, replacing the triadimefon previously used in some villages. A pink dye was added to the treatment to avoid the consumption risks.

During the growth, pesticides are nowadays used on field. During a mission in 2000 we mainly registered insecticides (deltamethrine, parathion, dichlorovos and other organophosphorous insecticides), herbicides like triallate 40%, and nematicides. But we did not notice any fungicide for treating barley on field, except thiophanate methyl, but this product is mainly used for wheat, and yet, not frequently in the villages.

According to the areas, harvest (spring barley) is made at the end of August or September (Nyemo for instance)

till October (Rimpung for instance). After harvest, barley is generally kept in bundles up to maximum 20 days on the field, for drying. Then, ears are traditionally beaten with flails, but nowadays, this operation is sometimes mechanized (Plate 3.2). After harvest, naked grains are put in bags or other containers, often the same day, and stored at home. Different traditional sorts of containers are used, as yak wool bags or large baskets. These days, plastic bags are most often used. These bags often weigh about 50 kg and are put in the store-room, generally raised on stones or pieces of wood. Rich families have the possibility to store grain during 2 or 3 years, but generally villagers run out of grain during the first year. The conditions of the storage may be very different according to the families (Plate 3.3).

Since the land reform in 1960, the government has introduced and encouraged in T.A.R. new cultivations like winter and spring wheat, corn, and colza (Plate 3.4), less adapted to high altitude conditions. On table 3.1, information collected on different cultivations made in a little village of Medrogongkar in 1995 are presented.

Table 3.1. Seed results obtained during a survey in 1995, and the cultivation timing, in a little village on a SE slope in the county of Medrogongkar. Colza was primarily produced to be sold.

Cultures	Seeds planted (kg)	Planting	Harvest	Use
Barley	2500	April 15 th /May	October	c
Colza	2400	June	October	s, c
Beans	750	April	October	s, c
Wheat*	750	before barley	October	c
Potatoes	500	June	October	c
Radishes		June	October	c

*= first year; c = consumed; s = sold

3.2. Other cultivations

The kitchen garden also occupies a significant place in the rural economy. The villagers cultivate potatoes, beans, and some green vegetables like radishes, spinach and Chinese cabbage. Wild plants are also a frequent food supplement.

Potatoes and radishes have an important part in the Tibetan diet. Radishes are actually big turnips. After harvest, these two vegetables are stored for winter, in holes of about 1 meter deep, dug in the ground. Storage can last maximum 7 or 8 months in these conditions. Beans are also cultivated. Sometimes they are planted with barley and later harvested and stored together. In some villages, beans are only cultivated for animals. In others, villagers explain they appreciate *tsampa* (see § 3.4) taste prepared with beans.



Plate 3.3. The traditional store-room. After harvest, naked grains are put in bags or other containers, often the same day, and stored at home. For grain, different traditional sorts of containers are used. A. Large baskets – B. Wool yak bags – C. Presently, plastic bags are mostly used – D. The store-room is generally very cluttered up with a variety of stored products. These comprise: E. Potatoes – F. Dried turnips – G. Dried meat. – H. A room where the family gathers for various activities. Women are sieving pea crop.



© Matisse François

Plate 3.4. Colza is the second cultivation in South Central Tibet. A. Field – B. Ass transport – C. Silques beaten with flails – D. A flail – E. Grain winnowing – F. Seed drying.

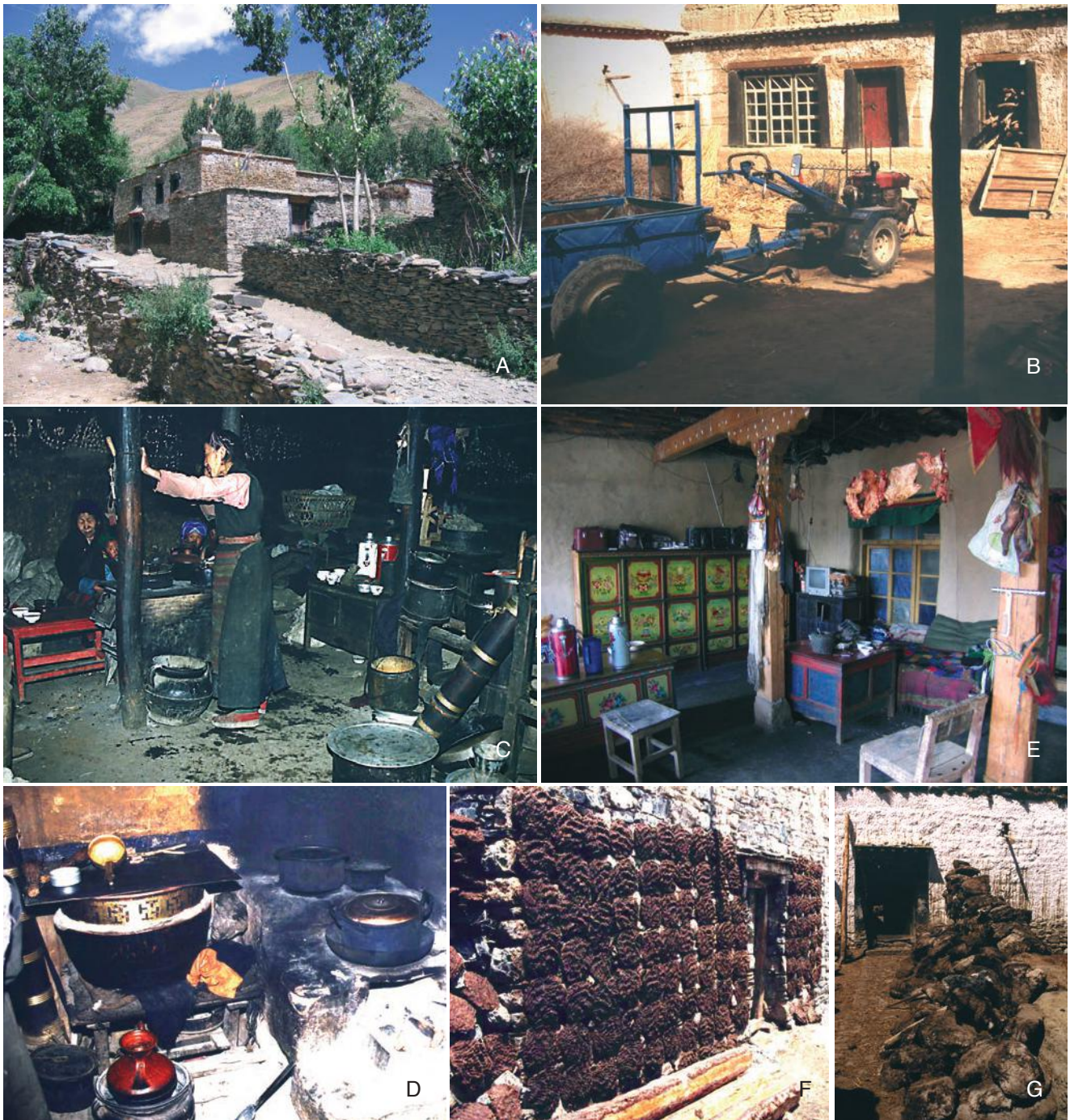
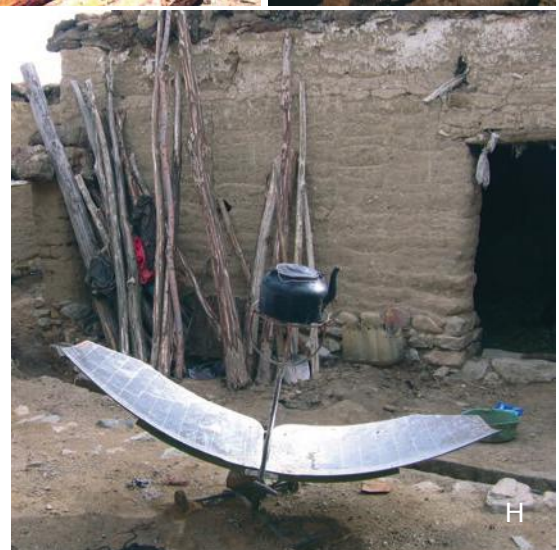


Plate 3.5. The Tibetan dwellings in the villages. A & B. Little villages in the valleys far from urban areas, are often composed of 20-30 families living in traditional buildings built around an inside courtyard. The living conditions are sometimes still similar to those of T.A.R. before 1960. The kitchens, without only a hole in the roof for chimney, were very smoked out, and inhabitant make drawings on the walls blackened by smoke – C & D. Among the principal kitchen utensils, the traditional churn and the beautiful water cupper tank – E. In the richest families, the dwelling is more spacious, with beautiful traditional furnitures – F. Wood is rare and expensive, and animal dejections are used for heating and food cooking, like yacks dung which are collected and dried in the sun on the walls – G. In some regions, peat is used for cooking – H. Presently, we also see in the inside courtyards a simple and astute solar heating which allows to boil water.



3.3. Breeding

Traditionally, nomadism is quite common in T.A.R. Nomad activities are focused on breeding and trade. The livestock consists mainly in yaks, well adapted to altitude, sheep and goats. In the villages, farmers have a small livestock. In the large square houses, families live on the first floor, the ground floor being kept for the cattle mainly yaks, *dris* (the female of the yak), oxen and *dzos* (*zho*). *Dzo* is the hybrid female yak with a bull. Stronger and especially quieter than the yak, it is more adapted for tillage or transport of loads. The female *dzo* is called *dzomo* or *zhom*, and gives more milk than *dris*. Inside farms courtyards, we can also see goats, horses, donkeys, sheeps, pigs and poultry. When the season allows it, the cattle of the village is gathered and taken to the pastures outside the village by children carrying a sling and accompanied by a dog to protect the herd (see also chap. 2).

3.4. Food habits in the villages

When visitors arrive in a village and are invited to enter a house, a cup of butter tea is immediately offered. And tradition wants the cup to be unceasingly filled. Butter tea is the national drink in the South Central Tibet, and villagers do not hesitate to drink scores of cups per day. Formerly, the various ways of preparing tea and the different ways to serve it according the social rank were explained by the explorer Alexandra David-Néel (1993). In the villages we visited, tea is prepared in a simple way. The tea, after being boiled, is poured into a churn to be mixed vigorously with butter and salt (Plate 3.6). The result does not taste bad but is more similar to a soup than to the tea we drink in Europe.

Tsampa is the staple food for Tibetans. To make *tsampa*, first the small stones are removed from barley grain (Plate 3.6). Then, barley is washed, dried, and poured in large frying pan to be slightly roasted. Sand is often added in the pan to prevent the grain from burning. Then, it is ground in the village mill or with a small individual mill. *Tsampa* is generally stored in large yak skin bags. In the kitchen, beautiful ornamented *tsampa* boxes can be seen (Plate 3.6.D). *Tsampa* is mainly consumed in the form of dough balls, rather dry, obtained by mixing with fingers the *tsampa* and a little butter tea. But *tsampa* is also used in other dishes such as soups with radishes or turnips (Plate 3.8).

To avoid *tsampa* spoilage, grains are ground in the village mill generally 3 times per year on the average. Sometimes, some families have their own mill in the kitchen. Villagers of Kargya (Medrogongkar county) for instance explained that *tsampa* sometimes smells bad. First, *tsampa* stored in the kitchen smells like «ground». Second, *tsampa* kept in the storage room for a longer period smells «old». This spoiled *tsampa* is given to animals in rich families, but is often eaten by poor people.

Momos are also a traditional dish of the Tibetan cooking. In the Yarlung Tsampo valley, *momos* are sometimes just a steamed dough made with flour and water, and eaten with sauces and vegetables. But in T.A.R., *momos* are traditionally small steamed pies stuffed with meat, vegetables or sometimes also with cheese (Plate 3.7).

Soups are also appreciated and diversified. They are generally meat-based (except for religious), thick and may constitute a meal alone. Among them, the most popular in T.A.R. is the noodle soup (*gyatoup*) (Plate 3.7), and the *tsampa* soup with radishes or turnips.

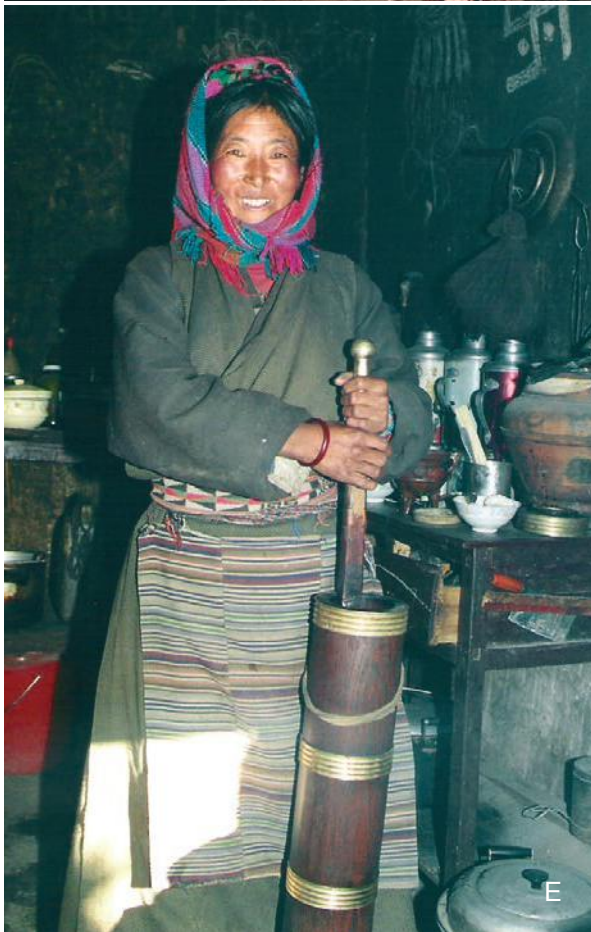
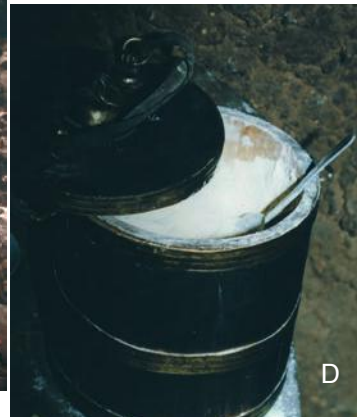
Nevertheless the diet of Tibetan peasants is far from diversified (Plate 3.8).

In the villages, breeding allows to complete food with animal products, mainly eggs, or meat which is preserved in drying it in air. Milk, permits to make the butter, abundantly used for the tea (but butter mixed with ashes is also used as face and hand-cream. Butter is also coloured to make statues for religious festivals, and lamps on the altars in the temples are filled with butter). Tibetans also appreciate cheese (Plate 4.9), desiccated curdled milk. Various forms are found and in particular small cubes of surroundings 2 to 3 cm large, and often bored in the medium and joined together in chain with a string. This extremely hard cheese due to a prolonged desiccation is used as a delicacy by children or travellers who let it melt patiently in their mouth for not being able to crunch it.

This short enumeration of the main Tibetan dishes would not be complete without the *chang*, a beer made with fermented barley that Tibetans sometimes drink in an immoderate way.



© Chasseur Camille



© F. Malasse

Plate 3.6. Rural food habits: *Tsampa* and butter tea. The *tsampa* is the main essential food for Tibetans. To make *tsampa*, barley grain is initially removed from the small stones. A. Then, it is washed, dried, and poured in large frying pan to be slightly roasted before being ground – B. Village mill – C. Small individual mill – D. The *tsampa* is generally stored in the kitchen, in beautiful ornamented *tsampa* boxes – E. The butter tea is the national drink in the South Central Tibet. The tea, after being boiled is poured in a churn to be mixed vigorously with butter and salt – F. Bent stomachs of sheep for butter conservation – G. Bent stomachs of yak for same use.

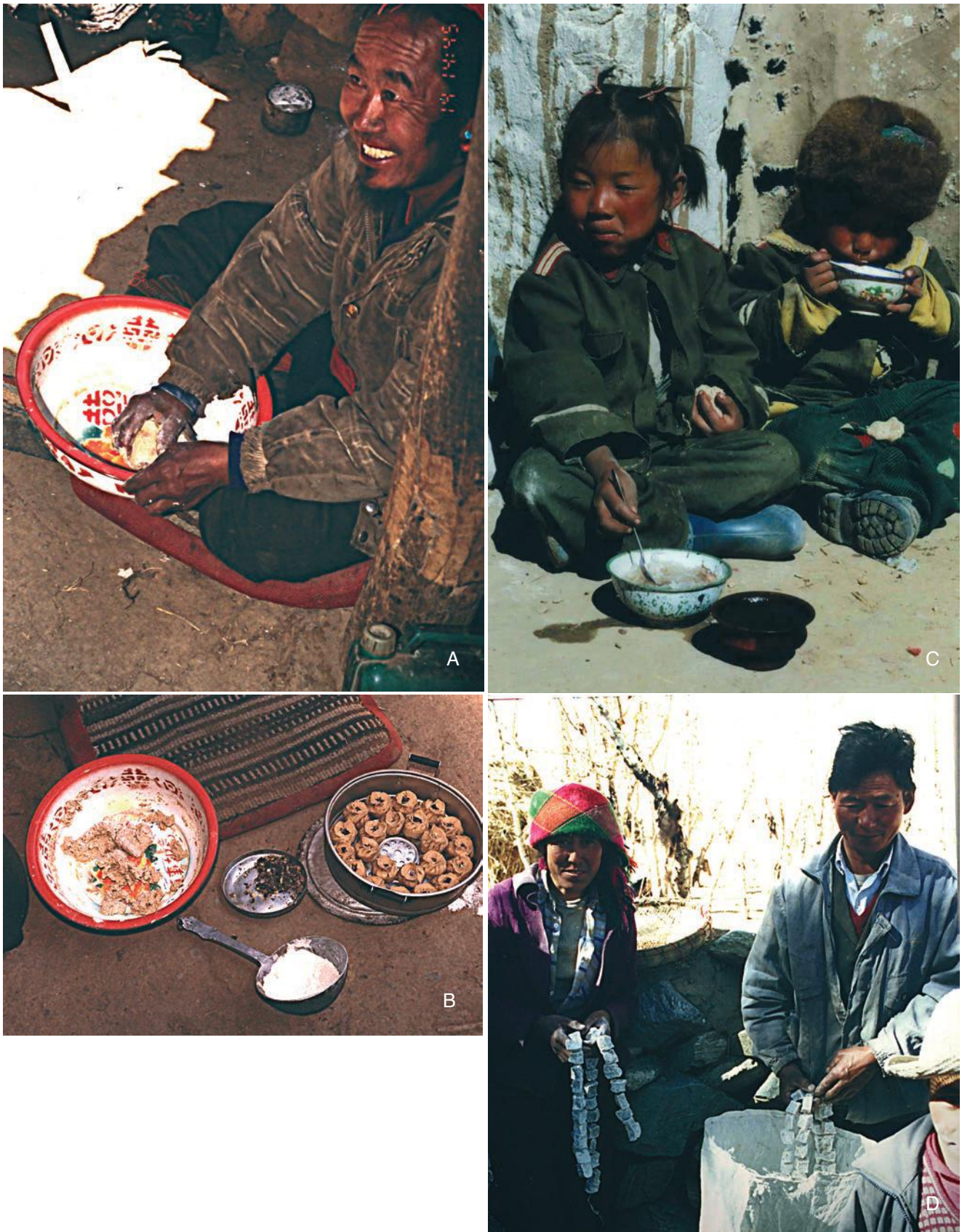


Plate 3.7. Food habits: the *momos*, noodle soup and cheese. A & B. In the Yarlung Tsampo valley, the *momos* are steamed pies stuffed with meat and sometimes with cheese – C. Soups are also appreciated, especially the most popular in South Central Tibet, the noodle soup – D. Small cubes of cheese, 2 to 3 cm large, bored in the medium and joined together in chain with a string. This cheese is extremely hard because of a prolonged desiccation.



Plate 3.8. Some foods increasing the low diet diversity. A. Turnips drying – B. Fresh garden peas – C. Grilled peas – D. Parsnips – E. Walnuts – F. White peaches.



Plate 3.9. Cheese... in all its states. A. Whole milk with rennet added to produce curd – B. Summer drying – C. Winter stockage – D. Small cubes.

Chapter 4 Kashin-Beck disease in T.A.R.: 15 years of programmes

- 4.1. Clinical study
- 4.2. Radiological study
- 4.3. Physical therapy study
- 4.4. Prevalence study
- 4.5. First epidemiological study
- 4.6. Second epidemiological study: prevention trial
- 4.7. Nutritional issues in KBD endemic rural areas
- 4.8. Third epidemiological study: clinical trial based on nutritional issues

Introduction

Kashin-Beck disease (KBD) is common in T.A.R. In popular tradition, it has always existed and is regarded as a fatal illness. The literal translation of Kashin-Beck disease is «*rutim*» (big bone disease) in Tibetan and «*ta gu ji ping*» (big joint disease) in Chinese.

From 1992 to 2002, Médecins Sans Frontières-Belgium (MSF) has been working in the field of Kashin-Beck disease in Tibet Autonomous Region. The different programmes covered 3 spheres of activity: curative health care and training, physical therapy study and epidemiological study.

Some of these programmes and studies are summarized in this chapter, focusing on the medical aspects of the disease.

At the end of 2002, MSF withdrew from T.A.R. However the whole KBD team (scientists in Belgium, national staff

in T.A.R. (Figure 4.1) and the Regional Health Bureau of T.A.R.) decided to continue the programmes under the name of the new-born non-profit organisation: Kashin-Beck Disease Foundation (KBD F).

Nowadays different programmes are still implemented and new initiatives are coming up.

Since 2003, the Centre for Disease Control and Prevention (CDC) of Tibet Autonomous Region (T.A.R.) has been the direct partner of Kashin-Beck Disease Foundation. The 2 partners are supported by the Regional Health Bureau of T.A.R.

All the programmes are directly implemented by the national staff. The scientists are given a technical support from Europe and are coming from time to time to the field.



Figure 4.1. KBD F national staff in Lhasa together with Françoise Mathieu.

4.1

Clinical study

Françoise Mathieu, Carl Suetens, Françoise Begaux, Lopsang Rinchen,
Lakpa Wangdu, Viviane De Maertelaer, Maurice Hinsenkamp

As KBD affects the form and the mobility of the joints of the limbs, the growth and the length of the long bones at maturity and, in some cases, the global stature, it seemed necessary to make a specific anthropometric description in order to better identify the clinical pathology (Figure 4.2) (Mathieu, 2001; Mathieu et al., 2001a). It had previously appeared essential to have a reference base for the different parameters that we wanted to study within a non-pathological Tibetan population.

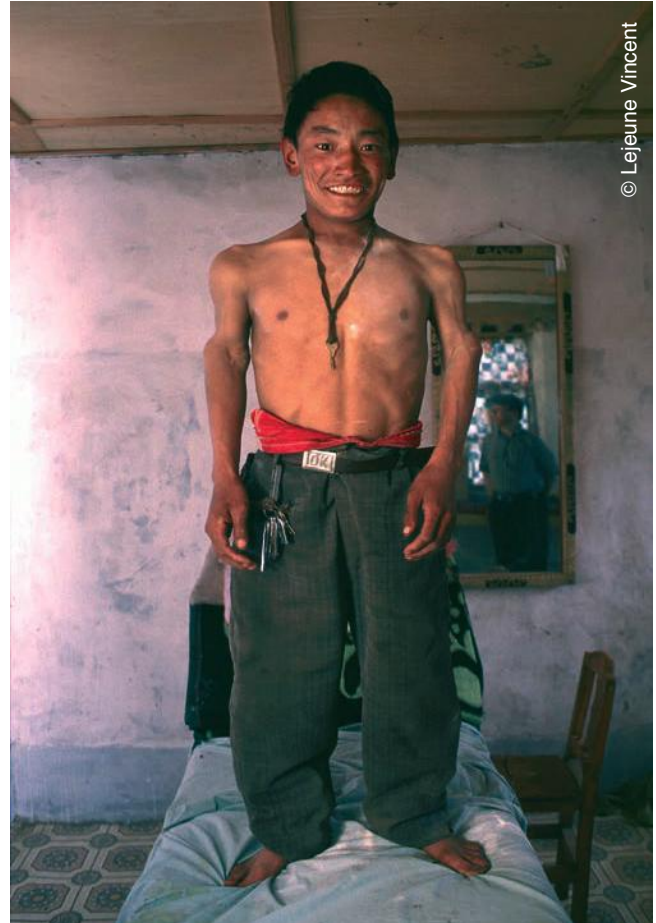


Figure 4.2. A 13 year-old boy suffering from KBD.

4.1.1. Objectives of the study

There was a two-fold objective to the study:

- to establish the standards for different anthropometric parameters within a rural population living in South Central Tibet, not affected by KBD;
- to study those parameters in a Tibetan rural population suffering from KBD.

In addition, a rigorous clinical description enabled us to make a better evaluation of the effects of prevention and treatment programmes of KBD.

4.1.2. Population and methodology

The study covered two different groups of subjects, both composed of farmers and semi-nomads. All were living in a similar environment, with the same living conditions and the same dietary habits. They were at least 5 years of age and all of the Mongoloid type.

Group 1: control group (n = 815) from non-endemic areas, living in the large county of Rimpung, in Shigatse prefecture.

Group 2: affected by KBD and coming from endemic areas (n = 1246), distributed over 8 counties in the prefectures of Lhasa, Lhoca and Shigatse (the counties of Medrogongkar, Taktse, Lundrup, Tolung and Nyemo in the prefecture of Lhasa, the county of Shetome in the prefecture of Shigatse, the counties of Sangri and Neudong in the prefecture of Lhoca) (Figure 4.3).

The following parameters were measured: height, weight, lengths of segments of the limbs (arm and forearm, thigh and leg) (Figure 4.4), the circumference and diameter of the joints (Figure 4.5) of the limbs (elbow, wrist, proximal and distal interphalanges, knee and heel) (Figures 4.6, 4.7 and 4.8) as well as their mobility.



Figure 4.4. A 16 year-old boy affected by KBD.



Figure 4.3. Seven prefectures of Tibet Autonomous Region and the different counties where the clinical study took place.



Figure 4.5. Measuring the diameter of the elbow with the help of a calliper.



Figure 4.6. Enlarged diameters of the knees of a KBD patient.



Figure 4.7. Enlarged circumference of the wrist of a KBD patient.



Figure 4.8. Ankle affected by Kashin-Beck disease.

Only the right side was measured. The measurements were always taken with the subjects placed in the same position. The mobility measures were taken while the subject was performing the movement alone, without any assistance.

The differences (intra-observers and inter-observers) measured remained within an interval of ± 5 degrees.

We analysed if these parameters are different in the 2 groups. In order to take into account influence of gender, we constituted 4 sub-groups (control group of men, control group of women, a group of KBD men and a group of KBD women) and submitted each of these parameters to a two-way analysis of variance with the sub-groups and the age of the subjects as factors.

Subjective parameters were also measured: joint pain, feeling of general fatigue, rapid fatigue after physical activity, muscular weakness (patient's appreciation), limitation of ability to work. Morphological and functional characteristics such as joint deformities (visual appreciation of the examiner), muscular atrophy (appreciation of the examiner), dwarfism, *pes planus* and any waddling gait were also noted. The joint pain was scored; the answers to the other variables were recorded as «yes» or «no».

4.1.3. Some results

As in some places the local health agents have not mastered the clinical diagnosis of the disease, they under-estimate the number of KBD cases and the regions affected. This study attempts to point out the most significant parameters for the disease, allowing to refine the clinical diagnosis. In our opinion and in the present state of knowledge, this is the most appropriate diagnosis, given the conditions encountered in the field (the impossibility of carrying out radiological or biochemical examinations, etc.).

The most interesting parameters coming out of this study are the height (Figures 4.9, 4.10) and the joint mobility of the different peripheral joints, regardless the age of the subject (Figure 4.11). The joint range of motion is reduced in KBD patients. It should be noted that the joint range of each motion, even among the controls, decreases with age and the joint mobility is lower in women.



Figure 4.9. A 23 year-old patient age suffering from Kashin-Beck disease.

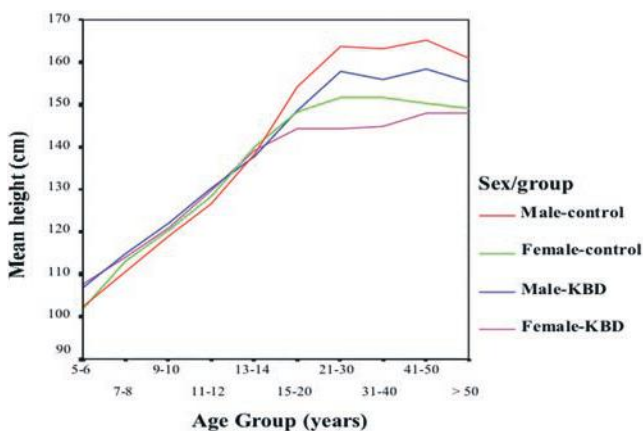


Figure 4.10. Mean of height by age group and by sex for a control group and a KBD group. Prefectures of Lhasa, Lhoca and Shigatse.

After statistical analysis, the measurement of the joint circumferences seems more interesting than that of the joint diameters, but this is only statistically significant in subjects over 15 years of age.

The evaluation of pain (Figure 4.12) is particularly interesting among children and young adults. This parameter should always be considered with caution because of the high level of subjectivity. The other subjective evaluations, or morphological or functional parameters, do not appear relevant for diagnosing the disease. They are regarded as accessory signs of KBD.

In the light of these different results, we proposed a new classification of the disease based on clinical examination, functional evaluation and accessory clinical symptoms.

Proposal of a functional classification of Kashin-Beck disease

Following our previous study, we proposed a new classification of the disease (Mathieu et al., 1997). Patients are classified in 3 stages according to a scale of severity based on clinical examination, functional evaluation and accessory clinical symptoms (Table 4.1).

Table 4.1. Classification of Kashin-Beck disease in 3 clinical stages.

Stage	Joint deformities	Joint pain	Degree of mobility	Associated symptoms
I	Yes (no*)	No (yes *)	0	No
II	Yes	Yes /no	1 or 2	No
III	Yes	Yes /no	1 or 2	Yes

* Children under 15 years with persistent joint pain without joint deformities and identified as suffering from KBD.

The 3 stages are defined as follows:

Stage I: At least one painful joint of a child or one joint deformity without reduction in the range of motion for an adolescent or an adult.

Stage II: Stage I associated with a reduction in the range of motion of at least one joint (Table 4.2).

Stage III: Stage II associated with at least one of the accessory clinical signs described further on.

The reduction in the range of motion of the joints of the appendicular skeleton, except the hand, is classified in 3 degrees (Table 4.2). The patient is characterized by the most severe loss of mobility measured on all the joints. The less-affected joints are not relevant for determining the stage of the patient.

The **accessory clinical symptoms** are:

1. general fatigue: tiredness when waking up;
2. acute fatigue: fatigue appearing immediately after work or during physical effort (e.g. children's play);

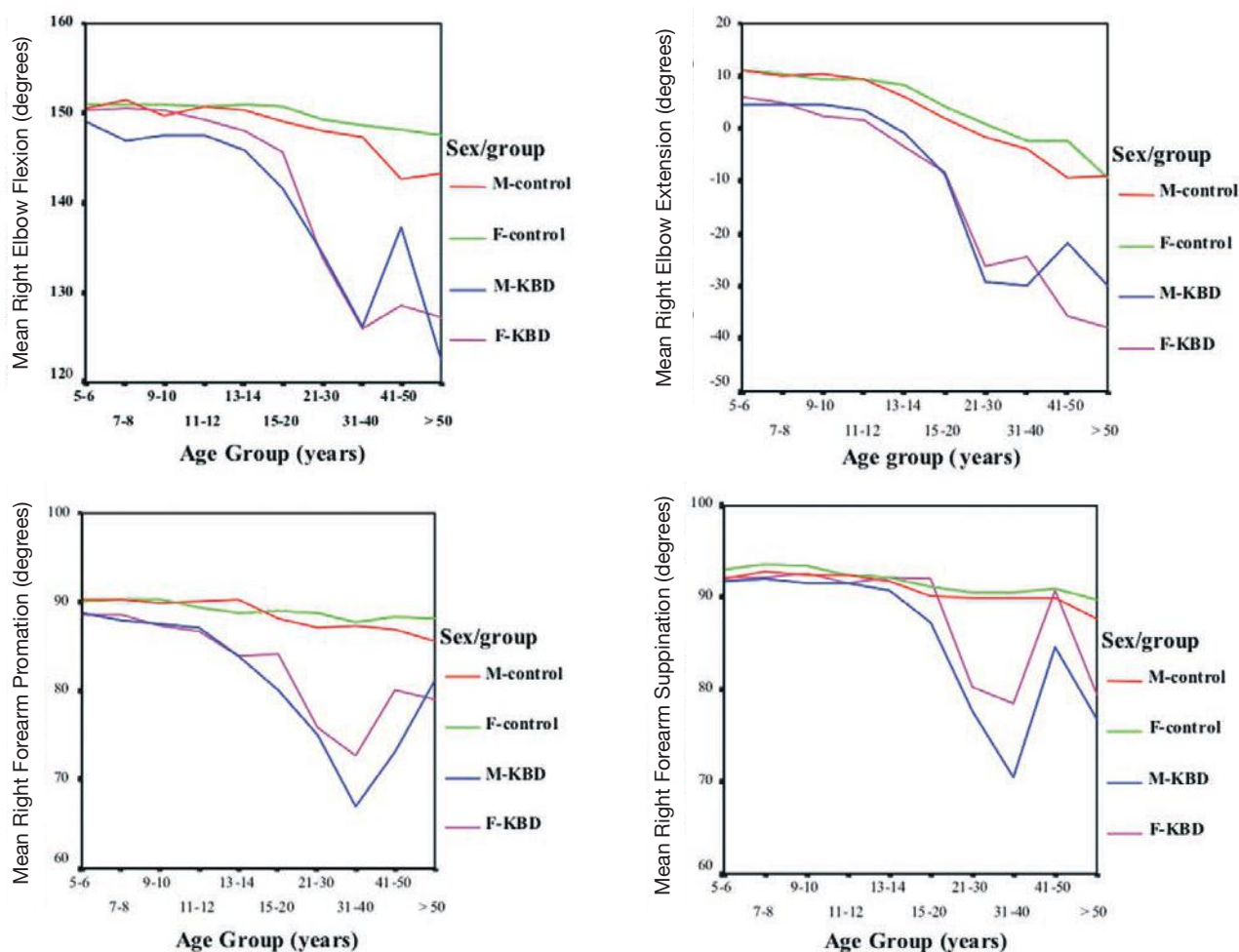


Figure 4.11. Means of the different ranges of motion for the right elbow and forearm by age and sex for a control group and a KBD group. Prefectures of Lhasa, Lhoca and Shigatse.

Table 4.2. Classification of each joint as a function of the limitation of joint range.

Articulation	Movement	Degree 0	Degree 1	Degree 2
Shoulder	Antepulsion	> 165	165 – 140	< 140
	Retropulsion	> 35	35 – 20	< 20
	Abduction	> 175	175 – 150	< 150
	Adduction	> 35	35 – 20	< 20
	Internal rotation	> 85	85 – 60	< 60
	External rotation	> 55	55 – 30	< 30
Elbow	Flexion	> 135	135 – 110	< 110
	Extension	≥ 0	-5 – -15	< -15
Forearm	Pronation	> 75	75 – 50	< 50
	Supination	> 75	75 – 50	< 50
Wrist	Extension	> 55	55 – 45	< 45
	Flexion	> 75	75 – 45	< 45
	Radial deviation	> 20	20 – 15	< 15
	Ulnar deviation	> 25	25 – 15	< 15
Hip	Flexion	> 120	120 – 100	< 100
	Extension	> 10	10 – 0	< 0
	Abduction	> 40	40 – 20	< 20
	Adduction	> 25	25 – 10	< 10
	Internal rotation	> 35	35 – 20	< 20
	External rotation	> 45	45 – 30	< 30
Knee	Flexion	> 115	115 – 90	< 90
	Extension	≥ 0	-5 – -15	< -15
Ankle	Dorsal flexion	≥ 15	<15 – 5	< 5
	Plantar flexion	> 35	35 – 25	< 25
	Inversion	> 45	45 – 25	< 25
	Eversion	> 25	25 – 10	< 10

3. muscular weakness: general feeling of muscular weakness;
4. performance at work: incapacity of carrying out usual work;
5. *pes planus* (flat foot);
6. waddling gait;
7. small stature.

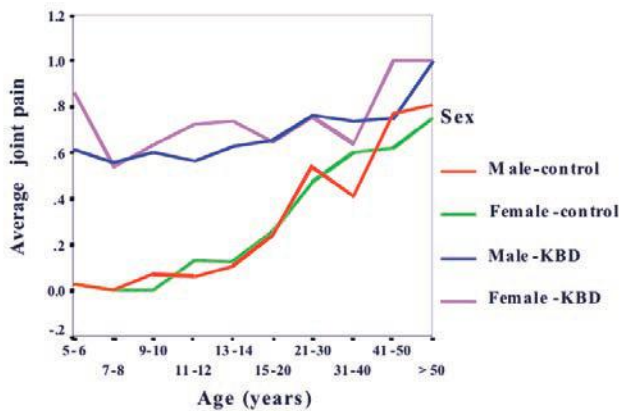


Figure 4.12. Average of joint pain by age and by sex for a control group and a KBD group. Prefectures of Lhasa, Lhoca and Shigatse.

Socio-economic impact

The limitation of joint motions among patients suffering from KBD begins usually at the age of 15-20 years, and had a significant socio-economic impact on the family and relevant consequences on the whole village, especially when the affected population is mainly composed of peasants and has to cope with heavy agricultural work.

From the clinical observations, we can say that KBD is a permanent and disabling osteoarticular disease involving growth cartilage and joint deformities. Clinical manifestations appear at the age of 5 years. Affected individuals present joint destructions which produces recurrent and often bilateral joint pain, with limited motion and joint enlargement. The most frequently involved joints are the ankles, knees, wrists and elbows. Severely affected cases are characterized by disproportionate «dwarfic» growth with associated joint deformity. For both adults and children, the resulting disability causes an important human and socio-economic burden in affected villages (Mathieu et al., 1997).

4.2

Radiological study

Maurice Hinsenkamp, Françoise Mathieu, Françoise Begaux,
Tashi Samdrup, Bernard Stallenberg

Introduction

The first systematic and detailed radiological description was made by Takamori and published in 1968. In China, during the following period, X-rays examination remained the reference for the diagnosis and evaluation of the KBD. Diagnostic criteria and classification of the disease was established by Ying et al. (1990) and Wang et al. (1996) but they focused their studies on foot and hand. Until now, no publication has reported the radiological study of the complete skeleton or at least of the appendicular skeleton of Tibetan patients suffering from KBD. In Central Tibet, our experience acquired in the field enabled us to observe that from a clinical point of view the patients are not suffering in all cases of their hand and foot but some major joints are affected representing the most severe handicap. We found that the description of the radiological alteration of the appendicular skeleton is important in the global evaluation of the patients. A more detailed analysis of these results was presented by Hinsenkamp et al. (2001) and Mathieu et al. (2001a).

4.2.1. Objectives

There were different objectives for this study:

- to describe the radiological lesions of the appendicular skeleton of patients suffering from KBD;
- to establish a radiological classification taking into account the lesions observed in the entire body of the patient;
- to study the relationship between the radiological and clinical stages;
- to define the most interesting clinical and radiological examinations for use on the field.

4.2.2. Population and methodology

One hundred sixty six Tibetan children between 6 and 15 year-old divided in three groups were studied:

- **Group 1:** 30 controls, healthy patients living in a non-endemic area;
- **Group 2:** 105 clinically affected patients living in endemic area;
- **Group 3:** 31 clinically healthy subjects living in endemic area.

The non-endemic area includes the Rimpung county (Shigatse prefecture) and the endemic area the Nyemo and Lundrup counties (Lhasa prefecture), Neudong county (Lhoca prefecture) and Shetome county (Shigatse prefecture). In each group there was an equal age distribution and an equal distribution of two-third of boys for one-third of girls (Table 4.3).

Table 4.3. Sex distribution according to the different groups. Prefectures of Lhasa, Lhoca and Shigatse, T.A.R.

	Group			Total
	1	2	3	
Gender Boy	20	72	20	112
Gender Girl	10	33	11	54
Total	30	105	31	166

Antero-posterior views of the appendicular skeleton were taken and in addition lateral views of the knee and ankle.

The radiological alterations appear first at the level of the growth plate: irregularity (blurring, weavy) of the bone margin, thickening followed by thinning of the metaphyseal cartilage, sclerosis, cone shaped metaphysis, early fusion, necrosis and fragmentation (Figures 4.13 to 4.15).



Figure 4.13. Lesions of the tarsus in a young 21 year-old man.



Figure 4.14. Subluxation probably due to a premature fusion of the epiphyseal plate in a 42 year-old patient.



Figure 4.15. Hypotrophy of the external condyle of the femur in an 18 year-old patient.

We established the following grading system:

- **Grade 0:** no radiological change;
- **Grade 1:** at least 1 radiological alteration of the epiphysis or the metaphysis;
- **Grade 2:** at least one radiological alteration of the metaphysis and the epiphysis without fusion;
- **Grade 3:** local fusion of the metaphyseal growth plate.

A detailed scoring for the whole skeleton was described by Hinsenkamp et al. (2001) with four stages of gravity (from 0 to 3).

The X-rays evaluation was done by two radiologists and one orthopedic surgeon. The skeletal maturity was determined according to the criteria of Greulich and Pyle (1959).

4.2.3. Results

The bone maturity compared to the actual age is significantly lower for the three Tibetan populations. The delay of maturity is between 2.3 years and 3.5 depending of the group but no significant difference was observed between the different Tibetan populations.

If the clinical symptoms are bilateral in 75% of the cases, the radiological alteration when present was observed on both sides in 99.4% (Figure 4.16).

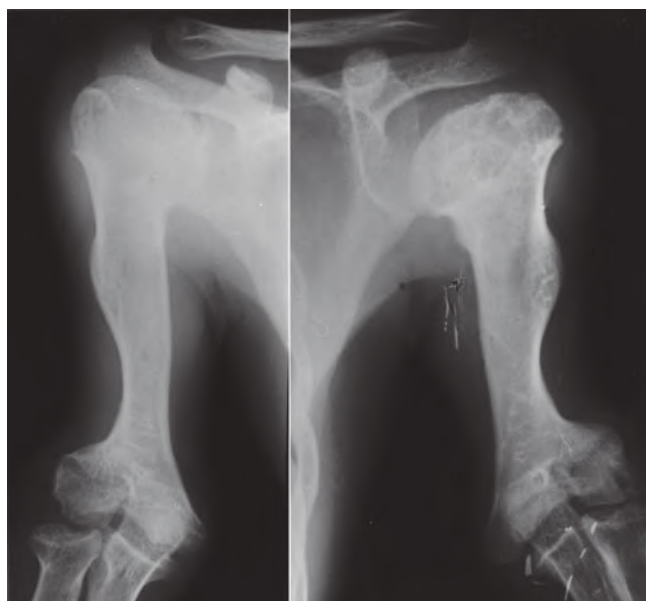


Figure 4.16. Characteristic shortening of both humerus in a 16 year-old boy.

Regarding the frequency of involvement of specific joint, the radiological screening confirms the distribution already observed in our previous clinical studies. A proximo-distal gradient is obvious along the limbs. The distal joints are more often altered than the proximal ones and the lower limb is more often and more severely affected than the upper limb.

Among the children with at least one radiological alteration, 51 out of 57 cases (89.5%) showed a lesion on the foot and/or the ankle and only 34 out of 57 cases (59.6%) presented a lesion of the hand and the wrist.

The involvement of the joint cartilage appears after the lesions of the growth plate and seems the consequence of the joint incongruence leading to the mechanical disturbance of the joint comparable to classical osteoarthritis degeneration.

The proximo-distal gradient, the more frequent alteration of the lower limb, the initial involvement of the growth plate and histological studies (Pasteels et al., 2001) seems in agreement with an inhibition of angiogenesis leading to focal necrosis of the growth plate cartilage. This could result for instance from the effect of one of the mycotoxin produced by the fungi present in the barley grain (Chasseur et al., 2001; Haubruge et al., 2001) which constitutes the main component of most of the local meal.

In addition, the distal joints are exposed to micro trauma, cold environment, hypoxia and heavy working conditions. Those environmental factors will be studied in our future programs. In the conclusion of his work, Takamori (1968) observed already a relation between the mechanical solicitation of the joint and the frequency and severity of the lesion.

There is a relationship between the clinical involvement and the gravity of the radiological alteration. The clinical diagnosis is more sensitive, it allows identifying 90% of the KBD patients against 56% of the radiological screening. However the radiological examination has a higher better specificity of 90%.

4.3

Physical therapy study

Françoise Mathieu, Lopsang Rinchen, Lakpa Wangdu, Françoise Begaux, Carl Suetens, Viviane De Maertelaer, Maurice Hinsenkamp

As the physiopathological mechanism of the disease is still not very clear, curative and preventive treatments remain uncertain. It seems to us that physical therapy treatment could alleviate the physical handicap of patients suffering from KBD.

From March 1993 till February 1997, we did a clinical study on physical therapy treatment (Mathieu et al., 1997; Mathieu et al., 2001b) (Figure 4.17).



Figure 4.17. Active exercises done by a KBD patient, with the help and the supervision of the physical therapist. Shume clinic. Nyemo county, Lhasa prefecture.

4.3.1. Objectives

There are two aims:

- to study the effects of physical therapy treatment on the clinical symptoms of the patients, more specifically on the pain, the joint mobility and their daily activities;
- to determine if the clinical evolution of the disease could be modified by a physical therapy treatment.

4.3.2. Population, materials and methods

The studied population ($n = 135$) was living in 3 communities (Lingkang, Shume and Shubu) in one valley of Nyemo county. This county is part of Lhasa prefecture. All the patients were suffering from KBD with various affected joints. Two groups were constituted: the treated group from Shume and Shubu ($n = 72$) and the control group from Lingkang ($n = 63$). Patients from the treated group received a physical therapy treatment twice a week. The patients were asked which joints they wished to be treated. The treatment consisted of massage, active and passive mobilization and exercises. The patient was encouraged to practice these exercises daily at home. Every 5 treatments, an evaluation of the pain and the mobility of the patient was performed. The mobility was assessed with a goniometer and the pain level with a questionnaire. Later on, a pain score was calculated. Also a record was done about their daily activities.

4.3.3. Some results

In the 135 patients, 372 joints were studied. Obviously, the patients chose more often the distal joints of the lower limbs for the treatment (Figure 4.18). They feel

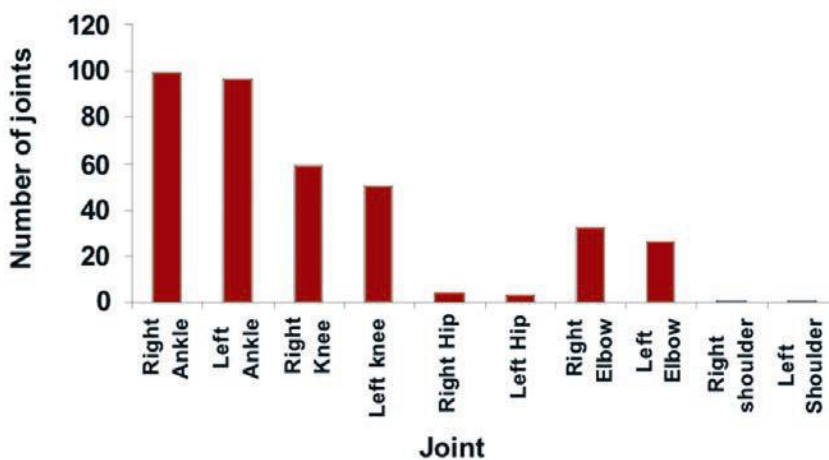


Figure 4.18. Distribution of the different studied joints ($n = 372$) for 135 patients. Nyemo county, Lhasa prefecture.

that the restriction of mobility of ankles and knee are more disabling.

The following results described by Mathieu (2001), Mathieu et al. (2001b), refer to the largest group of joints: the ankles ($n = 173$). Among this group, 97 ankle joints were treated with physical therapy and 76 were not.

At the beginning of the study (T0), the mean range of each of the 4 movements of the ankle is similar in the 2 groups. The 4 movements are the plantar and dorsal flexion, the inversion and the eversion (Figures 4.19 and 4.20).

Just after the physical therapy treatment (T1), there is an improvement of the range of mobility in the treated group. The difference is statistically highly significant ($P < 0.001$) for the 4 movements.

After almost 3 years of follow up, at the end of the study (T2), the difference between the 2 groups has slightly decreased but it still remains very significant ($P < 0.001$).

Concerning the pain, there is a significant difference between the 2 groups at the beginning of the study (T0), after the physical therapy treatment (T1) and during the whole follow up period (T2) (Figure 4.21).

At the beginning, the treated group seems to have more pain. But just after the physical therapy treatment, the pain score decreases.

We have no explanation for the difference at the beginning of the study.

Among the treated group, the pain is decreasing mainly during the second half of the treatment and seems to stabilize at the end of the study ($P < 0.001$).

For the control group, the pain is decreasing little by little during the whole study.

The physical therapy treatment did not improve any of the accessory clinical symptoms. But the treatment is more focusing on the joint mobility improvement.

In conclusion, physical therapy can help patients suffering from KBD. It can relieve pain, improve mobility and thus improve the socio-economic status of the patients. Of course earlier the treatment begins, better will be the results. The physical therapy treatment has to target mainly the children.

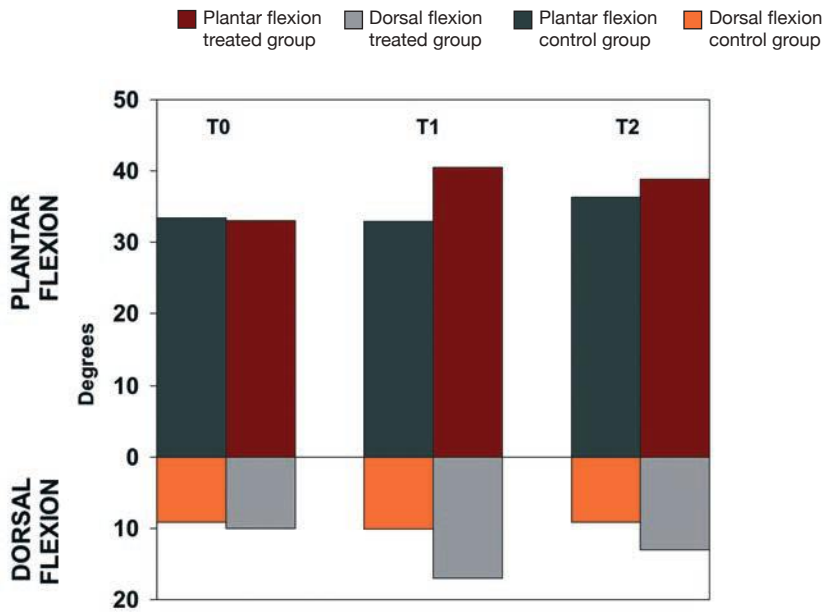


Figure 4.19. Mean value of dorsal and plantar flexion for 173 ankles at the beginning of the study (T0), after the physical therapy treatment (T1) and at the end of the study (T2). Nyemo county, Lhasa prefecture.

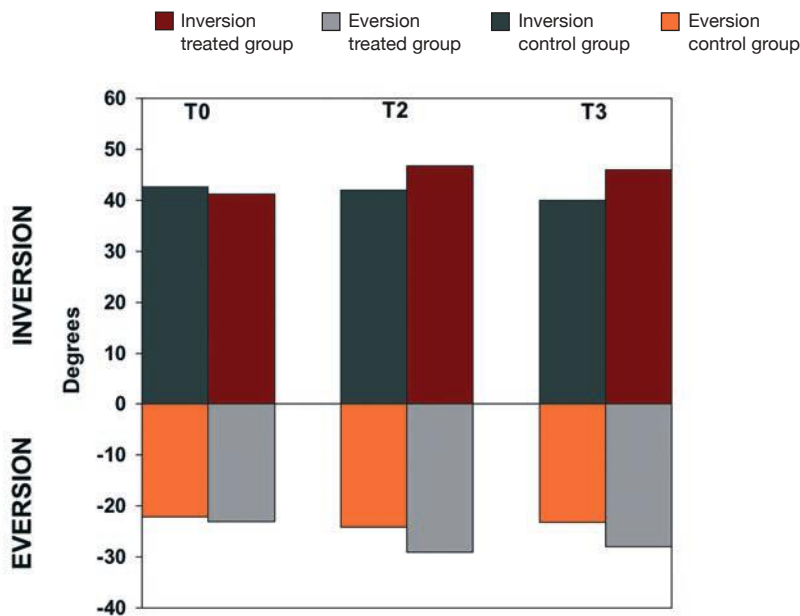


Figure 4.20. Mean value of inversion and eversion for 173 ankles at the beginning of the study (T0), after the physical therapy treatment (T1) and at the end of the study (T2). Nyemo county, Lhasa prefecture.

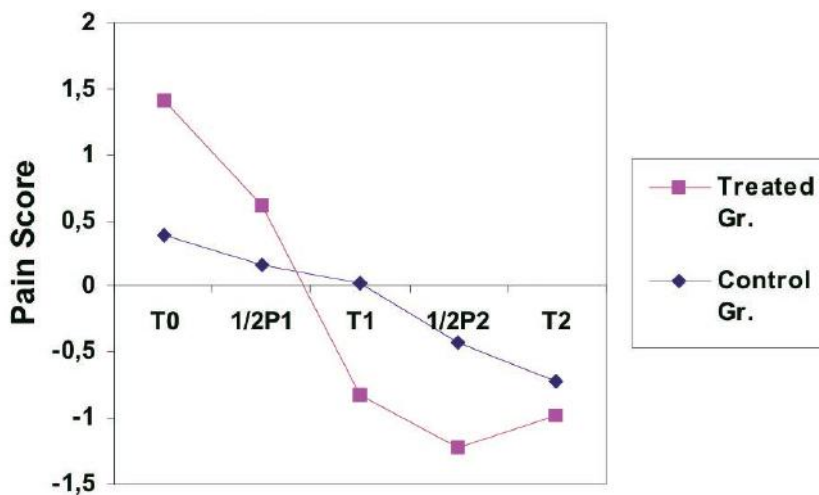


Figure 4.21. Evolution of pain score for the treated and control groups at the beginning of the study (T0), during the physical therapy treatment (1/2P1 and T1), and during the follow up (1/2P2 and T2). Nyemo county, Lhasa prefecture.

4.4

Prevalence study

Sheero Rapten, Tashi Samdrup, Lopsang Rinchen,
Lakpa Wangdu, Françoise Mathieu, Carl Suetens

In the early 1990's, there were very little data about the rates of prevalence. Although there have been some local surveys, their results could not be extrapolated to other regions given the very variable distribution of patients affected.

Thanks to the extension of the physical therapy programme by Médecins Sans Frontières, introduced between 1992 and 1998, we have been able to pinpoint the endemic regions in the other prefectures (Figure 4.22).



Figure 4.22. Distribution of Kashin-Beck disease in the 7 prefectures and their sub-divisions in counties of Tibet Autonomous Region.

In the endemic regions, KBD affects peasant and semi-nomadic populations. Urban populations and nomads seem to be spared.

According to our observations, the disease is usually concentrated along certain valleys, contrary to what has sometimes been stated in the literature (Tan et al., 1989). When it affects one village in a valley, we found that the whole valley is affected, but the prevalence rate can vary significantly from one village to another.

Since the start of our work, it has been extremely difficult to obtain precise information on KBD. The data supplied by the health authorities were often hardly reliable, most of the evaluations having been made without a precise methodology. If the data came from health officials working at the central level, the figures have usually been artificially raised in order to influence operational decisions. If the data came from surveys carried out in the field, they were also not very reliable, given the subjectivity of the people making the examinations and their inter-individual variations.

All the prevalence data were therefore considered very cautiously.

Presently, the official data say that KBD is still highly prevalent in large areas of Tibet. The disease is present in 417 villages of 133 townships. They are included in 39 of the 74 counties in the 7 prefectures of Tibet Autonomous Region (T.A.R. CDC sources, 2007).

An update of the KBD prevalence in each prefecture is currently on going with the natural village as the smallest checked unit.

This work is done in close collaboration between T.A.R. CDC and KBD Foundation. It should be finished by the end of 2008.

4.5

First epidemiological study

Carl Suetens, Françoise Mathieu, Françoise Begaux, Lakpa Wangdu,
Lopsang Rinchen, Marie-Claire Durand, Camille Chasseur

Introduction

From 1995 till 1996, MSF and coll. carried out an epidemiological study. The aim of this study was to confirm the pertinence of the 3 main risk factors described in the literature: selenium deficiency, the grains and their culture, the water.

Selenium. The objectives were to characterize KBD regarding its clinical, biochemical and radiological status and to identify KBD cases for a clinical trial of selenium supplementation, and to test the hypothesis of a relation between selenium deficiency and KBD. The ultimate objective was to propose preventive measures.

Grains and culture. The objectives were to test the hypothesis of a contamination of the grains during the harvest time and the storage period and to study the means of storage and the origin of the different grains. Following the results, the aim was to propose a preventive programme.

Water. The objective was to check the hypothesis that water is a risk factor for KBD because of the presence of too many organic materials (fulvic acid).

4.5.1. Population and methodology

Six hundred children were enrolled in the study. They were from 5 to 15 year-old, living in 12 rural villages of Lhasa prefecture.

Transversal and longitudinal (clinical trial and selenium supplementation) studies were performed.

4.5.2. Some results

- Clinical and radiological signs of KBD increase with the age. The most frequent clinical signs are joint deformities and pain. Later on, there is also a mobility

restriction, which can be very severe. The most affected joints are the ankles, the elbows and the knees.

- Selenium deficiency is extremely severe in the children but is not significantly different in cases and non-cases (Moreno-Reyes et al., 1998). The results of the selenium supplementation do not show any effect on the main symptoms and signs of KBD either on growth or thyroid function once iodine deficiency has been corrected (Moreno-Reyes et al., 2003).
- All the children are also severely iodine deficient. KBD is correlated with this deficiency.
- Concerning the grains, there is a very strong correlation between the presence of 3 fungi in the barley grains and KBD (Chasseur et al., 1997).

There are 3 critical periods for the fungal contamination:

- during the growth period of the plants, fungal contamination can occur by infected seeds or by the field itself;
- during the harvest time, when the bundles remain on the fields;
- after the harvest time, during drying of the grains before storage in bags.

Families with KBD have a higher proportion of dark barley grains in the samples collected in their houses, indicating a possible role of barley varieties; some varieties may be more sensitive to fungal contamination.

- Concerning the water, the KBD families use more frequently smaller water containers. The organic content (TOC) of the water in these containers was significantly higher than TOC in larger containers, suggesting that smaller containers do not allow sufficient time to deposit organic matter (La Grange et al., 2001).
- Children from families with higher income and higher access to different nutrients are relatively protected from the disease, probably reflecting the access to more diversified food and possibly other anti-oxidant nutrients such as vitamin C or E.

4.5.3. Conclusion

In conclusion, the findings are compatible with the multifactorial environmental theory.

4.6 Second epidemiological study: prevention trial

Carl Suetens, Françoise Mathieu, Françoise Begaux, Philippe Gillet,
Pascal Kanyandekwe, Philippe Goyens, Dawa Tserin, Lixing He,
Marjorie Lagrange, Marie-Claire Durand, Camille Chasseur

Introduction

Given the high public health impact of the disease in rural T.A.R., and based on previous findings, it was hypothesized that a comprehensive prevention programme focused on a combination of defence and noxious factors will result in significant decrease of KBD in affected areas:

- reducing fungal contamination of grains: optimal drying of grains before storage, improve storage conditions, seeds and plants disinfection;
- monitored iodine supplementation, in addition to the national iodine supplementation programme;
- antioxidant therapy: vitamin C, vitamin E, selenium supplementation as a combination;
- reducing organic content in drinking water: health education with regard to the use of drinking water from brooks or irrigation channels and to the use of appropriate water storage containers.

In order to assess the efficacy of different combinations of preventive measures on the evolution of KBD, a prevention trial was implemented to 5 to 15 year-old children from 22 villages distributed over 3 prefectures (Lhasa, Shigatse and Lhoca) from 1998 till 2002.

4.6.1. Population and methodology

Nine hundred ninety seven children aged from 5 to 15 years were enrolled in the study.

Five study groups were set up to test different elements of the multifactorial hypothesis of KBD. Two of these were constituted in the same population and differed by the random allocation of either combined antioxidant therapy or placebo in a random clinical trial (RCT) embedded in the study design.

The 5 groups (G1 to G5) benefited from different interventions. Here below, it is a summary of these interventions.

Intervention category:

- G1: g/i/ao: grain measures, iodine supplementation, antioxidant supplementation (selenium, vitamin C, vitamin E);
- G2: g/i/p: grain measures, iodine supplementation, placebo; G1 and G2 were randomized within the same 9 villages;
- G3: g/w/p: grain measures, water measures, placebo;
- G4: g/p: grain measures + placebo;
- G5: control: only placebo.

KBD symptoms were evaluated at baseline and outcome by the same physical therapist, without awareness of the intervention to which the children were submitted.

Clinical evolution was both assessed by the KBD stage assigned by the physical therapist and by two clinical scores composed of biometric parameters.

Based on the overall clinical judgment of disease stage and the differences in measured biometric parameters, a prediction of stage improvement was performed by logistic regression in order to build a **clinical improvement score**.

The score was composed of following weights and independent predictors:

clinical improvement score = 2 x difference in ankle flexion (dorsal+plantar) + 2 x difference in ankle rotation (eversion+inversion) + 3 x difference in elbow rotation (supination + pronation) - 16 x difference in pain score (sum of joint pain) + 6 x age in years.

The resulting scores ranged from - 184 to + 288 with a mean of 31 (\pm 67.5).

A higher score indicates a more pronounced clinical improvement.

A second score was made to predict stage 3 (same score for income and outcome) following the same method. The resulting **disease severity score** was:

800 (constant) - 3 x ankle flexion (dorsal + plantar) - 3 x elbow sagittal range (flexion+extension) + 60 x distal phalanx perimeter - 14 x elbow perimeter + 12 x painscore + 800.

Serum bone alkaline phosphatase (BALP) was assessed as **biological marker**.

Data from 67 children from a non-endemic area (parallel study in 2 other villages: Tsingda and Keypa) were used to compare biological values at baseline.

Grain samples were obtained in 1997, 1998, 1999, 2000 and 2001, after harvest, shortly after storage of the grains in bags, and analysed in the KBD laboratory unit in Lhasa.

4.6.2. Some results

Description of the study population

The main demographic and baseline clinical characteristics of the study population are given by study group in table 4.4.

Their mean age at baseline was 9.7 years. According to the family questionnaire, these children represented 91.6% of the total 5 to 15 year-old population (n=1088) of the 22 villages, who at their turn represented 34% of the total population of 3,174 of these villages. Outcome data were obtained for 810 children (81.2%).

In 1998, a number of clinical and biological parameters were measured as well in a group of children living in a non-endemic area. One of the most striking findings was the fact that plasma selenium levels were considerably higher in the non-endemic area ($p < 0.001$), but not different between cases and non-cases in endemic areas, as documented previously (Figure 4.23).

Table 4.4. Distribution of selected demographic and clinical variables at baseline by intervention category.

	G1 (g/i/ao)	G2 (g/i/p)	G3 (g/w/p)	G4 (g/p)	G5 (control)	Total
N enrolled	196	200	214	184	203	997
Mean age (year)	9.9	9.8	9.7	9.7	9.4	9.7
% male	52.5	47.5	53.3	52.2	52.7	51.7
Family size	6.3	6.7	7.1	6.5	6.2	6.6
Reported ability to read (%)	39.8	39.0	36.4	38.4	44.3	39.6
% High food diversity	35	36	10	51	32	31.0
KBD stage 1 (%)	28.8	28.8	25.7	26.6	24.1	26.8
KBD stage 2 (%)	31.8	31.3	30.8	34.8	31.5	32.0
KBD stage 3 (%)	12.1	10.6	15.9	10.3	9.4	11.7
KBD all stages (%)	72.7	70.7	72.4	71.7	65.0	70.5
N with outcome data	165	158	178	153	156	810
Lost to follow-up (%)	31 (16%)	42 (21%)	36 (17%)	31 (17%)	47 (23%)	187 (19%)

g: grain measures; i: iodine supplementation; ao: antioxidant supplementation; p: placebo; w: water measures; control: only placebo.

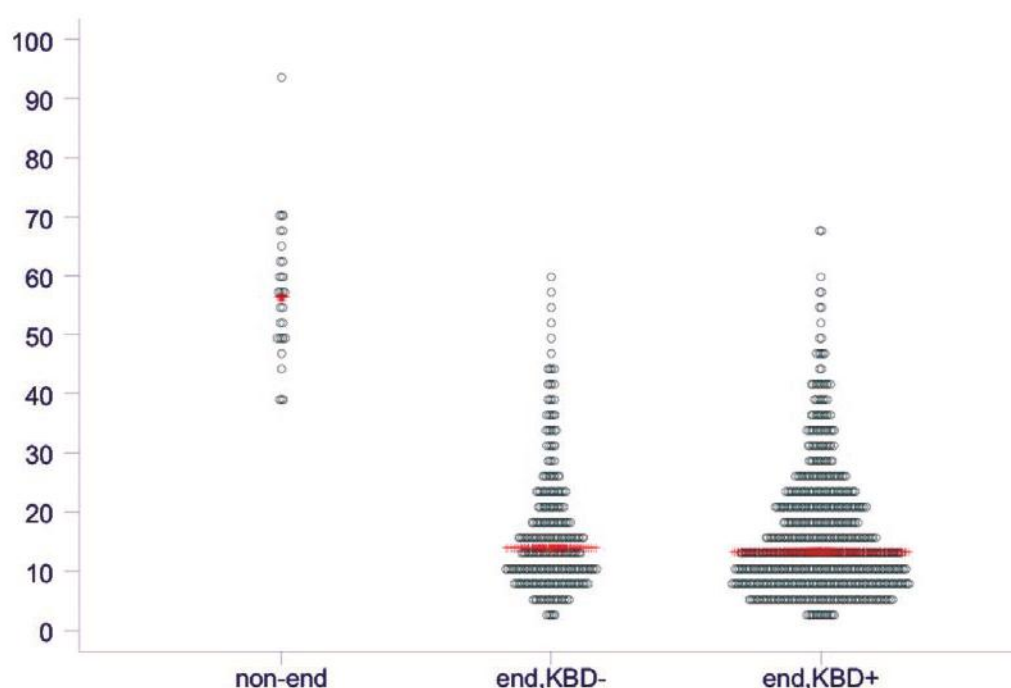


Figure 4.23. Plasma selenium levels at baseline in KBD cases and non-cases in endemic areas (Lhasa, Lhoca, Shigatse prefectures) and in a non-endemic area in Shigatse prefecture, 1998.

Age- and sex-adjusted BALP (a-BALP) levels were higher in stage 2-3 cases, both at study intake ($p = 0.04$) and at study outcome ($p = 0.001$).

Effect of implemented measures on the risk factors

1. Antioxidant supplementation

The effect of antioxidant supplementation was evaluated by plasma selenium levels at baseline and at the end of the study. Figure 4.24 shows that selenium levels were increased successfully in the supplemented group, although the median value of the non-endemic area was not reached (Figure 4.23). However, some increase was also noticed in children receiving placebo in the villages where the randomized clinical trial was set up, indicating that some «contamination» of the placebo group occurred (and vice-versa in the supplemented group). The increase in the placebo group was unlikely to be due to other sources of selenium supplementation since such a supplementation was not reported to the field investigation team and there was no increase in selenium levels in the other intervention groups.

2. Iodine supplementation

Iodine supplementation was evaluated by differences in serum thyrotropin (TSH) levels at baseline and at the end of the study. TSH levels decreased significantly both in supplemented as non-supplemented groups (Table 4.5). Although the decrease was slightly more pronounced in children supplemented during the study ($p = 0.02$), the fact that the non-supplemented group was not withheld from the routine iodine supplementation programme explains the decrease in this group.

3. Grain disinfection measures

Figure 4.25 shows that grain disinfection (treatment of seeds and pulverisation of fungicides on the field) and storage measures (controlled drying before storage, new storage bags, elevation of storage bags, treatment of storage rooms) had a slow but significant effect on the overall fungal contamination of stored grains as expressed by the percentage of grains contaminated by any fungus (except *Cladosporium* species, reflecting background contamination). The difference between the intervention and non-intervention group was highly

Table 4.5. Prevalence of mild and severe hypothyroidism before and after intervention (no supplementation = children not supplemented within the trial but with access to routine iodine supplementation programme).

	Iodine supplementation		No supplementation	
	1998	2001	1998	2001
n children	251	251	410	410
TSH ≥ 5.0 mU/L	97 (38.6%)	19 (7.6%)	152 (37.1%)	56 (13.7%)
TSH ≥ 10.0 mU/L	46 (18.3%)	1 (0.4%)	46 (11.2%)	2 (0.5%)

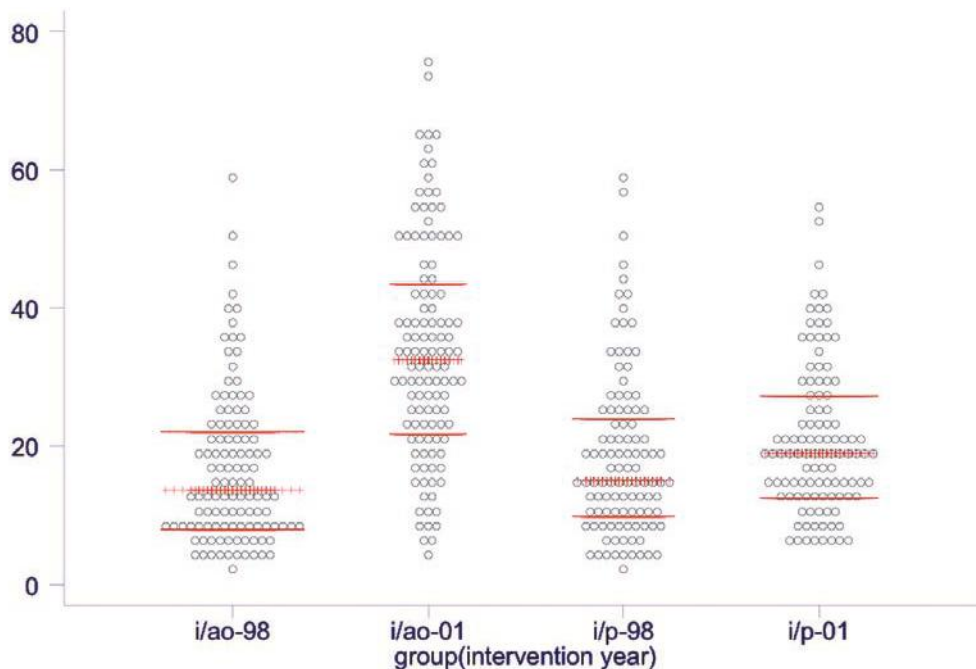


Figure 4.24. Plasma selenium levels before and after antioxidant supplementation, 22 study villages, 1998-2001 (n = 661 of 810 children).

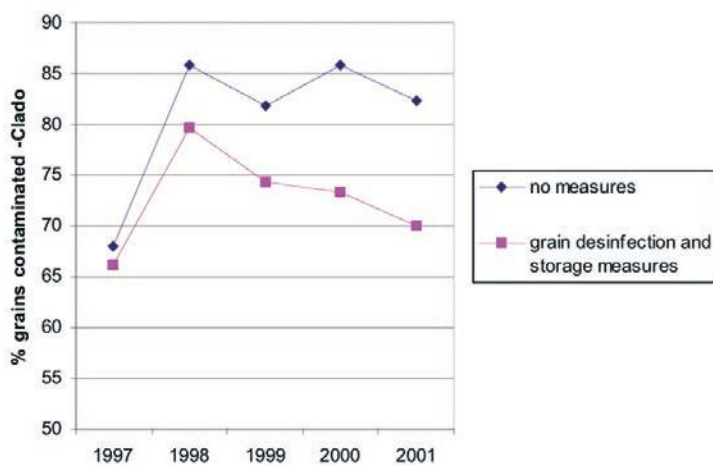


Figure 4.25. Evolution of mean percentage of grains contaminated by any fungus (except *Cladosporium* species) in newly stored grains (after harvest) in families with and without grain intervention, KBD prevention pilot project, T.A.R., 1997-2001.

significant ($p = 0.002$), but contamination levels in the intervention group still remained quite high until the end of the study, so that the overall effect of grain disinfection on the evolution of Kashin-Beck disease could probably not be fully observed in our study. However, these results indicate that the continued efforts are capable of reducing the fungal load of self-produced storage grains in this population and justify the prolongation of the measures in these highly endemic villages.

Note: 1997 samples were stored in the laboratory refrigerator much longer before analysis; therefore 1997

results may not be directly comparable to those of subsequent years.

Effect of implemented measures on Kashin-Beck disease

Table 4.6 shows the clinical diagnosis at income and at outcome (stage 0 = no KBD, 1 = stage 1, 2 = stage 2, 3 = stage 3). In italic are cases whose stage diagnosis remained unchanged over the 3 years (n = 381, 46.5%). The right upper half of the table shows cases who's clinical stage worsened (n = 279, 34.4%), while the stage of cases in left lower half of the table improved (n = 150, 18.5%).

The percentage improved cases was 2.1 (95% CI 1.2-3.6) times higher in the antioxidant supplemented group as compared to the placebo group ($p = 0.006$). However, it was not higher in the supplemented group as in the other groups (Table 4.7). Since the other 3 groups were not randomized (or only at the village level), other factors such as the higher food diversity in this group (see table 4.4) may account for the improvement.

The clinical improvement score was significantly higher in iodine + antioxidant-supplemented cases as compared to the placebo, iodine-supplemented group ($p = 0.03$), but it was the highest in the group who received only grain disinfection measures (Table 4.7). The mean increase in disease severity was also lowest in the latter group and highest in the iodine/placebo group, but not significantly higher in the latter group as compared to the iodine/antioxidant group.

Table 4.6. Overall comparison between Kashin-Beck disease stage at study enrolment and outcome, KBD prevention trial, 1998-2001.

		KBD stage 2001				Total
		0	1	2	3	
KBD stage 1998	0	138	85	11	20	254
	1	35	127	31	41	234
	2	21	49	69	91	230
	3	3	11	31	47	92
	Total	197	272	142	199	810

Table 4.7. KBD stage improvement and clinical scores in different intervention groups, KBD prevention trial, 1998-2001.

	g/i/ao	g/i/p	g/w/p	g/p	Control
% improved stage	21.6%	10.3%	19.7%	23.5%	17.3%
Mean clinical improvement score	31.1	8.0	29.6	45.4	26.3
Mean clinical severity score 1998	174.8	163.3	178.0	166.0	154.4
Mean clinical severity score 2001	208.0	201.8	215.2	191.1	184.4
Mean increase severity score 1998-2001	34.3	38.5	37.3	25.3	31.3

Clinical improvement was significantly associated with a decrease in serum bone alkaline phosphatase (BALP) levels between 1998 and 2001 ($p = 0.012$). At study outcome, age- and sex-adjusted BALP levels were slightly lower in supplemented children ($p = 0.03$) but the decrease in BALP levels was not significantly different. The difference in BALP levels (2001-1998) was however marked in children with access to more diversified food. On average, the age- and sex- adjusted difference in serum BALP levels between 1998 and 2001 was 26.5 ng/ml lower in 370 children with access to 4 or more crop types (as compared to 3 or less, $n = 430$) ($p < 0.001$, Table 4.8 and Figure 4.26). The clinical score increased by 17.1 points more in the high access group while the disease severity score increased less ($p < 0.001$, table 4.8). The percentage of children that improved their stage on direct clinical assessment was also higher in the high food diversity group, but this difference was at the limit of statistical significance.

4.6.3. Conclusion

Although the most important improvement of KBD indices was expected in the intervention group that combined 3 measures (grain disinfection, iodine supplementation and antioxidant supplementation), our prevention trial could only demonstrate significant improvement of some clinical indices in the antioxidant supplemented group as compared to the randomized placebo group that only received grain disinfection and iodine. The findings in the other intervention groups were not consistent with the prior working hypothesis.

However, clinical improvement in the study was consistently highest in children with access to more diversified food, suggesting that more micro-nutrients play a role in KBD pathogenesis and that by consequence the beneficial effect of a supplementation could still be enhanced. The causal model of the disease based on antioxidant stress (Peng et al., 1992; Suetens et al., 2001) could still hold, but more antioxidants are probably involved than just selenium, vitamin E and vitamin C. Further studies with more comprehensive micronutrient supplementation are needed.

Table 4.8. Difference in bone alkaline phosphatase (BALP) levels and clinical KBD indices according to differences in food diversity at study intake, 1998-2001.

	Food diversity		p-value
	Low	High	
Difference in BALP levels (ng/ml) 2001-1998 (\pm SD)	-30.6 (\pm 50.0)	-57.1 (\pm 56.5)	< 0.001
Mean clinical improvement score (\pm SD)	15.1 (\pm 56.9)	32.2 (\pm 53.5)	< 0.001
Difference stage 3 score (\pm SD)	261 (\pm 59)	242 (\pm 57)	< 0.001
% improved stage	16%	22%	0.09

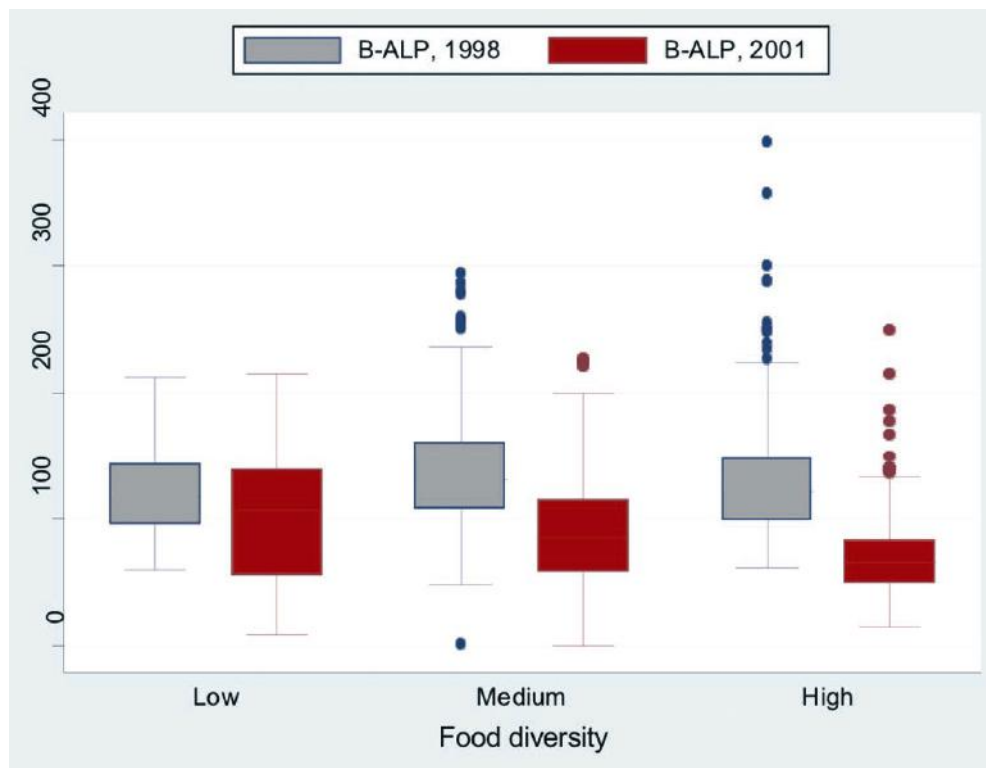


Figure 4.26. Evolution of serum bone alkaline phosphatase levels as a function of food diversity at baseline, 1998-2001.

4.7

Nutritional issues in KBD endemic rural areas

Philippe Goyens, Pascale Bally, William Claus, Dikki Yangzom, Françoise Mathieu

Introduction

One of the hypotheses that has been put forward is that nutrition might play a role in the etiology of the disease (Moreno-Reyes et al., 2003). Quite rapidly, severe iodine and selenium deficiency have been recognized in areas where KBD is highly prevalent (Moreno-Reyes et al., 1998). Later on, more general questions were raised concerning the nutritional status of populations in rural areas of the Tibetan Autonomous Region (T.A.R.).

Few publications address the nutritional status of children in T.A.R. (Beall et al., 1981; Beall et al., 1987; Kolsteren et al., 1995; Harris et al., 1996; 2001). These publications essentially describe growth retardation, anemia, vitamin A deficiency and rickets.

Therefore, the nutritional status of children living in KBD endemic areas, in the prefectures of Lhasa, Lhoca and Shigatse, is continuously evaluated since 2001 and their nutritional intake throughout the year assessed. More recently, nutritional interventions have been set up by the KBD Foundation (KBD F).

4.7.1. Exploratory nutritional survey

An exploratory nutritional survey was conducted in October and November 2001. The nutritional status of 818 subjects, between 8 and 18 years of age, was evaluated. These subjects were enrolled three years earlier in a study designed to assess the effect of various interventions on the appearance and the evolution of KBD. One should underscore that this cohort comprised only children aged more than 8 years, while it is well known that the most vulnerable age group, from a nutritional point of view, are the under fives.

The survey showed that many older children present with a short stature. A high prevalence of linear growth retardation (low height-for-age or stunting) indicates long term, cumulative effects of inadequacies of nutrition (chronic malnutrition) and/or diseases (ill health).

Chronic malnutrition was complicated, in a small number of children, by acute malnutrition (low weight-for-height or wasting). Weight loss is the consequence of acute starvation and/or severe disease. Prevalence of wasting at the time of the survey (beginning of the winter season) was low. However, among populations living in a system of subsistence agriculture, food availability and prevalence of wasting may vary according to the seasons. In T.A.R. therefore symptoms of more severe nutritional deprivation (Plate 4.1.A) might be more prevalent when food availability is lower (end of spring and summer, «hungry season» before harvest).

This exploratory nutritional survey has shown that many older children, besides a short stature, also presented with a series of symptoms suggestive of specific deficiencies:

- clinical signs or sequelae of rickets (Plate 4.1.B and C) as well as hyper-reflexia, polykinetic osteotendinous reflexes and clonus, suggestive of neuromuscular hyper-excitability and compatible with hypocalcemia;
- dental abnormalities (mottling and enamel defect) (Plate 4.1.D);
- goiter (12,7% of the subjects) (Plate 4.1.E), which, as previously mentioned, was already investigated and shown to be the consequence of iodine deficiency (Moreno-Reyes et al., 1998). The same authors have also documented selenium deficiency in this population;
- bitot spots, with a prevalence of 0.6%, evidence of severe vitamin A deficiency (WHO, 1996). Indeed, hemeralopy and night-blindness were frequent complaints;
- various abnormalities of the skin (dryness, mosaicism, depigmentation, hyperkeratosis) (Plate 4.1.F), hair (abnormal texture and depigmentation) (Plate 4.1.G) and mucous membranes (tongue, gums, lips) (Plate 4.1.H) suggestive of protein-energy malnutrition, riboflavin deficiency, pellagra, and deficiencies of other water soluble vitamins as well as trace element deficiencies;
- some other unexplained symptoms were observed as well, e.g., tenderness of muscular masses and osteotendinous hyporeflexia.

Since during this exploratory nutritional survey only clinical signs were observed, the various nutritional deficiencies suggested by clinical observations had to be confirmed by longitudinal observations of at risk groups (infants and young children, pregnant and lactating women), by food consumption analyses, biological evaluations and supplementation studies.

4.7.2. Food consumption

Breast-feeding habits are evaluated through mother and child health (MCH) activities, which were set up by the KBD-F team in 2005. The vast majority of infants in rural areas are breast-fed. Exclusive breast-feeding however is exceptional, since most infants, very early in life (in many cases from the third day of life onwards), receive complements made up of barley, the local staple food. Exclusive breast-feeding does not occur anymore between 12 and 24 months of age, but 3/4 of these children are still partially breast-fed (Plate 4.1.I). Very few breast-fed infants receive cow's milk products. More important, only half of the children, after weaning from the breast, receive cow's milk products (Plate 4.1.J), although in very small amounts.

Food enquiries, conducted on a regular basis by MSF-Belgium and since 2004 by the KBD-F, and a prospective food consumption analysis among a cohort of 39 teenagers (2002), have shown that food intake of this farmer population surviving from subsistence farming, is characterized by poor food quality (very small amounts of milk, very small amounts of meat, no fruit...) but also by an extreme monotony of the diet. Besides, the quality of the soil, with regard to mineral and trace element content and bioavailability, is to be questioned as well. These characteristics imply a high risk of nutritional deficiencies and/or imbalances, especially among the high risk groups, i.e. young children below 5 years of age and pregnant and lactating women. Indeed, as already mentioned, growth retardation, vitamin A deficiency, severe iodine and selenium deficiency and rickets have been described in this population (Beall et al., 1981; Beall et al., 1987; Kolsteren et al., 1995; Harris et al., 1996; 2001). Rickets is most probably the consequence of reduced sun exposure, but calcium deficiency quite certainly also plays a role, since milk consumption is very low, especially in winter.

4.7.3. Anthropometric surveys

A series of cross sectional anthropometric and clinical surveys have been conducted since 2001, involving more than 1900 children between 3 and 18 years of age, whereby weight, height, head circumference, arm circumference, and triceps and subscapular skinfolds were measured. About half of these children were evaluated three times, by the same investigators (FrM and PhG) over a 3 years period. These data confirmed the information obtained during the exploratory survey conducted in 2001, regarding growth retardation and the occurrence of specific nutritional deficiencies. The last anthropometric evaluation was conducted in October and November 2007. It is to be foreseen that the whole set of data gathered since 2001 will soon be analyzed.

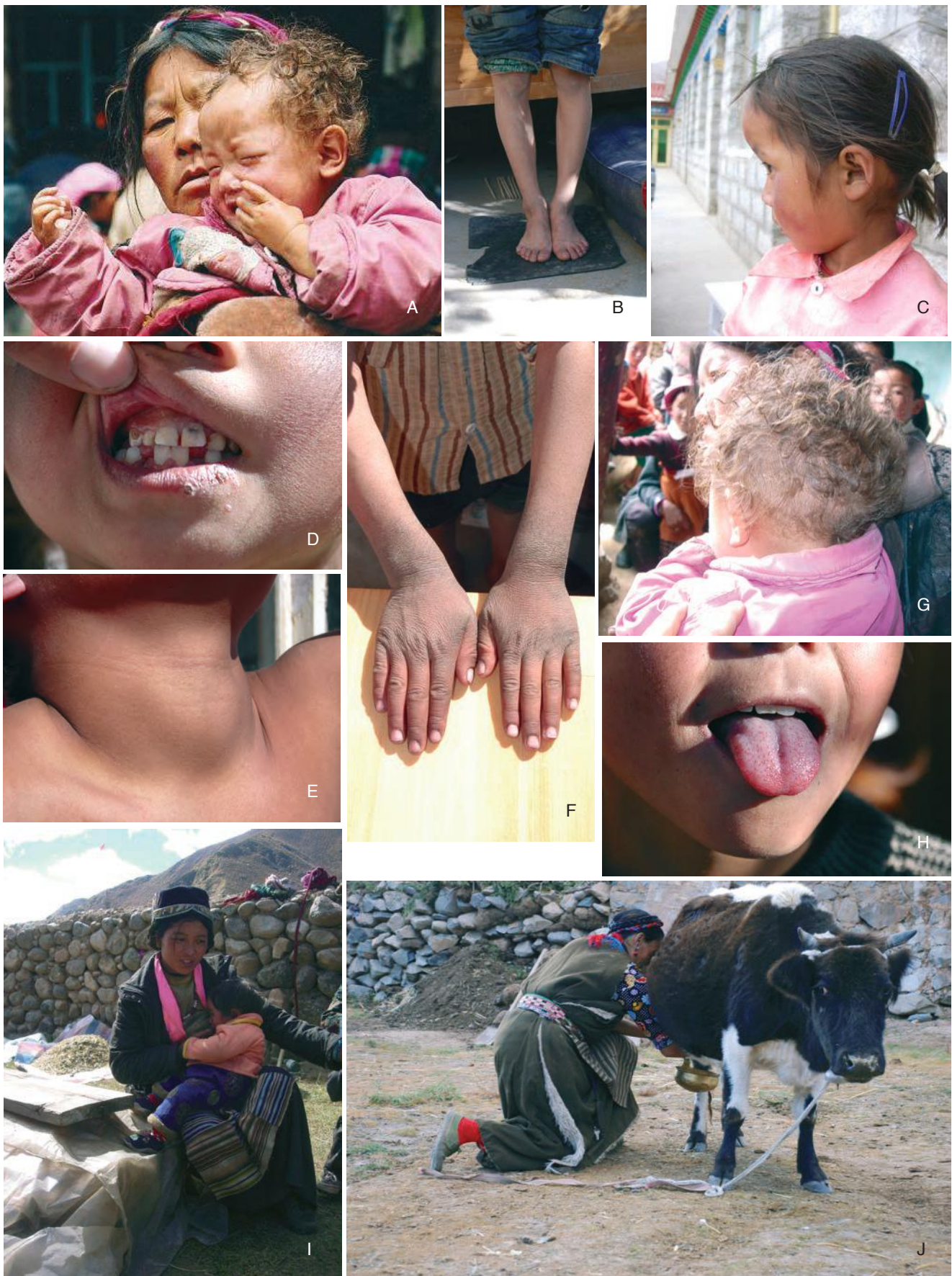


Plate 4.1. Clinical signs of nutritional deficiencies. A. Severe malnutrition – B. Bowing of the legs – C. *Caput quadratum* – D. Enamel defect – E. Goiter – F. Hyperkeratosis of the skin – G. Hair depigmentation – H. Tongue: redness of the tip and lateral margins and swelling of the papillae – I. Prolonged breast-feeding – J. Milking the cow.

Besides, the growth of a cohort of 500 infants and children below 5 years of age is prospectively followed on a monthly basis through MCH activities at the village level (Plate 4.2). Few children present with acute malnutrition (low weight for length). Z-score for arm circumference is nevertheless around -1, suggesting some wasting. On the contrary, most – if not all – young children suffer from severe linear growth retardation (mean height for age Z-score around -2.4), of the same intensity in boys and girls. Growth retardation is worsening between 12 and 24 months of age, suggesting that breast-feeding offers some protection.

4.7.4. Specific nutritional deficiencies

A series of investigations and intervention studies are presently conducted to confirm the occurrence and the severity of nutritional problems in young children living in KBD-endemic rural areas of South Central Tibet. Biological confirmation of some nutritional deficiencies could already be obtained and, in some cases, the efficacy of nutritional interventions proven as well.

Iodine deficiency

Many children present with goiter (Plate 4.1.E), suggesting that iodine deficiency is inadequately prevented by the salt iodination strategy. Indeed, although many children are found with normal urinary iodine concentrations, very low and extremely high values are observed as well. Consequently, blood TSH values are frequently found to be normal, but TSH levels on many occasions are very high, suggesting thyroid insufficiency either because of iodine deficiency or due to excessive iodine intake. These observations raise questions, as in many other less developed rural areas, regarding the adequacy of the strategy of salt iodination to control iodine deficiency.

Rickets

Clinical signs or sequelae of rickets are highly prevalent. In view of the reduced exposure to sunlight and of the low calcium intake, it is not surprising that the prevalence of clinical rickets in rural areas of South Central Tibet is extremely high. These clinical observations have been confirmed by measurements of vitamin D, calcium and parathyroid hormone.

All children between 12 and 24 months of age, evaluated in 2005, before the implementation of a vitamin D supplementation programme by the KBD F, presented with hypocalcemia, low vitamin D concentration and extremely high parathyroid hormone. Hypocalcemia is corrected in part by vitamin D supplementation, with, consequently, lowering of the extremely high

parathyroid hormone values. It is to be foreseen that calcium supplementation as well will be necessary to fully correct the hypocalcemia.

Vitamin A

The clinical evaluation of the children suggests that other micronutrient deficiencies also occur in this population. Some are currently already confirmed, others are still under investigation.

Vitamin A is found in some foods of animal origin (liver, fresh liver oil, whole milk, egg yolk); provitamin A (carotenoids) is found in vegetables, yellow fruits and vegetables. Food enquiries suggest that the intake of vitamin A in rural areas of South Central Tibet do not meet recommendations. Bitot spots, pathognomonic of severe vitamin A deficiency, with high risk of blindness, are observed in T.A.R., although unfrequently. Mean plasma retinol values of a cohort of 48 children between 12 and 24 months of age, were below the reference range, and, more importantly, 13 out of these 48 children presented with values below 0.70 μmol per liter. WHO guidelines suggest that vitamin A deficiency is to be considered as an important public health problem when more than 10% of the population present values below this cut-off (WHO, 1996). The best known symptoms of vitamin A deficiency are ocular lesions and blindness and impaired resistance to infection, but it is worth mentioning that vitamin A deficiency also causes a series of symptoms which are frequently observed in rural areas of South Central Tibet: keratinization of mucous membranes and skin (Plate 4.1.F), defects of tooth enamel (Plate 4.1.D), and retarded growth. Besides, vitamin A deficiency also causes faulty epiphyseal bone formation. This observation suggests that vitamin A deficiency, besides rickets, might play a role as well in the pathophysiology of KBD.

Vitamin E

Vitamin E is found in germ oils of various seeds, green leafy vegetables, nuts and legumes. Plasma vitamin E has also been measured in the same cohort of children between 12 and 24 months of age. All children presented with values well below the third percentile of the reference population. Vitamin E deficiency causes red blood cell hemolysis in premature infants and loss of neural integrity.

Pellagra

Pellagra was suspected on clinical grounds. However, this hypothesis was rejected after a supplementation trial with niacin, which proved to be unable to prevent the very peculiar dermatologic lesions presented by the children on cheeks, hands (Plate 4.1.F) and ankles, and around the neck. The cause of these lesions still needs to be elucidated.



Plate 4.2. Vitamin D programme. A. Poster about vitamin D issues – B. Height measurement during the monthly consultation for children aged below 5 year-old – C Sunbath for a baby – D. Dikki, the KBD field assistant, carrying the coolbox containing the vitamin D. She is accompanied by the community doctor. Both of them are going to the clinic for the monthly visit – E. Dikki is giving the monthly vitamin D supplementation to the child.

Trace elements

Evaluation of the nutritional status for trace elements is complex. Concentrations of trace elements in blood, for instance, provide very imprecise information with this regard. Nevertheless, very low concentrations of trace elements in plasma or serum are suggestive of deficiency, caused by inadequate intakes or excessive losses. This is certainly the case when children present with growth retardation, and, hence, reduced requirements. It is therefore noteworthy to mention that although the mean serum zinc value in the same cohort of children between 12 and 24 months of age was normal, 13% presented with pathological values below 60 μg per dl. On the contrary, the serum copper concentration was normal in all children.

4.7.5. Intervention studies

Thus, a series of nutritional deficiencies have already been documented by the investigations conducted since 2001 in rural areas of South Central Tibet. Others still need to be evaluated. These investigations are conducted in parallel with intervention studies. The KBD team is presently offering large scale vitamin D (Plate 4.2) and calcium supplements to at risk groups. Vitamin A supplementation is planned soon. Selenium supplementation is offered as well. The KBD F also participates in efforts made by local authorities to prevent iodine deficiency.

Results obtained in multiple micronutrient supplementation trials in various countries are contradictory (Smuts et al., 2005); growth acceleration has seldom been observed (Bui Dai Thu et al., 1999; Le Thi Top et al., 2005). However, a pilot trial, for a period of 12 months, whereby a full set of vitamins and trace elements was provided daily to children between 12 and 24 months in KBD endemic rural areas of South Central Tibet gave extremely promising results, e.g., in terms of linear growth and well being.

4.7.6. Conclusion

Evidence is accumulating that the population living from subsistence farming in KBD endemic rural areas of South Central Tibet present with a series of severe nutritional deficiencies and/or imbalances. Whether these nutritional problems play a role in the pathogenesis of KBD remains to be proven by randomized clinical

trials whereby the efficacy of nutritional interventions for the prevention of KBD will be evaluated. However, it is tempting to speculate, since vitamin A deficiency is highly prevalent and since 100% of young children in a KBD endemic area present with rickets and hypocalcemia, that vitamin A, vitamin D, and calcium deficiency play a role in the pathogenesis of KBD.

Besides the possible link between KBD and nutrition, one should keep in mind that nutrition is a major risk factor for disease (Lopez et al., 2006). Stunting, severe wasting, and intrauterine growth retardation are among the most important problems in child nutrition. Stunting is highly prevalent in rural areas of South Central Tibet; severe wasting does occur; information on birth weight in these populations is very scarce. The occurrence of wasting throughout the year and intrauterine growth retardation need to be evaluated.

Further research is necessary to fully investigate the causes of severe growth retardation in children of rural areas in South Central Tibet, to identify specific deficiencies and analyze their severity and consequences, and to evaluate the effects of interventions. These should occur early in life. After 2 years of age, undernutrition has caused irreversible damage for future development towards adulthood. There are proven effective interventions to reduce stunting and micro-nutrient deficiencies, e.g., breast-feeding counseling, vitamin A supplementation, and zinc fortification. The children in rural areas of South Central Tibet would certainly benefit from a series of other micronutrients, e.g., vitamin D and calcium. But the fact that multiple micronutrient deficiencies have already been documented (iodine, selenium, vitamin D, calcium, vitamin A, vitamin E) and that a series of others are suspected, certainly warrant multiple micronutrient supplementation trials, in order to evaluate cost and benefit in terms of general well being and, possibly, for the prevention of KBD as well.

Other interventions are also likely to provide value, such as improving dietary intake during pregnancy and maternal supplementation with iron, folic acid and possibly other micro-nutrients, vitamin D and calcium, for instance. Moreover, long-term investment in the role of women as full and equal citizens through education, economic, social, and political empowerment, is the only way to deliver sustainable improvements in maternal and child nutrition, and in the health of women and children in general.

4.8 Third epidemiological study: clinical trial based on nutritional issues

Carl Suetens, Camille Chasseur, William Claus, Pascal Bally, Sheero Rapten, Rinzin Wangla, Lopsang Rinchen, Lakpa Wangdu, Pelma Drolkar, Lixing He, Dawa Tserin, Nima Tserin, Philippe Gillet, Miriam Eddyani, Pascal Kanyandekwe, Philippe Goyens, Françoise Mathieu

Food diversity was previously described as an independent protective factor of Kashin-Beck disease (Suetens et al., 2001) and may essentially act through 2 mechanisms, either alone or in combination:

- increased intake of protective factor(s), both in variety as in quantity (e.g. micro-nutrients);
- decreased exposure to causal factor(s) present in a single or few basic food item(s), e.g. self-produced barley contaminated by fungi.

Besides, observations made during previous studies (clinical and biological data) showed that the studied population suffered from a series of micro-nutrient deficiencies and that the supplementation with antioxidants alone did not show an important preventive effect on KBD. However children with access to more diversified food (involving more than just the selected antioxidants) did consistently show a better evolution of their clinical and biological parameters.

We therefore proposed to set up a randomized clinical trial (Plate 4.3) in order to evaluate the effect of a combined micro-nutrient supplementation including all micro-nutrients known to be deficient in the population studied and hypothesized to participate in the pathophysiology of KBD.

The objectives of the current randomized clinical trial are to evaluate the effects of a combined micro-nutrient supplementation on the incidence and evolution of Kashin-Beck disease. The study started in July 2004 and the clinical outcome was done in October 2007. The cohort included 1,064 children, aged 3 to 10 years. The sample has been randomized in 2 groups (iodine/selenium + micro-nutrient supplement versus iodine/selenium + placebo) and supplemented daily during

3 years. The micro-nutrient supplement includes vitamin A, vitamin E, vitamin C, manganese, zinc and copper.

At the outcome of the study, the occurrence and evolution of clinical parameters and biological markers

of KBD were compared in both groups. At present, biological analyses are performed. Results are expected by end of year 2008.



Plate 4.3. Randomized clinical trial. A. KBD clinical examination – B. Paediatric clinical examination – C. Urine sample collection – D. Randomized micro-nutrient supplementation – E. End of the check during the outcome of the study – F. Children receiving apples and tea at the end of the check up.

Chapter 5

Studies and actions concerning several hypotheses

- 5.1. The fungal hypotheses
- 5.2. The mineral deficiency hypothesis
- 5.3. The alternative food path or the very little diversified diet hypothesis
- 5.4. Protinet



Plate 5.1. Surveys and samplings in the villages. A. Cereal grain are sampled in sacks or containers in the family store-room with a grain trier – B. This trier is conceived for sampling grain at different levels in bags or containers. It is important because fungal contamination may be different according the depth – C. Humidity of grain is also measured with a Samap device.

5.1

The fungal hypotheses

Camille Chasseur, Georges Lognay, Carl Suetens, Sheero Raptan, 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 moniliforme* 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), Heilongjiang (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.

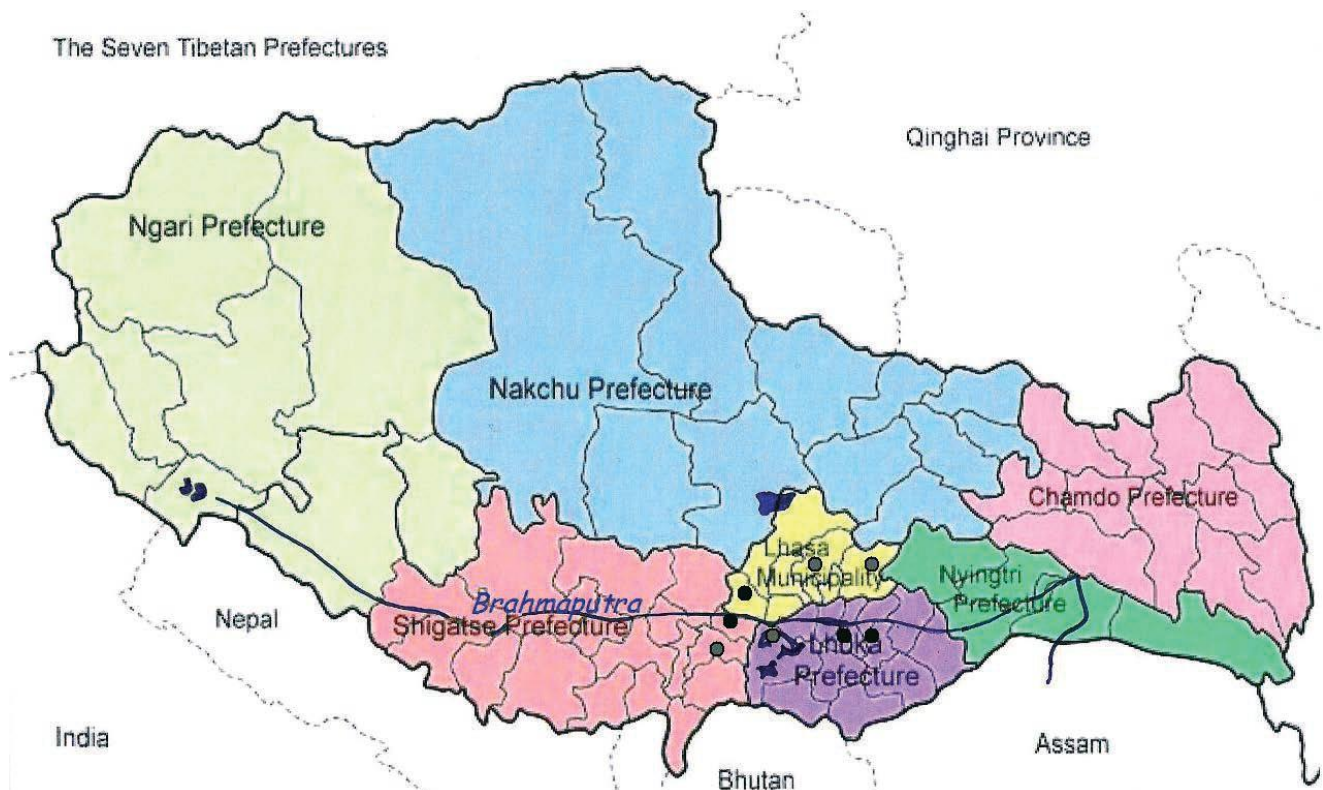


Figure 5.1. Tibetan Autonomous Region area with prefectures, counties and the Brahmaputra. A first mycological study was carried out in 2002 in 4 counties (black dots). A second study from 2004 to 2006, with 20 additional villages in 4 new counties (grey dots) (From Mapinfo geographic – version 4.1,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

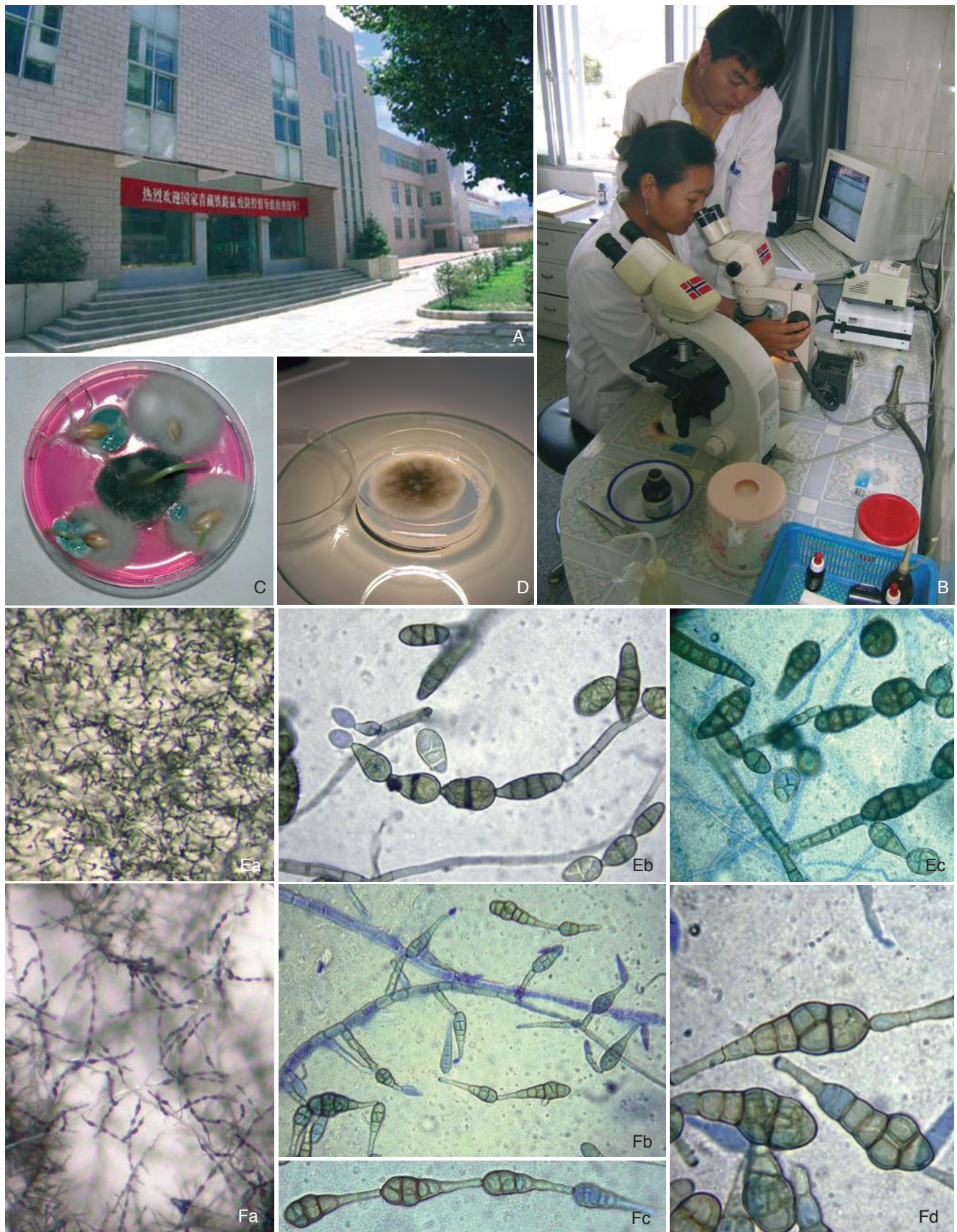


Plate 5.2. The fungal analyses: laboratory of mycology in Lhasa (CDC). A. laboratory of mycology installed in the CDC building in Lhasa in 1998, where specific trainings in mycology are regularly given – B. The team analyses every year several hundreds of grain samplings – C. Grains are analysed by direct 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* lto (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*).

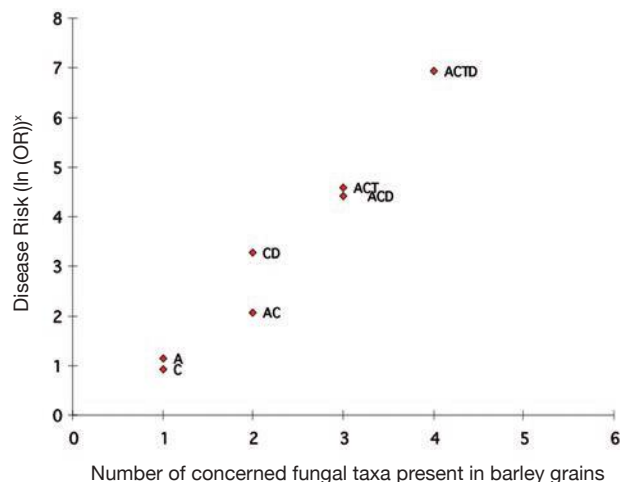


Figure 5.3. Evolution of KBD risk in Tibetan children as function of fungal taxa detected in barley grains (Chasseur et al., 1997) (C: *Cladosporium*; A: *Alternaria*; D: *Drechslera*; T: *Trichothecium*).

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.

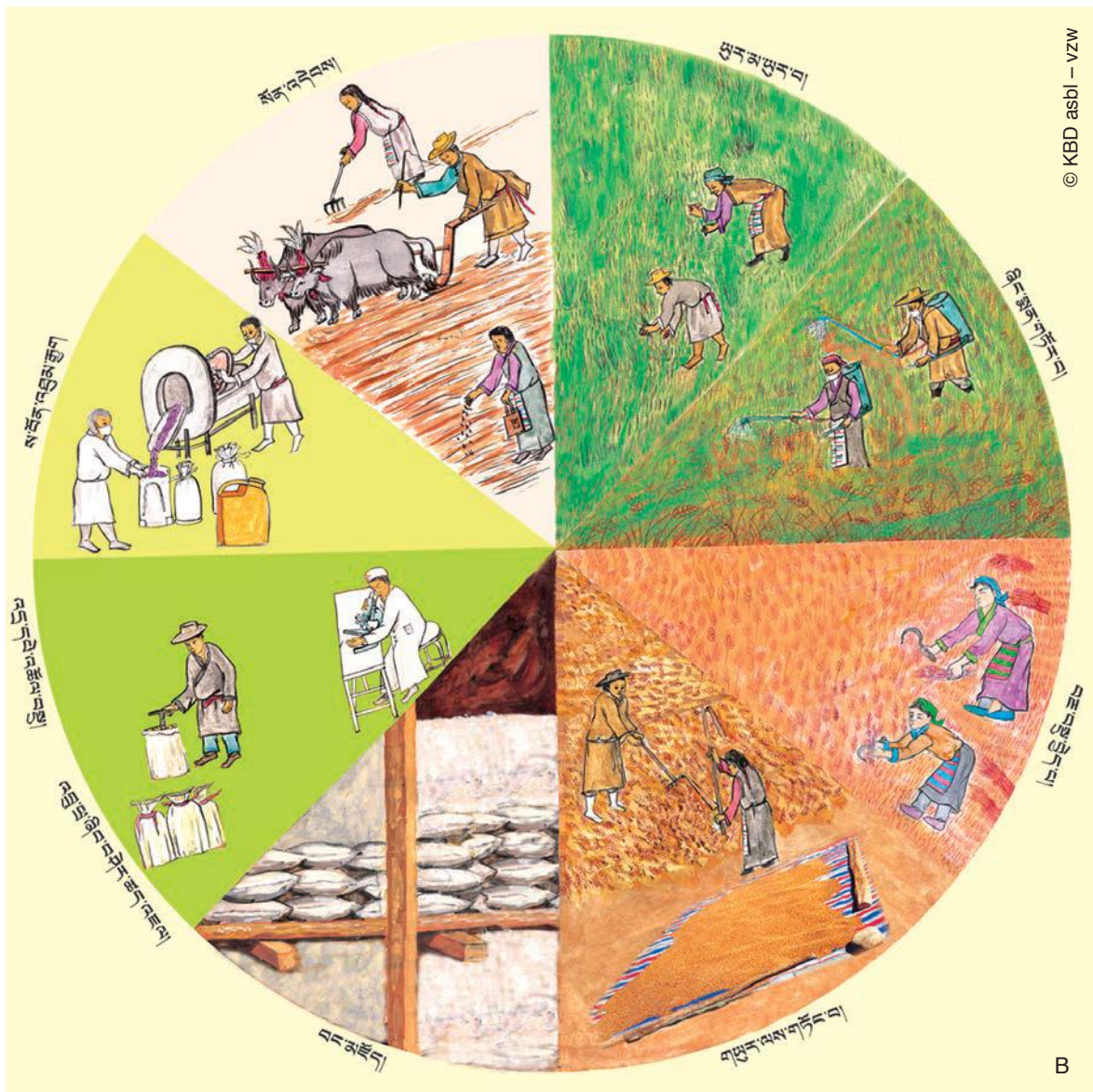
Studies after 2001 were firstly focused on the reproducibility of the mycological results obtained on both sides of Brahmaputra. The significant difference ($p < 0.01$) between the two areas was confirmed during 3 years (2004-2006), and we noticed a difference ($p < 0.05$) between affected (92%) and 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.



© Wangla Rinzen

A



© KBD asbl – vzw

B

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.



© Chasseur Camille

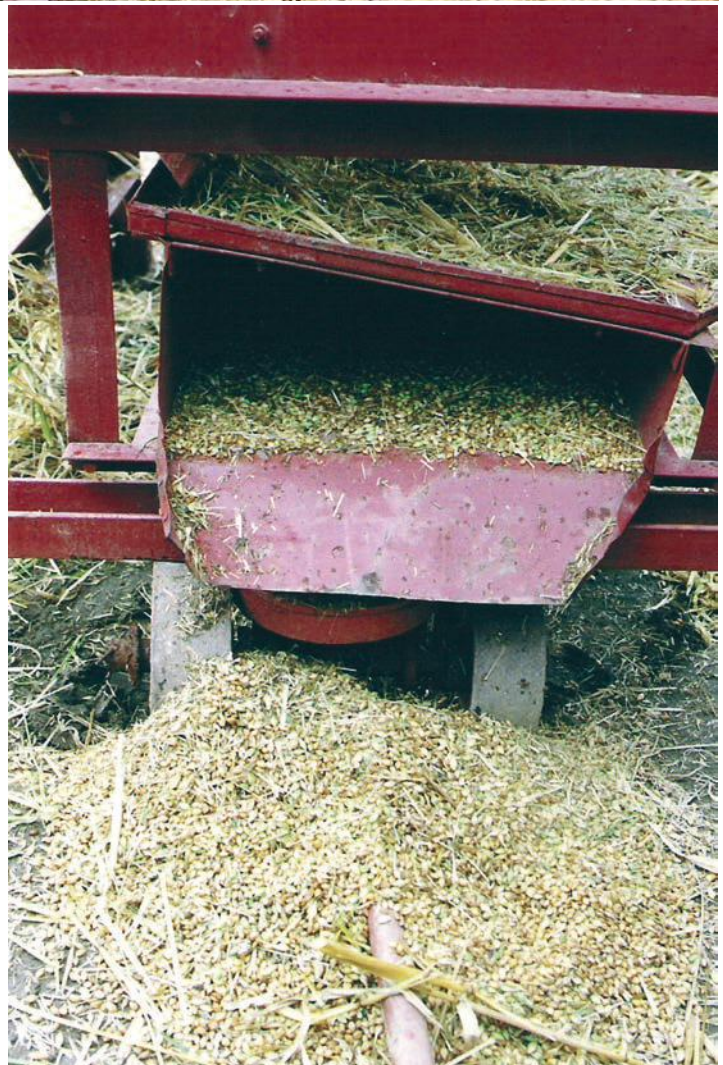


Plate 5.5. Threshing machines distribution. Threshing machines are an important help for peasants. Forty one machines were distributed by MSF in the prefectures of Lhasa, Lhoca and Shigatse between 2000 and 2002.

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

- Although significantly different ($p < 0.05$) the ergosterol concentrations in barleys from NEA ($1.2 \pm 1.1 \mu\text{g/g}$) and EA ($2.8 \pm 2.2 \mu\text{g/g}$) did not indicate acute mould proliferation. These results were in line with the mycological counting approach ($341 \pm 408 \text{ CFU/g}$ and $1253 \pm 1874 \text{ CFU/g}$ 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 Gansu confirmed our observations (95.8% of them showed ergosterol level ($< 5 \mu\text{g/g}$). 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 $> 10 \mu\text{g/g}$!
- No difference ($P > 0.05$) could be made between KBD affected ($2.8 \pm 2.4 \mu\text{g/g}$) and non affected ($2.8 \pm 2.0 \mu\text{g/g}$) 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 $30 \mu\text{g/kg}$). 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 biosynthesize the four mycotoxins of interest but in different proportions. This observation can be considered as a proof of their ability to synthesize 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 ($25 \mu\text{g/kg}$) 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 Heilongjiang (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 Heilongjiang, 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 Heilongjiang 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 significant 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.

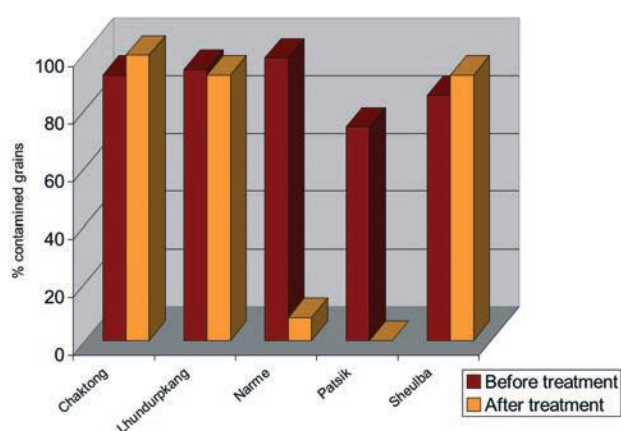


Figure 5.4. Total hygrophilic fungal contamination of seed before and after disinfection.

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 $\mu\text{g/g}$ 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 $\mu\text{g/g}$
				%	
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





© Wangla Rinzen



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.



© Wangla Rinzen

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.

Chapter 5.2

The mineral deficiency hypothesis

Camille Chasseur, Carl Suetens, Françoise Begaux, Eric Haubruge, Rinzen Wangla, Lobsang Rinchen, Lakpa Wangdu, William Claus, Françoise Mathieu

As we have seen above, in T.A.R., the weather conditions prevent from cultivating a large diversity of species, and food is generally rather poor and little diversified. Nutritional studies realised in T.A.R. showed signs of malnutrition in the rural populations which present growth difficulties and micronutrients deficiencies responsible for anaemia (Harris et al., 2001), gouter and cretinism (Kolsteren, 1995).

A selenium deficiency which is geographically distributed rather like the endemic area of KBD was considered as a serious hypothesis of KBD etiology. Some experiments on mice (Yang et al., 1993a; 1993b) revealed that a selenium deficient diet induced degeneration of the articular cartilage in the knee joints; and development of fibrocartilage at the articular surface of knee joints resembling the early stages of osteoarthritis. Sasaki et al. (1994) performed histological and biological studies on bone and articular cartilage specimens obtained from rats fed with a low-Selenium diet. Microscopically, no clear changes in the articular chondrocytes were apparent, whereas with the electron microscope, chondrocytes in the deep layer showed degeneration of nuclei and endoplasmic reticular ballooning. A decrease of bone mineral density was also noted. Anti-oxidant effect of selenium is generally suggested to explain the role of this element in cartilage and bone formation (Suetens et al., 2001).

From September to November 2002, during a mycological survey conducted in 40 families distributed on both sides of the Brahmaputra (Lhasa, Lhoca, Shigatse prefectures), earth samples were collected in the field of the studied families.

In a first time, the experiment was focused on selenium concentration in soil and in corresponding barley grain. For each sample, 3 core samples were taken in the upper 15 cm with a stainless steel to be combined into a composite sample, and dried at room temperature

before being brought back for laboratory analyses. Selenium was also quantified on grain coming from the same collected samples of barley used for fungal analyses. Measures were made by ICP, after wet destruction (HClO₄), followed by a treatment by HCl in a warm water bath.

Concerning selenium, the earth analyses results showed (Figure 5.5) a significant difference between EA and NEA. In EA, the concentration was very low everywhere (< 0.05 ppm; background level 0.5 ppm). Measured concentrations in earth sampled in NEA were higher than in EA, but with values lower than 0.5 ppm. In corresponding barley, a strong selenium deficiency was noted everywhere (≤ 0.02 ; background level 0.1–0.2 ppm) which supposes other etiologic parameters, combined or not. These results corroborate other findings which have brought scientists to a consensus in favour of a multifactorial origin, such as different aspects of complementary micronutrients deficiencies, micronutrients antagonisms, or links between micronutrients deficiencies and mycotoxins.

considered in relation to selenium and iodine status (Moreno-Reyes et al., 1998).

Concerning iron, in the thirties, the Japanese Hiyeda and other authors draw the attention to the role of this element (Levander, 1987; Sokoloff, 1989). They found a high iron rate in drinking water of the endemic zones like in the blood of KBD patients (Yamamuro, 2001). On the basis of various experiments on rabbits, they affirm that iron excess is the cause of Kashin-Beck disease. At present, the assumption of iron excess is no longer taken into account whereas among mineral interactions, manganese-iron looks quite interesting. Within the intestinal mucosa, manganese has been found to compete with iron for common binding sites for absorption, manganese having far less affinity to the carrier proteins than iron.

Concerning zinc, Wang research (2002) showed a direct effect deficiency on proliferation, differentiation, and apoptosis of chondrocytes in the epiphyseal growth

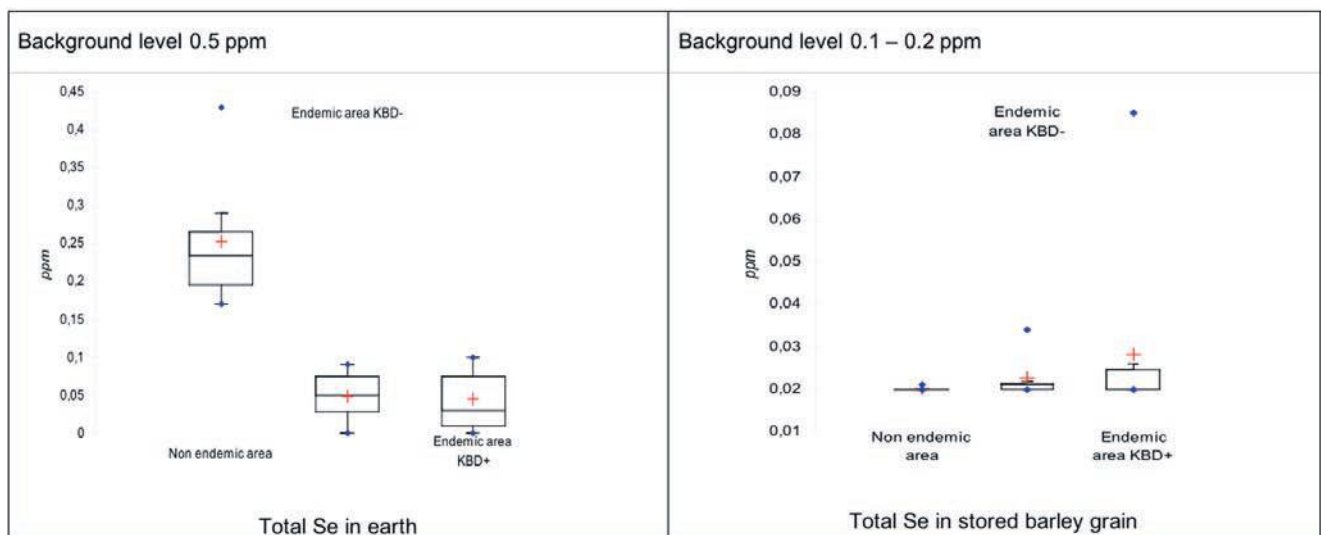


Figure 5.5. Selenium concentration in cultivated soil and in corresponding barley grain. Comparison between families in non endemic area and families with (KBD+) and without (KBD-) the disease in endemic area (not published).

Among other micronutrient deficiencies, manganese, zinc, copper, and nickel play an important role in the ossification process. On the contrary, iron may have an antagonist role in some micronutrient assimilation.

Manganese deficiency results in abnormal skeletal development in a number of animal species (Tuormaa, 1996). For example, in rats, mice, pigs, chicks, rabbits and guinea pigs, manganese deficiency, during gestation, has been associated with retarded and defective bone growth, enlarged hock joints, crooked and shortened legs and a variety of other leg deformities. Manganese has also been found to play a part in the formation of thyroxine, the active principle of the secretion of the thyroid gland, and this is interesting when KBD is

plate of young chickens. Severe growth plate lesions characterized by reduced cellularity and abnormally shaped cells were formed in areas remote from blood vessels after 7 days of zinc deficiency feeding. Zhang et al. (2003) suggested that zinc deficiency impaired proliferation and differentiation of chondrocytes and balance between osteoblast and osteoclast function by reducing the growth hormone levels in sera. Meriardi et al. (2004) observed that femur diaphysis length was greater in foetuses whose mothers received zinc supplements, and the difference tended to increase with gestational age, which suggests the potential importance of maternal zinc status for foetal bone growth in human. Munday (1985) also showed the ability of zinc to inhibit intestinal absorption of copper.

For these reasons, several experiments concerning measures of different micronutrients in cultivated soils, in the food, in different regions of China (T.A.R., Gansu,

Heilongjiang, Inner Mongolia) were started (Plate 5.9). Result analyses are in progress.



© Chasseur Camille

Plate 5.9. Some sites where surveys took place. A. T.A.R.: Targye village, Rimpung county, Shigatse prefecture – B. Gansu province: Pouwei village, Qingshui county, Tianshue prefecture – C. Heilongjiang province: Kunshin village, Huanan county, Jamusi district – D. Inner Mongolia Autonomous Region: Yao Xiao Qian Zi, Moli Dawa county, Zhala Tung prefecture.

5.3 The alternative food path or the very little diversified diet hypothesis

François Malaisse, Georges Lognay, Eric Haubruge, André De Kesel, Emile Delcarte, Bernard Wathelet, Patrick Van Damme, Françoise Begaux, Camille Chasseur, Pelma Drolkar, Philippe Goyens, Maurice Hinsenkamp, Béatrice Leteinturier, Françoise Mathieu, Lopsang Rinchen, Christiane Van Marsenille, Lakpa Wangdu, Rinzen Wangla

5.3.1. Background

For a long time, the welfare of Tibetan populations suffering from the Kashin-Beck disease has been the target of a team supported firstly by «Médecins sans Frontières» (MSF) – Belgium, later by the Kashin-Beck Disease Foundation (KBD F).

In 1998, F. Malaisse joined the KBD team. During a first survey in summer 1998 he recognized four major macro-ecosystems in South Central Tibet. These are the urban (Plate 5.10), the sub-urban (Plate 5.11), the rural and the nomad macro-ecosystems (Plate 5.12). Presence of KBD is restricted to the rural group. One major difference between the livelihood of inhabitants of this unit versus livelihood in the others groups consists in the diet. Moreover, as developed in previous chapters, within this unit, prevalence of KBD differs from one valley to another. Progressively the importance of diet has retained more attention by the KBD team. Therefore a research programme was set up in order to obtain a better knowledge of the local diet of peasants, and in particular the diversity and the importance of alternative foods.

Such approach has much to gain from environmental ethnological studies or «Ethnoecology». Indeed, increase of local population welfare through a better understanding of their ecological knowledge is one of the main concern of ethnoecology. Ethnoecology is a young science. Its first utilisation goes back to 1954, during a study carried out by Conklin and dealing with the Hanunoo, a Philippine people. Ethnoecology may be defined as the extension of two disciplines, ethnography and ecology. Other authors consider that it is the natural confluence of four precursor rivers:



Plate 5.10. Lhasa city, an example of Tibetan urban macro-ecosystem. A. The Norpu Linka road – B. The 3 golden yaks monument on the round point of Central Beijing road – C. The Potala palace – D. Barkhor street – E. Tibetan street ambiance.

ethnology, agroecology, ethnography and environmental geography (Malaisse, 1997a). It brings together the description of the different nations, from their material and cultural manifestation (ethnology) to the science of the milieu (ecology) (Berlin, 1992; Toledo, 1992; Gragson et al., 1999; Nazarea, 1999; 2006; Malaisse, 2001; Martin, 2001). Ethnoecology is regarded as the study of the management by cultures (customs, beliefs, social forms and material traits of a radical, religious or social group) of natural and human-modified ecosystems. It is an ecological approach to understand and appreciate traditional knowledge of land and living organisms (notably plants and animals).

As developed in several papers, ethnoecology underlies the real support of «Lessons of the Past for a better Future», the theme of a symposium organized in Brussels by the Royal Academy of Overseas Sciences of Belgium in coordination with UNESCO in August 2001.

5.3.2. Alternative foods

Human foods belong to diverse sources, including mineral products and living products. The last is to be found within the 5 kingdoms, namely Protista, Monera, Fungi, Plants and Animals.

No alternative foods belonging to the two first kingdoms were quoted during the surveys conducted in South Central Tibet. Human foods belong to the three last kingdoms and are commented below.

Links between Man and Fungi probably appeared at the dawn of mankind (humanity), but detailed accounts on this matter are relatively recent. According to Hawksworth et al. (1995) the term «ethnomycology» was first used by Wasson and Wasson in 1957 in their «Mushrooms, Russia and History». Today it is an established and currently used term to designate the study of the local people's knowledge of Fungi and their uses. Ethnomycology is a multidisciplinary field of research with a worldwide growing interest among mycologists as well as ethnologists, linguists, pharmacologists, food chemists and others.

The local importance of mycophagy varies greatly from one nation to another. As far as the Tibetan plateau is concerned few ethnomycological accounts have yet to be published. Sacherer (1979) studied the Sherpa's mycological knowledge in the Rolwaling valley and reports the local names of 15 edible species. Bhandary (1985) presents a synthesis for Nepal, listing 107 edible mushrooms (latin, english and nepali names), whilst for the same country, Adhikari and Durrieu (1996) report the consumption of 57 fungi (latin names). The

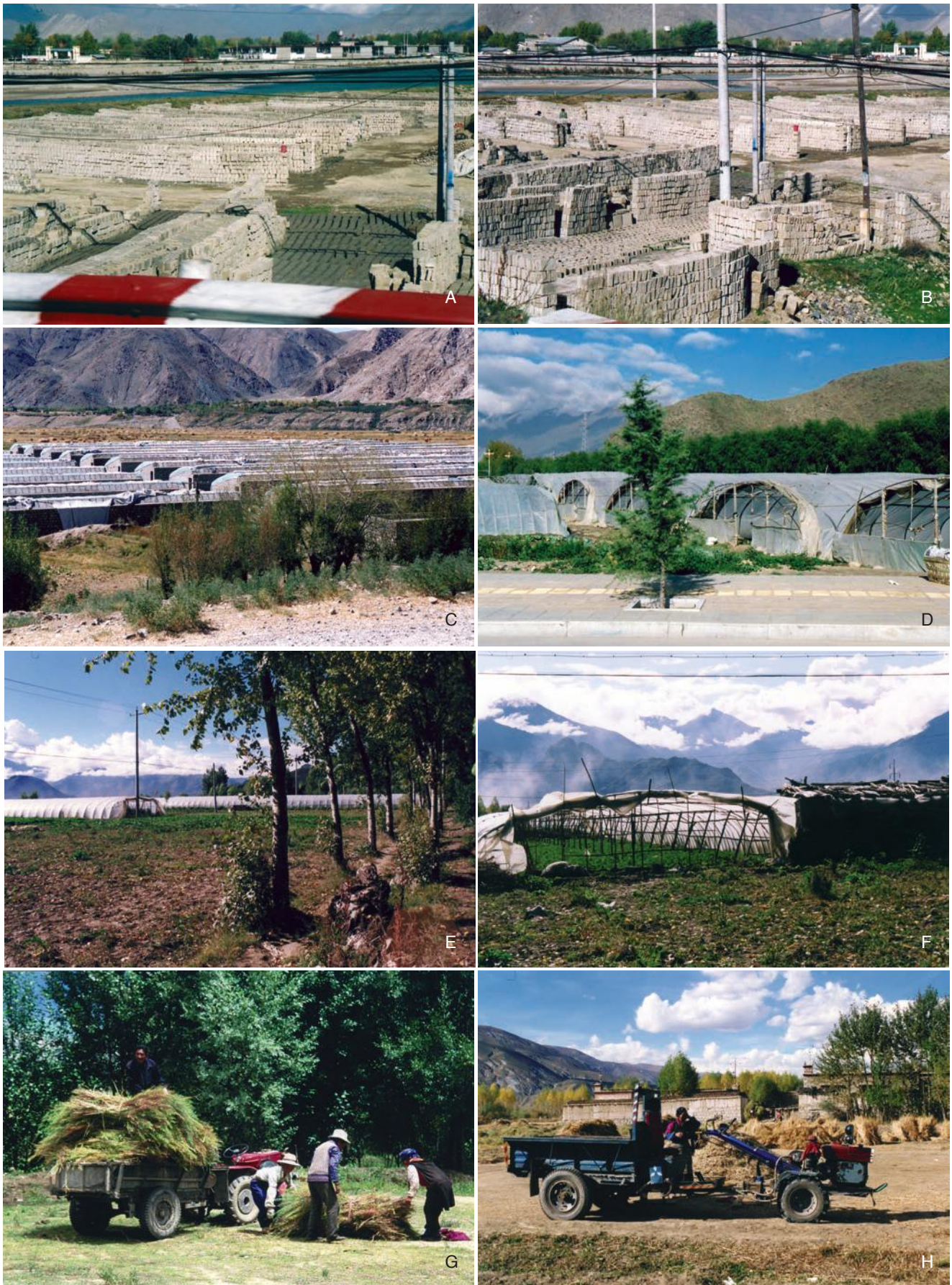


Plate 5.11. Around Lhasa city, the sub-urban macro-ecosystem. A. & B. Cement works – C. Market gardening – D. Tree nursery – E. & F. Canvas sheet covered greenhouses – G. & H. Tractors.



© Malaisse François

Plate 5.12. The pastoral zone or nomad macro-ecosystem. A. High plateau – B. Camping site during summertime – C. Nomads on Nam plateau – D. Camping site – E. Woollen catapult – F. Grandmother greeting – G. Young girl – H. Inner tent ambiance – I. Tent furniture (table) – J. Tibetan nomad tent – K. Inner tent ambiance – L. Top opening of a tent.

knowledge regarding fungi, as well as the consumption of mushrooms by Ü people, is hardly known and restricted. In spite of this, the Ü recognize about ten edible mushrooms and some other fungi for other uses. Usually mushrooms are collected for domestic consumption, mostly during field work or while tending the livestock (cows or others). Collecting for commercial purposes exists as mushrooms pickers head to the roads or the towns to sell their goods.

Ethnobotany has been defined as the study of people's classification, management and use of plants. This approach faces the challenging task of not only recording knowledge of the plant world, but also applying the results of their studies to biodiversity, conservation and community development. One of their goals is to ensure that local natural history becomes a living, written tradition in communities where it has been transmitted orally for many years (Martin, 1995). They are working against time, because local knowledge of the environment is fast disappearing in the face of worldwide destruction of natural areas and transformation of traditional cultures, which is, under others, the case in T.A.R. Good examples are the book of Martin (1995) that provides a fine methods manual, whilst Cunningham (2001) develops applied ethnobotany.

Several ethnobotanical surveys have been conducted regarding peoples inhabiting the Himalaya range and plateau. Most of them were located on the Southern slope of the range (Jest, 1972; Sacherer, 1979; Gaul et al., 1983; Toffin et al., 1985; Shrestha, 1988; Manandhar, 1989; 2002; Pohle, 1990; Johsi, 1991; Sundriyal et al., 2001). Less information concerns the Tibetan plateau, and mostly deals with medicinal plants (Tsarong, 1994; Dga' ba'i rdo rje, 1995; Kletter et al., 2001 as examples).

Alternative plant foods have received less attention. As far as South Central Tibet is concerned, preliminary results concerning Ü and Tsang peasant's knowledge should nevertheless be mentioned (Malaisse et al., 2002). This study leads to list 36 wild edible plants. Presently we have quoted the consumption by Ü of some 15 potherbs, 6 plants provide edible underground organs, 3 offer edible fleshy fruits, the flowers of 3 plants are sucked for their nectar, 4 plants are used as spices, 6 as aromatic herbs, one as a condiment.

Other aspects of ethnobotanical knowledge of Ü are tremendous; for instance they concern plants used in the phytotechnology of the main crops or also for domestic uses. Underrated targets are notably incense and incense sticks or also plants used in dyeing as well as food colourings.

The term ethnozoology was used for the first time in 1914 by Henderson and Harrington studying the Indians tribes of the Great Plain, but interest turned to ethnozoology arises later (Chevallier et al., 1988). In 1966, ethnozoology was developed at the Museum National d'Histoire Naturelle of Paris by Pujol.

Ethnozoology is the study of the past and present interrelationships between human cultures and the animals in their environment. It includes classification and naming of zoological forms, cultural knowledge and use of wild and domestic animals.

Ethnoecological enquiries were carried out in 1998, 2000, 2001, 2002, 2004, 2005 and 2007 (Plate 5.13). They took place in 3 prefectures, 12 counties, 22 communities, 75 villages and 7 sites. All together, 116 families and some 215 peoples were involved (Plate 5.14). In fact, the number of people involved varies according to the targets. They were asked

- to enumerate the edible species they consume,
- to recognize taxa illustrated in an atlas,
- to rank the species eaten in decreasing order of preference,
- to comment on their habitat,
- to estimate the extent of time they ate those products last summer.

These enquiries allow to establish a citation index as well as to obtain an approached estimation of consumption.

Reference materials (collection B. Leteinturier and F. Malaisse for fungi, collection F. Malaisse for higher plants) are deposited in the diverse sections of the National Botanical Garden of Belgium (BR acronym according to Holmgren et al. 1990), notably Mycology Herbarium and Higher Plant Herbarium. Authorities of mushroom names are according to Brummit and Powell (1992), of higher plants according to the Flora of China (1994-2008).

5.3.3. Edible mushrooms

Local naming of ethnotaxa

In T.A.R., mushrooms are referred to as «*chamo*», but several ethnospecies are given a particular (unique, specific) name. This last name is frequently built from the syllable «*cha*», meaning mushroom, preceded by an attribute. It should be noted, on the other hand, that the word «*cha*», and no more the syllable, means «*meat*»; and consequently that the radical «*cha*», related to mushroom also brings to think about meat, as texture of certain mushroom is fleshy! No surprise therefore regarding the Tibetan proverb: «*cha mebo, chamo sa gui*», or «when there is no meat, mushrooms are eaten!».

As stated above, a precise nomenclature relative to diverse mushrooms exists. It is mainly based on the colour of the fruiting bodies or sporocarps. In this case, two radicals are put together, one of the colour and the second being «*cha*». It should be kept in mind that *karmo* means white, *serpo* yellow, the radical *ser* corresponding to gold, *marmo* means red, *djangpo*

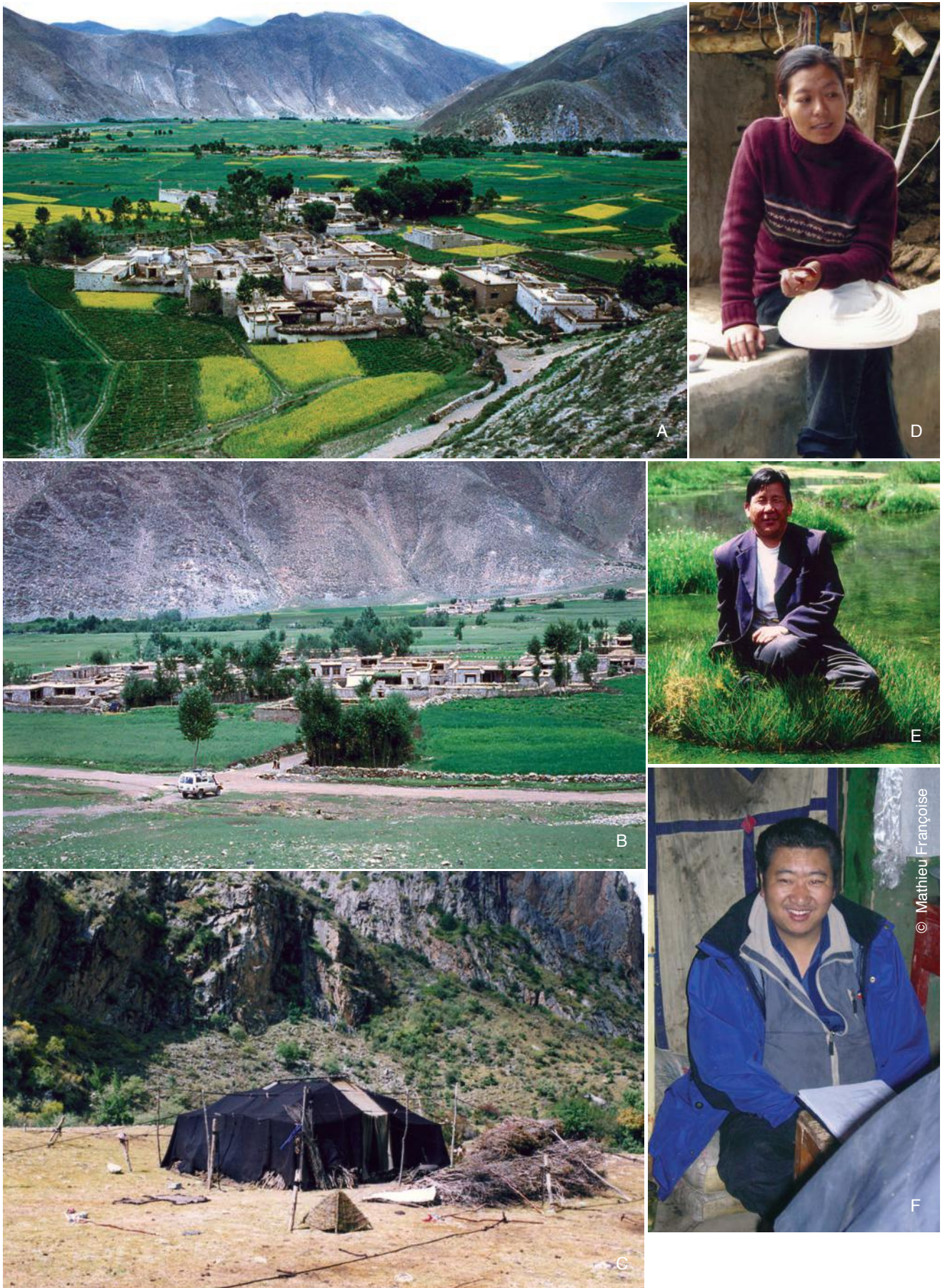


Plate 5.13. The ethnoecological survey. Ethnoecological inquiries took place in 75 villages and 7 sites. A. Lamotse village – B. Porokang village – C. Site in the vicinity of Tsingda – D. to F. Translations were carried out by 3 collaborators: D. Pelma Drolkar – E. Lopsang Rinchen – F. Lakpa Wangdu.



Plate 5.14. Some locutors of the environmental ethno-enquiries.

green, *wangpo* blue, *gyammu* brown, and *nakpo* means black, in such a way that some mushrooms are known as *karcha*, *sercha* and *marcha*.

A number of *Agaricus* with whitish colours are named «*karcha*». After heavy rains they are frequently encountered in fields and meadows. They are observed in abundance between 3,600 and 4,000 m a.s.l. and they are quite commonly eaten. *Agaricus* with a white cap are known as *pangcha*; another *Agaricus* (sect. *Flavescentes*), with a yellow cap, is known as *langchu*.

Sercha, or *Floccularia luteovirens*, is a yellow Tricholomatoid mushroom observed in July, especially in years with heavy rainfalls. It is mainly observed between 3,900 and 4,600 m in green short (dwarf shrub) vegetation of the *pangri* type. It appears as the most appreciated mushroom, and is frequently offered for sale in the town markets. It is eaten raw or preferably fried.

Chucha, also erroneously locally called «*mokro*», has several times been quoted during interviews. It refers to a representative of the Auriculariales, namely *Auricularia polytricha*, and it is sometimes sold on the markets. Its name, «water mushroom», refers to its occurrence in humid or very wet environments. When growing in polluted streams, *chucha* is not collected. It must not be confused with *Auricularia auricula-judae*, the Jew's ear, in fact *mokro* which is imported from other provinces of China and frequently offered for sale in town markets. The latter is mostly eaten by Chinese peasants, and only sometimes by Tibetans.

Some shaggy ink caps, among others *Coprinus comatus*, are also eaten from time to time, by few peasants. They are usually referred to as «*pungu kacha*», meaning «donkey mushroom». This naming is given according to the resemblance of the fruiting bodies with the erected penis of donkeys. They are available in June and July.

Marcha, a reddish mushroom outside, is more rare and collected in July. This Boletineae is only eaten after cooking.

In general trees and wood are linked with the name «*ching*», while «*djang*» refers to trees that were planted by man, like willows (*Salix* spp.) and poplars (*Populus* spp.). *Chingba* literally refers to «the tree goutre», because of the tumour this polypore (Polyporaceae) causes on its host. *Chingba* is on several *Salix* spp.; it is more and more eaten, offered for sale along roads and in town markets; one pound is sold for 4 yuans. *Djangsha* or «tree-mushroom», on the other hand, refers to *Lentinula edodes*.

It should be noted that a whitish to cream coloured pleurote is frequently sold in town markets. These *Pleurotus pulmonarius* are most often bought by Chinese people coming from other provinces and neglected by

most Tibetans. In the same way it was observed that *Lentinula edodes*, the Japanese shii-take, becomes also frequent in town markets.

Finally we observed that the nomads from the surroundings of Shigatse identify *Calvatia* sp. as *porok-nata* or «crow-snuff» (*vide* Ritschen); although edible, this puffball is not eaten. Around Lhasa, in the rocky outcrops, *Calvatia cyathiformis* was found and named *pokoudi*, but not eaten.

The importance of collecting *yazagonbu* or caterpillar fungus, should not be underestimated. This fungus, *Cordyceps sinensis*, extends in spring a thread-like to club-shaped fruiting body from a below ground caterpillar. *Cordyceps sinensis* is a parasitic fungus and it attacks and kills the hibernating larvae of *Aenetus (Hepialus) virescens* (Doubleday) an Hepialidae. The use of this fungus is primarily medicinal, it is known as a tonicum and appreciated for its energetic properties (Pegler et al. 1994). *Cordyceps sinensis* fruiting bodies are to be found in the Asian-alpine meadows, above 4,000 m altitude (Jingwei 1982). In T.A.R., the collecting occurs mainly in the vicinity of Chamdo and Nachku prefectures, in May and June; then (farmers) peasants seek through mountains in search of this manna, which provides an important financial injection. In the region of Amdo Golok, in eastern Tibet 9,105 kg of *yazagonbu* were extracted from 1987 to 1992 (<http://www.tew.org/wildlife/wildlife.biodiversity.html>); this product is still of importance in Kumaon Himalayas (Garbyal et al., 2004). In South Central Tibet people are also looking for this mushroom. Even better, sometimes they eat half of the specimens they collect and sell the others.

Diversity and appreciation of fungi recognized

Table 5.2 lists the edible mushrooms recognized by Ü people and by Tsang farmers (Plate 5.15). If most of these peasants name, at first, two or three edible mushrooms, nearly a tenth are recognized. A few others are of interest for diverse reasons, mainly medicinal use. Each person interviewed reported to know 1 to 6 mushrooms, with a mean value of 2.68 taxa per person. Ethnospecies *sercha* and *karcha* emerge, with respectively 173 and 106 quotations (Table 5.2) and a citation index value of 321 and 106. Of lesser importance is *chucha*, with 95 quotations and a citation index of 87. Other mushrooms eaten are *djangcha* (41 quotations, citation index of 38), *pangsha* (42 quotation, citation index of 21), *langshu* (43 quotations, index of 21) and *tashopoungri* (12 quotations, citation index 4.5).

In suburban areas the consumption of *Pleurotus pulmonarius* progresses. Frequencies of citation, of preference and of consumption of *sercha* versus *karcha* increase with the higher altitude of the villages.

Importance of mushroom consumption

The 178 interviewees stated they use one to six different mushrooms for consumption, one man refuses all mushrooms. The average number of ethnospecies consumed per person is 2.68 ± 0.52 .

Chemical composition of Tibetan edible mushrooms

• Oligoelements

Four species have been studied regarding their mineral content (Table 5.3.). They concern the three most eaten species as well as the major species commercialized in town markets.

Besides the interspecific variability, the measured concentrations of the potentially toxic elements like As, Cd, Pb and Hg are low for mushrooms which are renowned as bioaccumulators. Only *Floccularia luteovirens* shows high concentrations of Cd and Hg. Indeed the European norms for food products are 0.2 mg/kg fresh weight for both elements.

• Proteins

The same species have been studied regarding their protein content (Table 5.4) and amino-acid score (Table 5.5).

Lysine is a limiting amino-acid or nearly limiting all these mushrooms. Leucine is also limiting especially

Table 5.2. Mushrooms eaten in South Central Tibet.

Nr	Species	Translitteration of Tibetan name	Voucher L=Leteinturier M=Malaisse	Litteral translation	Number of citations (178 individuals)	Preference index	Rank
1	<i>Agaricus</i> (sect. <i>Agaricus</i>) <i>campestris</i> var. <i>squamulosus</i> (Rea) Pilát	karcha	M 15809, 15812	White mushroom	106 (58%)	143.5	2
2	<i>Agaricus campestris</i> L. s. l.	pangcha	M 15827	Steppe mushroom	42 (24%)	31.8	5
3	<i>Agaricus</i> sp. 1 (sect. <i>Flavescentes</i>)	langchu	L&M 540		43 (24%)	20.8	6
4	<i>Auricularia auricula-judae</i> (Fr.) Quél.	mogru	L&M 428, 538		(1, 2)		
5	<i>Auricularia polytricha</i> (Mont.) Sacc.	chucha, mogro	L&M 249	Water mushroom	95 (53%)	87	3
6	<i>Calvatia cyathiformis</i> (Bosc) Morg.	pokoudi			4	1.2	9
7	<i>Coprinus comatus</i> (O.F. Müll.) Gray	pungukacha, tashopungri	L&M 248, 544	Dunkey mushroom	12 (7%)	4.5	8
8	<i>Cordyceps sinensis</i> (Berk.) Sacc.	yarzagonbu	L&M 429	Winterworm, summer grass	5	(4)	
9	<i>Flammulina velutipes</i> (Curtis) Singer	zingzengu	L&M 543		(1)		
10	<i>Floccularia luteovirens</i> (Alb. & Schw.) Pouzar.	sercha	L&M 428, 431, 432, 462	Golden mushroom	173 (97%)	321.3	1
11	<i>Inocybe dulcamara</i> (Alb. & Schw.: Pers.) Kummer	djincha	L&M 426	Piss mushroom	(3)		
12	<i>Lentinula edodes</i> (Berk.) Pegl.		L&M 430, 463, 545, 546		16 (9%)	9.9	7
13	<i>Lentinus cyathiformis</i> (Schaeff.) Bres.	?	L&M 424	?	1		10
14	<i>Paxillus involutus</i> (Batsch.) Fr. s. l.	djangcha, marcha	L&M 547	Tree mushroom	41 (23%)	38.0	4
15	<i>Pholiota nameko</i> (T. Itô) S. Ito & S. Imai		L&M 552		(1)		
16	<i>Pleurotus ostreatus</i> (Jacq.) P.Kumm.		L&M 548		(1, 2)		
17	<i>Pleurotus cf. pulmonarius</i> (Fr.) Quel.	pingu	L&M 247		(1, 2)		
18	<i>Tyromyces</i> sp. 1	chinba	L&M 423	Tree goiter	1	0.3	10

(1) cultivated by Chinese peasants of other regions; (2) rarely eaten by Ü; (3) information to be confirmed; (4) mostly considered as medicine rather than food



Plate 5.15. Some edible mushrooms . A. *Floccularia luteovirens* – B. *Agaricus campestris* – C. *Paxillus involutus* – D. *Auricularia polytricha* – E. *Auricularia auricula-judae* – F. *Coprinus comatus* – G. *Paxillus involutus* – H. *Lentinula edodes* – I. *Pleurotus pulmonarius* – J. *Agaricus campestris* var. *squamulosus* – K. *Floccularia luteovirens*, for winter consumption – L. *Aenetus virescens* caterpillar parasited by *Cordyceps sinensis* – M. *Flammulina velutipes*.

Table 5.3. Mineral content of four major Tibetan edible mushrooms (in % of 100 g D.M. or in mg/kg D.M. (Dry Matter)).

Mineral	<i>Agaricus campestris</i> <i>var. squamulosus</i>	<i>Auricularia polytricha</i>	<i>Floccularia luteovirens</i>	<i>Pleurotus pulmonarius</i>
P (%)	0.769	0.398	0.775	1.359
K (%)	3.112	1.612	4.630	6.460
Ca (%)	0.086	0.711	0.106	0.083
Mg (%)	0.141	0.452	0.150	0.223
Na (%)	0.052	0.172	0.040	0.160
S (%)	0.107	0.019	0.383	0.409
Cl (%)	0.032	< 0.001	0.014	1.670
Fe (mg/kg)	138	977	393	823
Mn (mg/kg)	8.3	164	31	31
Zn (mg/kg)	99	31	97	107
As (mg/kg)	0.80	1.45	0.35	0.86
Se (mg/kg)	1.26	0.27	0.72	0.92
Cu (mg/kg)	18	6.2	60	48
Cd (mg/kg)	< 0.01	0.03	2.96	< 0.01
Co (mg/kg)	< 0.01	3.73	2.12	0.34
Cr (mg/kg)	0.49	17	13	8.1
Ni (mg/kg)	0.83	8.7	3.9	3.9
Pb (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01
Hg (mg/kg)	0.05	0.04	1.18	0.63

in *Agaricus campestris* in which all the amino-acids are limiting. If we compare the relative proportions of essential amino-acids, they are best correlated to leguminous plants than to cereals. If we except *Agaricus campester*, the amino-acid score is near that of pea.

• Lipids

The lipid composition of ten species have been studied (Table 5.6).

The total lipid content of the investigated Tibetan edible mushrooms is quite low and ranged between 1.6 to 5.3% (DM). The fatty acid profile revealed constituents from C14 to C24 and highly unsaturated oils. Excepted in *Cordyceps sinensis* it was found that the major component was linoleic acid (C18:2), an essential fatty acid from the ω -6 family.

Discussion

The use of only one term for naming all the mushrooms in a local language is a common practise and mentioned in numerous ethnomycological studies. Adhikari and Durrieu (1996) point out that it is also the fact for nearly all the Nepalese languages (nine are listed). The same observation was made by Malaisse (1997b) concerning a number of languages from South Central Africa. It should be noted that the substantif «shamu», that is to say «hat» and by extension, «fungi with hat», is a general term used in Nepal (Sacherer, 1979); in Tamang «syamo» and in Nepali «chyau» are used in the same way (Bhandary, 1985).

The choice of the attribute for particular names originates from several aspects of the mushroom, such as

macroscopical features (colour), ecological characters (mainly habitat), organographic analogies and so on.

Concerning *Cordyceps sinensis*, it should be noted that the vernacular name in Sherpa language (Nepal), is «yer tsa gum bu» (Adhikary et al., 1996), literally meaning «winter larvae, summer grass. The same is noted in Chinese «dongchong xiacao» (Jingwei, 1982), as well as in Tibetan; they all express the metamorphosis of the caterpillar in winter into a blackish filament, which resembles a little bit to a blade (Plate 5.15.L). The Nepalese name «yer tsa gum bu» strongly resembles the Tibetan name being «yarzagonbu».

In comparison to the ethnomycological literature from Nepal and the Himalayas, our records add a number of edible species which typically belong to high altitude open habitats. Adhikari and Durrieu (1996) mention 28 species, commonly found on the markets in Nepal. Most of these taxa, i.e. more than 50%, are exclusively ectomycorrhizal or symbiotic (including Termitomyces) suggesting that these come from forested areas at lower altitudes (below 3500 m). The saprophytic species they mention indicate that a number of species is cultivated, i.e. *Volvariella volvacea* and some *Pleurotus* spp. From the non-cultivated species mentioned in Adhikari et al. (1996), it seems only *Agaricus campestris*, *Coprinus comatus*, *Auricularia auricula-judae*, *Lentinula edodes* and *Cordyceps sinensis* are also found in Tibet. Given their large ecological amplitude, it is probably right to suggest that these taxa are common on markets from higher altitude villages and settlements. The list of edible species from Nepal, presented by Bhandary (1985), is more extensive and adds up to over a hundred edible species, including a high number of taxa like *Scleroderma* spp., *Mycena galericulata*, *Hygrocybe*,

Table 5.4. Composition in proteins of four major Tibetan edible mushrooms (in % of 100 g D.M.).

Protein	<i>Agaricus campester</i> var. <i>squamulosus</i>	<i>Auricularia polytricha</i>	<i>Floccularia luteovirens</i>	<i>Pleurotus pulmonarius</i>
N(%)	9.000	1.693	6.682	3.474
Proteins (%)	56.250	10.580	41.760	21.710
Asp	1.880	0.932	3.222	1.935
Thr	0.922	0.497	1.716	0.859
Ser	0.947	0.516	1.887	0.922
Glu	3.903	1.244	5.206	3.251
Pro	0.868	0.435	1.250	0.702
Gly	0.938	0.500	2.160	0.841
Ala	1.387	0.640	2.007	1.201
Cys-Cys	0.250	0.108	0.417	0.204
Val	1.111	0.527	1.577	0.957
Met	0.274	0.139	0.458	0.280
Ile	0.823	0.348	1.273	0.687
Leu	1.373	0.678	2.082	1.170
Tyr	0.455	0.221	1.039	0.536
Phe	0.732	0.374	1.260	0.692
Glu-NH ₂	5.432	0.773	3.711	2.236
His	0.611	0.223	0.743	0.355
Orn	0.204	0.019	0.630	0.143
Lys	0.978	0.493	1.915	0.949
Arg	0.997	0.510	2.038	1.029
Trp	0.39	0.19	0.42	0.28
Total	24.473	9.363	35.009	19.234

Table 5.5. Amino-acid score of Tibetan edible mushrooms (see Appendix II).

Amino-acid	mg/g prot.	<i>Agaricus campester</i> var. <i>squamulosus</i>	<i>Auricularia polytricha</i>	<i>Floccularia luteovirens</i>	<i>Pleurotus pulmonarius</i>
Thr	34	48	138	121	116
Val	35	56	142	108	126
Met + Cys-Cys	25	37	93	84	89
Ile	28	52	117	109	113
Leu	66	37	97	76	82
Tyr + Phe	63	33	89	87	90
His	19	57	111	94	86
Lys	58	30	80	79	58
Trp	11	63	159	91	119
Score		30.00	80.34	75.53	75.43

Tremella sp. and others, which are not consumed in Europe. The common taxa in our and Bhandary's lists are again the same, almost cosmopolitan species. The most important species for Tibet which is missing in the accounts on Nepalese mushrooms seems to be *Floccularia luteovirens*. This taxon is badly known in Europe, ectomycorrhizal and associated in T.A.R. with shrub trees above 4000 m altitude. *F. luteovirens* is recognized by its stout tricholomatoid silhouette, a pale yellow to cream-coloured, 10 cm wide pileus, becoming paler with age, having at first yellowish and then paler slightly reflexed and fairly large squamules; the cap's margin is devoid of any striation and at least appendiculate when young. The lamellae are white with concolorous edge, adnexed and becoming yellow with age. The stipe is exceptionally cylindrical but most often strongly tapering to the base, full (rarely fistulose) and relatively short compared to the diameter of the

pileus, measuring 2-3 cm long by 0.5-1.5 cm thick. It is annulate, whitish-yellowish and glabrous above the ring, yellowish and covered with reflexed squamules below the ring. Annulus is fixed half way the stipe, membranous, simple, whitish, becoming more yellowish and completely shrunk with age. The presence of *Inocybe dulcamara* among the edible taxa is unique and surprising, as this taxon belongs to a notoriously toxic genus *Inocybe* causing a Sudorian syndrome, i.e. due to muscarine (Bresinsky et al., 1985) and causing transpirations, excessive salivation, lacrymation, nausea, diarrhoea, eyesight problems, asthma. Hallucinations are not reported nor the interactions with alcohol. More studies are urgently needed to check whether poor people indeed consume this «pee mushroom» and if so, how they prepare it.

Table 5.6. Composition in lipids of Tibetan edible mushrooms (in % of total lipids).

Fatty acid		<i>Agaricus campestris</i> var. <i>squamulosus</i>	<i>Auricularia polytricha</i>	<i>Coprinus comatus</i> var. <i>ovatus</i>	<i>Coprinus comatus</i>	<i>Cordyceps sinensis</i>	<i>Flocularia luteovirens</i>	<i>Inocybe dulcamara</i>	<i>Lentinula edodes</i>	<i>Lentinus cyathiformis</i>	<i>Pleurotus pulmonarius</i>
Myristic acid	C14:0	0.16	0.54	0.65	0.32					0.35	7.37
Pentadecanoic acid	C15:0	0.22	1.09	0.38	0.22			0.12	0.93	2.62	0.40
Palmitic acid	C16:0	7.37	11.64	11.20	12.75	17.12	12.3	10.06	12.52	15.11	15.86
Palmitoleic acid	C16:1	1.7		0.97	0.61	1.59	1.03	0.48	0.76	0.97	1.35
Margaric acid	C17:0	0.29								0.50	
Stearic acid	C18:0	1.71	1.27	0.42	0.76	0.84	1.7	2.07	0.99	0.83	3.56
Oleic acid	C18:1 cis-9	2.69	13.79	5.50	6.37	52.77	13.2	35.63	3.39	8.31	26.14
	C18:1 isomer	1.07		1.40						1.84	2.52
Linoleic acid	C18:2	81.24	70.66	77.35	76.80	25.06	71.3	45.22	79.08	67.59	35.32
Linolenic acid	C18:3			0.93	1.26	1.93		0.71		0.22	3.03
Arachidic acid	C20:0	1.27									0.36
	C20:1										0.41
Behenic acid	C22	0.78									0.82
	C22:1									0.44	
Lignoceric acid	C24	0.45						0.34			tr
Others *		1.05	1.01	1.2	0.91	0.69	0.47	5.37	3.43	1.22	2.86
Total Lipids**		3.7	2.3	3.2	4.5	5.3	3.1	2.8	1.6	1.9	2.6

* Unidentified compounds; ** g lipids/100d Dry Matter (DM).

Conclusion

In conclusion, it appears:

- the popular knowledge of edible mushrooms in South Central Tibet is mainly limited to three ethnotaxa: *sercha*, *karsha* and *chucha*, sometimes also erroneously called *mokro*. Whilst the two first are eaten for themselves, *chucha* is always served with other food, mainly meat;
- more rare, even exceptional, is the consumption of 5 to 8 other taxa, of which five are certified by reference material;
- a reduced local consumption of fungi is noted for adults (1 to 5 time in summer), but is more frequent for children, mainly for those that tend cattle;
- a local distribution and consequently also a local consumption which is largely controlled by climatologic conditions (yearly temperatures and rainfall) as well as the ecosystems and land use (farming, grazing);
- *karsha* is frequent between 3,600 and 4,000 m altitude and mainly observed in fields and meadows; *sercha* is

frequent between 3,900 and 4,600 m altitude and occurs in the bangri vegetation; finally *chucha*, which develops in aquatic conditions, presents a more wide altitudinal distribution, but is neglected when growing in polluted water bodies;

- there exists a clear gustative preference for *sercha*;
- *sercha* is frequently dried and conserved for winter consumption.

5.3.4. Edible potherbs

Previous studies

Some results on wild edible plants of South Central Tibet have already been published, namely a table of 36 plant taxa, their uses, as well as their Tibetan names (Malaisse et al., 2003). Those plants comprise 14 potherbs (39%), 9 spice plants (25%), 6 stem-legumes (17%), 4 edible underground plants (tubers, roots or corms) (11%) and 3 fleshy fruits (8%). Wild edible leafy plants or potherbs were in this way the most important group. Potherbs

are frequently one of the main item of alternative foods, even if in several regions, such as in tropical Africa, fruits take the top position (Malaisse, 1997b). Nevertheless diversity of potherbs vary greatly according to ethno-linguistic groups. High values have been quoted for several countries of Africa (Irvine, 1952; 1956) as well as South-East Asia (Christensen, 2002).

Diversity of potherbs

A fifteen or so potherbs were listed (Table 5.8, Plate 5.16). The relative importance of them was approached through a citation index score. Table 5.7 presents those values.

Sapo, the young nettle leaves (*Urtica* spp.), takes the top position. Anybody knows the plant – in fact several species are concerned – and this food is highly appreciated, collected and stored (Chap. 5.4). A soup, *suptuk*, is prepared from nettle young leaves.

The tender leaves and shoots of *koni*, *Carum carvi*, came in second position. It should be noted that this herb is primarily known for the use of its fruits as spice (Németh, 1999) and medicine (Tsarong, 1994), but leaves are locally also used notably for importing aromatic flavours to supps and sauces (Bhattacharjee, 2000). We have quoted its presence during July and August at the Lhasa markets.

Cham-pah, *Malva verticillata*, is also a well known medicinal plant (Tsarong, 1994) and potherb, but less wanted.

The importance accorded by local populations to touchong, an adder's tong fern, became obvious. Indeed, *tuchung* has three times been presented, during the interviews as a valuable legume entering a local soup. It took the fourth place as potherb. This fern produces one or two fronds, 5 to 15 cm long, at mid-July.

Some eight other items appear on table 5.7. Finally leafstalks and stems of rhubarbs, young shoots of asparagus are also eaten (Malaisse et al., 2003); but neither rhubarbs, neither asparagus are here considered as potherbs.

The lipid composition of ten plants is presented in table 5.9. As shown, except *Lancea tibetica* (7.0% oil), all the investigated plants have low lipid content ranging from 2.8 to 5.3% by weight. Their fatty acid methyl ester profiles determined by GC-FID of all of them show interesting levels of essential polyunsaturated fatty acids (from 29.7 to 70.7%). As in other common green vegetables α -linolenic acid (9,12,15-cis,cis,cis-octadecatrienoic acid) is the major constituent and plays a very relevant role on a nutritional point of view (precursor of biologically important eicosanoids with specific functionalities). The consumption of the different investigated potherbs contributes to the supply in ω -3 fatty acids for the Ü and Tsang peoples. Consumption of these potherbs should be encouraged.

Discussion

Young shoots and leaves of diverse nettles, including *Urtica dioica*, *Urtica triangularis* and *Urtica hyperborea*, are wanted in spring and consumed after boiling in water as spinach, the so-called *sapo*, or also air dried in view of further consumption as soup, called *suptuk* in South Central Tibet. The use of young leaves of the stinging nettle as green has been quoted for other populations within the Himalayan range, notably for the Tamang (Toffin et al., 1985), the Sherpas of the Rolwaling valley in Nepal (Sacherer, 1979), in Garhwal Himalaya (Gaul et al., 1983) as well as Sikkim Himalaya (Sundriyal et al., 2001). It is still observed elsewhere (see chap. 5.4). Leaves of several nettles may be boiled and eaten, or used young and uncooked as spinach. They are appreciated by local population in several countries, notably in Kenya for *Urtica massaica* Mildbr. (Friis, 1989; Maundu et al., 1999), in Simien mountains of Ethiopia for *Urtica*

Table 5.7. Relative importance of wild edible potherbs of South Central Tibet.

Species	Tibetan transcription	Number of recognition (37 interviews)	Number of citation (5 top plants)	Citation index
<i>Urtica</i> spp.	zapo	37	28	87
<i>Carum carvi</i>	koni, toniu, sharotange	29	20	60
<i>Malva verticillata</i>	champa	28	8	27
<i>Ophioglossum polyphyllum</i>	tuchung	3	3	14
<i>Taraxacum</i> spp.	khumo, mo, yo, khurmong	23	5	13
cf <i>Angelica</i> sp.	cha	23	3	12
<i>Codonopsis bulleyana</i>	sukpa metok	14	4	12
<i>Fagopyrum tataricum</i>	tragö, koyowa, torcherawa, pagö	24	1	3
<i>Chenopodium album</i>	neu (lego, nubre)	12	0	0
<i>Plantago depressa</i>	tharam, (alathapo, popkera)	10	0	0
<i>Sonchus brachyotus</i>	gyakhur nagpo, gyegungwa, anggum, umguma	6	0	0
<i>Lancea tibetica</i>	didigonggu, payagpa	2	0	0

Table 5.8. Wild edible potherbs of South Central Tibet according to Ü and Tsang (after Malaisse et al. 2003, modified).

Family	Species	Voucher (L & M)*	Organ eaten	Use	Translitteration of Tibetan names (Wylie, 1959)	Transcription of Tibetan names (Tournadre, Dorje, 2003)	English names
Apiaceae	<i>cf. Angelica</i> sp.	342, 443	young upper part	green vegetable		cha	
Apiaceae	<i>Carum carvi</i> L.	278, 435	tender leaf and shoots	green vegetable	goh-nyöd	koniü, toniu	caraway
Asteraceae	<i>Cortia depressa</i> (D.Don) C.Norman		young leaf	green vegetable			
Asteraceae	<i>Sonchus brachyotus</i> DC.	535	young leaf	green vegetable	rgya-khur nag-po	gyakhur nagpo, anggum, umguma	
Asteraceae	<i>Taraxacum</i> sp. 1	277	young leaf	soup	khoor-m'ang	khurmo, mo, yo	dandelion
Asteraceae	<i>Taraxacum tibeticum</i> Hand.-Mazz.	499	young leaf	soup	khoor-m'ang	ngo, mokhumo	Tibetan dandelion
Campanulaceae	<i>Codonopsis bulleyana</i> Forrest ex Diels	507	leaf and tender stem	green vegetable		sukpa metok	
Campanulaceae	<i>Codonopsis</i> aff. <i>mollis</i> Chipp		leaf and tender stem	green vegetable		sukpa metok	
Chenopodiaceae	<i>Chenopodium album</i> L.	452	limb of leaf	soup	sne'u	neu, lego, nubre	fat-hen
Malvaceae	<i>Malva verticillata</i> L.	357, 449	limb of leaf	soup	lcam-pa, cham-pah	champa	Chinese mallow, cluster mallow
Ophioglossaceae	<i>Ophioglossum polyphyllum</i> A. Braun apud Seub.	371	frond	soup		tuchung	adder's tong fern
Plantaginaceae	<i>Plantago depressa</i> Willd.	450	young leaf	soup	tha-ram	tharam, alathabo, popkera	plantain
Polygonaceae	<i>Fagopyrum tataricum</i> (L.) Gaertn.	355, 451	limb of leaf	soup	bra-rgod	tragö, koyowa, torcherawa, pagö	Tatarian buckweat
Scrophulariaceae	<i>Lancea tibetica</i> Hook.f. & Thoms	500	leaf	green vegetable	pa-yag-pa	didigonggu, payagpa	Tibetan lancea
Urticaceae	<i>Urtica dioica</i> L.	483	young leaf	vegetable, soup	za-po	sapo, suptuk	stinging nettle
Urticaceae	<i>Urtica hyperborea</i> Jacq. ex Wedd.	514	young leaf	vegetable, soup	za-po	sapo, suptuk	white nettle
Urticaceae	<i>Urtica triangularis</i> Hand.-Mazz.	504	young leaf	vegetable, soup	za-po	sapo, suptuk	nettle

* Reference collection (L=Leteinturier, M=Malaisse) deposited at Belgium National Botanical Garden [BR].

Table 5.9. Lipids composition of ten edible potherbs.

LIPIDS	<i>Urtica hyperborea</i>		<i>Urtica triangularis</i>		<i>Malva verticillata</i>		<i>Taraxacum sp.</i>		<i>Ophioglossum polyphyllum</i>		<i>Angelica sp.</i>		<i>Codonopsis bulleyana</i>		<i>Fagopyrum tataricum</i>		<i>Chenopodium album</i>		<i>Lancea tibetica</i>	
	L & M 514	L & M 504	L & M 449	L & M 277	L & M 371	L & M 443	L & M 507	L & M 451	L & M 452	L & M 500										
Total Lipids (%)	3,2	4,0	5,0	4,5	2,8	2,8	3,3	4,4	5,3	7,0										
Fatty acid profile (Area%)																				
Myristic (C14)	0,8	0,7	1,1	1,7	traces		0,5	traces		1,0										
Myristoleic (C14:1)			0,7																	
Pentadecanoic (C15)	0,1						0,4			0,5										
Palmitic (C16)	11,4	11,3	15,3	19,3	15,8	18,7	13,3	12,0	16,8	15,5										
Palmitoleic (C16:1)	4,8	5,7	5,9	5,2	1,9	5,1	4,1	6,2	6,1	5,8										
Hexadecadienoic (C16:2)					0,8	1,5														
Margaric (C17)					0,2		0,3													
Stearic (C18)	1,5	1,4	3,0	1,8	3,5	1,4	1,3	1,2	1,0	3,4										
Oleic + isomer* (C18:1)	2,4	0,9	4,0	1,8	9,3	2,3	2,3	3,4	3,4	4,7										
Linoleic (C18:2)	11,7	11,0	6,6	14,8	15,2	31,3	23,2	13,6	13,4	14,5										
6-octadecynoic (C18:1 tr)					17,1															
Linolenic (C18:3)	43,7	50,7	58,8	47,1	13,7	21,1	35,7	57,1	54,0	49,9										
Arachidic (C20)	2,8	2,7			0,4	0,9	0,4			1,2										
Eicosenoic (C20:1)					1,8															
Docosanoic (C22)	0,2	0,4		0,7	2,1	1,8	1,3													
Docosenoic (C22:1)	tr	2,5			3,5	0,8	1,9													
Lignoceric (C24)	2,7	1,9		0,6	2,6	2,3	2,1													
Saturated	19,5	18,5	19,3	24,1	24,6	25,1	19,3	13,2	17,8	21,6										
Mono-unsaturated	7,2	6,6	10,6	7,0	16,5	7,4	6,4	9,6	9,5	10,4										
Polyunsaturated	55,4	61,7	65,4	61,9	29,7	53,9	59,0	70,7	67,5	64,4										
Acetylenic					17,1															

* cis-Vaccenic acid – tr = triple bound.



© Matisse François

Plate 5.16. Some wild edible potherbs. A. *Sonchus brachyotus*: spring habit – B. Bag of *Taraxacum tibeticum*, for winter consumption – C. Plate of *Ophioglossum polyphyllum* – D. *Ophioglossum polyphyllum*: habit – E. *Malva verticillata*: habit – F. *Lancea tibetica*: habit – G. *Fagopyrum tataricum*: habit – H. *Plantago depressa*, autumn habit.

simensis Hochst. ex A.Rich., in Sierra Nevada for *Urtica dioica* L. subsp. *holosericea* (Nutt.) Thorne and *Urtica serra* Blume (Woodland, 1982). Consumption of a thick soup nettle-based is also quoted from Nepal (Sacherer, 1979). Nettle leaves lose their urticant property after a few minutes immersion in boiled water.

Malva verticillata, cluster mallow or Chinese mallow, is a well known plant by Ü (76% of recognition), but ranks only 8 citations (22%), thus moderately appreciated as potherb. It should be remind that its seeds are used in Tibetan medicine for renal disorders, the retention of fluids, frequent thirst and diarrhoea, whilst leaves and stems are given to women in the advance stages of pregnancy. Moreover the plant furnishes cream, yellow and green dyes. It is well known in Europe, where it is cultivated as a salad plant (Polunin et al., 1997).

Ophioglossum reticulatum is used as a substitute of spinach in villages and towns of Uttar Pradesh and Madhya Pradesh in India (Sharma, 2000), being still appreciated in Indonesia were it is eaten as salad or cooked as a vegetable. In Nepal, its fronds as well as those of *Ophioglossum nudicaule* L.f. are cooked as a vegetable (Manandhar, 2002). Within a pilot study undertaken in Namibia it has been reported that *Ophioglossum polyphyllum* may be used as famine food when few other plants are available but the species is not popular or well known (Larsen, 2001).

Young leaves of at least two dandelions (*Taraxacum* spp.), called *kouma*, are currently eaten in villages (Plate 5.16). The sale in town is of little importance and is seasonal. Eating of leaves of dandelion has been quoted from other countries, for instance Nepal (Manandhar, 2002), Garhwal Himalaya (Gaul et al., 1983), notably by Bhotias, Gujars, Gaddis aboriginal groups (Negi et al., 1993), and Sikkim Himalaya (Sundriyal et al., 2001), but also Europe (Couplan, 1998).

The use of young shoots and leaves of *Codonopsis thalictrifolia* as vegetable was moderately quoted (38%). The same use is reported for the young leaves of a plantain, *Plantago depressa* Wild. as well as for those of *Fagopyrum tataricum*, the Tartary buckwheat, whose grain provides a nutty-tasting flour which remind one of buckwheat, once widely cultivated in Europe (Mabey, 1997).

Fat-hen or common lambsquarters (*Chenopodium album*), is a common weed in T.A.R. At a regional scale, its consumption is quoted from India (Low, 1992), notably Garhwal Himalaya (Gaul et al., 1983), Sikkim Himalaya (Sundriyal et al., 2001), Western Rajasthan (http://www.hort.purdue.edu/newcrop/Famine_Foods/), from Nepal (Manandhar, 2002), from China. Leaves are still eaten as a famine food, typically boiled with salt and chilli in several parts of India (Monghyr, Mirzapur, Dholpur, Alwar, Udaipur, Poona and Ahmednagar) (Manandhar, 2002). Moreover fat-hen is a well-known peasant potherb all over the world. Its mealy leaves have been harvested

in Western Europa, Russia, Canada, America, Africa (Couplan, 1998).

Rarely quoted, *Sonchus brachyotus* (16%) and *Lancea tibetica* (5%) are of less importance.

Finally it should be noted that the Tibetan potherbs have more plants in common with pistic, a traditional special dish prepared from 56 wild herbaceous plants from western Friuli, in North-Eastern Italy (Paoletti et al., 1995), as with plants listed for Nepal (Manandhar, 2002).

5.3.5. Spices, aromatic herbs and condiments

Wild edible plant used as spice, aromatic herbs or condiments occupy an important place in the diet of populations inhabiting the Himalayan plateau. Information on there diversity may be found in several books and papers, notably Manandhar (2002).

Diversity

Some 23 ethnospecies have been quoted during the survey conducted in South Central Tibet (Table 5.10). Four of them are spices (Plate 5.17).

Konieu or caraway ranks the top position. It is known from anybody in South Central Tibet. The citation index ranks a very high value. It should be remained that besides the fruit wanted as spice, young leaves and also roots are edible (Norman, 2002). It is used to flavour meat. It has a pungent aroma that is warm and bittersweet, sharply spicy, with a note of dried orange peel and a slight but lingering hint of anise (Norman, 2002). The culinary essence of caraway is a very odorante cetone, the d-carvone and a terpene, the d-limonene. In South Central Tibet the plant occurs in open situations, on well-drained soils. Stems are cut in July. *Konieu* is offered to purchase on Lhasa markets from early July to early November. In rural Tibet bags of *konieu* exist in most of the houses.

Tcharsil, field pennycress or Mithridate mustard (*Thlaspi arvense*) presents an eurasiatic distribution. This hardy annual or bisannual herb occurs in cultures and rubble. Seeds are used to flavour dishes.

The cultivation of *Coriandrum sativum* has been noted in few villages.

Seven aromatic herbs have been quoted (Plates 5.18, 5.19).

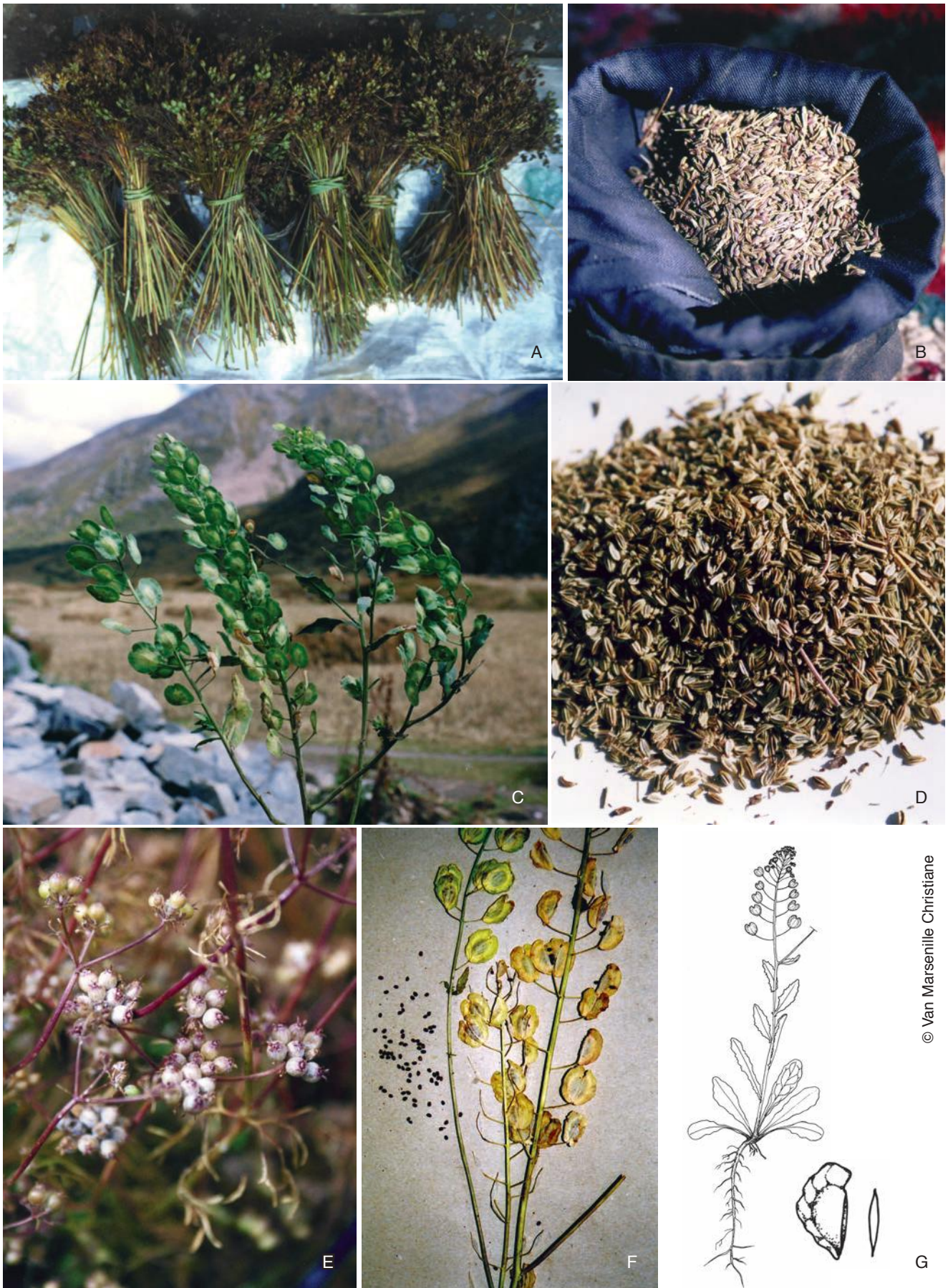
As well as in Garhwal Himalaya (Gaul et al., 1983), several garlic plants are appreciated in South Central Tibet. At least six wild species dealing with the genus *Allium* are eaten. They are mainly distinguishable according to their

Table 5.10. Diversity of spices, aromatic herbs and condiments in T.A.R.

Family	Species	Voucher *	Organe	Use	English Name	Translitteration Tibetan names
Apiaceae	<i>Carum carvi</i> L.	L & M 278, 435	fruit	spice for meat, with needle	caraway	konieu
Apiaceae	<i>Coriandrum sativum</i> **		fruit	spice for meal	coriander	sharotange
Apiaceae	<i>Cordia depressa</i> (D.Don) C. Norman		upper part	spice for meat, with needle		
Apiaceae	<i>Heracleum candidans</i> Wall. ex DC.	L & M 434	upper part	squach as aromatic herb	field pennycress,	shakoktengual
Brassicaceae	<i>Thlaspi arvense</i> L.	M 16012	seed	spice	mitrhidate mustard	tcharsil
Lamiaceae	<i>Dracocephalum tanguticum</i> Maximowicz	L & M 534 M 16011	above ground part	powered as aromatic herb for meat and cheese		ngopyiang
Lamiaceae	<i>Dracocephalum nutans</i> L.	L & M 448, 498	inflorescence	spice for cheese		lugulangstal
Liliaceae	<i>Allium atrosanguineum</i> Schrenk	L & M 513	upper part	aromatic herb		lugra
Liliaceae	<i>Allium caesium</i> Schrenk		upper part	aromatic herb		simbo
Liliaceae	<i>Allium fasciculatum</i> Rendle	L & M 440	upper part	aromatic herb for meat		gogpa
Liliaceae	<i>Allium henryi</i> C.H.Wright	L & M 445	upper part	aromatic herb for meat		simbo
Liliaceae	<i>Allium macranthum</i> Baker	L & M 509	upper part	aromatic herb for meat	Chinese red allium	gyagok, simbo
Liliaceae	<i>Allium prattii</i> C.H.Wright		upper part	aromatic herb		dzinak
Liliaceae	<i>Allium wallichii</i> Kunth		upper part	aromatic herb		simbo
Liliaceae	<i>Allium</i> sp.	L & M 464	upper part	aromatic herb for meat		mouktok
Polygonaceae	<i>Rheum acuminatum</i> Hook. & Thomson	L & M 441	leaf petiole	condiment	rhubarb	numdi, chuju
Polygonaceae	<i>Rheum australe</i> D.Don	L & M 441	leaf petiole	condiment	rhubarb	numdi, chuju
Polygonaceae	<i>Rheum globulosum</i> Gage		leaf petiole	condiment	rhubarb	
Polygonaceae	<i>Rheum inopinatum</i> Prain		leaf petiole	condiment	rhubarb	
Polygonaceae	<i>Rheum lhasaense</i> Li & Hsiao		leaf petiole	condiment	rhubarb	numdi, chuju
Polygonaceae	<i>Rheum moorcroftianum</i> Royle		leaf petiole	condiment	rhubarb	chuju
Polygonaceae	<i>Rheum palmatum</i> L. **	L & M 460	leaf petiole	condiment	Chinese rhubarb	tchum
Polygonaceae	<i>Rheum pumilum</i> Maxim.		leaf petiole	condiment	rhubarb	
Polygonaceae	<i>Rheum rhomboideum</i> Losinskaja		leaf petiole	condiment	rhubarb	
Polygonaceae	<i>Rheum tanguticum</i> (Maxim.) Maxim.	L & M 508	leaf petiole	condiment	Chinese rhubarb	tchum
Polygonaceae	<i>Rheum webbianum</i> Royle		leaf petiole	condiment	rhubarb	

* Collection L & M = Leteinturier & Malaisse, M = Malaisse

** Locally cultivated



© Matisse François

© Van Marsenille Christiane

Plate 5.17. Some spices used in T.A.R. A. *Carum carvi*: fresh brunches – B. *Carum carvi*: fruits packed into bags during winter – C. *Thlaspi arvense*: habit – D. *Heracleum candidans*: fruits – E. *Coriandrum sativum*: fruiting plant – F. *Thlaspi arvense*: fruiting plant – G. *Thlaspi arvense*: habit: fruit and seed.

inflorescence colour (white, yellow, pink, purple, mauve, violet), but the leaves, broad or narrow, as well as the shape of the inflorescence are of interest. At the Lhasa markets, *Allium macranthum*, or *gyakok*, is available in July and August; in rural areas *Allium fasciculatum* (white globose inflorescence, *kokpa* or *kotste*), *Allium* sp. (elongated lilac inflorescence, *muktok*), *Allium atrosanguineum* Schrenk (yellow inflorescence) and *Allium pratii* (mauve inflorescence, broad leaves) are much appreciated.

Several species issued as condiment. These plants belong to the genus *Rheum* (Plate 5.20). *Rheum*, is a highly diversified genus with about 60 species. Rhubarbs are mainly confined to the mountainous and desert regions of the Tibetan plateau and adjacent areas. This genus represents a good example of the extensive diversification of the temperate genera in the Tibetan plateau, in which the forces to drive diversification remain unknown. To date, the infrageneric classification of *Rheum* has been mainly based on morphological characters but molecular studies are in course (Wang et al., 2005).

Consumption of leafstalk and stem of several rhubarbs appear frequently. This fact was already quoted by Bell

(1928) who wrote «rhubarb grows wild and is of various kinds, some of great height». We have noted the use of *Rheum officinale*, *Rheum australe*, *Rheum palmatum* and *Rheum tanguticum*, but others authors have quoted *Rheum emodi* (Jest, 1972 in Sacherer, 1979) and *Rheum mootcroftianum* (Gaul et al., 1983).

Discussion

Several Tibetan species are from a long time used outside this area and their history, present range and cultivation will be commented on.

Caraway has a mountain Asiatic origin. It is to be found in Mediterranean kitchen and medicine since some 5,000 years.

Coriandre is already quoted in the Old Testament under the name of «gad». It was used to flavour bread by Hebrews. Leaves are sometimes used as potherb, even roots are edible (Viard, 2004). It occurs from Orient to Mediterranean countries. When drying the fruits loose their bug scent whilst their perfume increases, revealing orange zest.



© Malaisse François

Plate 5.18. Two garlics of South Central Tibet. A. Basket of *Allium* sp. or *muktok* – B. Basket of *Allium* sp. or *muktok*: detail – C. Basket of *Allium* sp. or *muktok*: the elongate mauve inflorescence – D. *Allium fasciculatum* or *gogpa* in the wild – E. *Allium fasciculatum* or *gogpa*: habit – F. *Allium fasciculatum* or *gogpa*: the spherical white inflorescence.



Plate 5.19. Diversity of aromatic herbs stored at home for winter consumption. A. *Allium henryi* or *simbo* – B. *Allium macranthum* – C. String of *Allium henryi* cubes – D. String of *Allium fasciculatum* cubes – E. *Allium caesium* – F. *Allium* sp.



© Matisse François

Plate 5.20. Some rhubarbs of South Central Tibet. A. *Rheum tanguticum* – B. *Rheum emodii* – C. *Rheum palmatum* – D. Collecting a leaf of *Rheum tanguticum* – E. *Rheum* sp.

5.3.6. Other plant organs' foods

Some wild fruits, underground parts (rhizomes, roots, tubers, etc.), stems and flowers as well as receptacle are also eaten from time to time (Table 5.11). Two of them take a major importance, namely the tubers of a silverweed and the fruit of a rosebush.

Five wild fruits have been quoted (Plate 5.21). The fleshy mesocarp of the orange to red rosehips, locally known as *sindou* or *simbou*, of a dog rose called *sewa*, *Rosa omeiensis* Rolfe, are highly appreciated. *Sindou* are offered for sale in town markets in July and August (Malaisse et al., 2003). The scarlet berries of *Podophyllum hexandrum* called *momsussu*, *gyatse thumlo* or *abulu* are frequently collected and wanted; they are sometimes stored for winter consumption. *Momsussu* is also an important medicinal plant, the rhizome containing podophyllin, a possible drug used in treatment of cancer (Polunin et al., 1997). The berries of some *Ribes* spp. are also from time to time eaten. Several barberries occur in the area under study; ripe fruits of some are eaten. They have a slightly acide taste. The dark crimson fruits of *Berberis asiatica* Roxb. are eaten in Nepal (Toffin et al., 1985, Polunin et al. 1997), those of *Berberis asiatica* and *Berberis chitora* in Sikkim Himalaya (Sundriyal, pers. com.), those of *Berberis dictyophylla* from time to time in the studied area. Two other plants delivering edible fruits are kuyuk-thal-chak and *Asparagus curillus*; this information needs nevertheless confirmation.

Five underground organs have been quoted. *Potentilla anserina* subsp. *anserina* is the most important (Plate 5.22). It is a perennial herbaceous plant, whose dark

brown tubers, toma, are a highly prized foodstuff, an observation already quoted by Grenard (1904). They are sometimes eaten raw, notably by nomad children, but come mostly into diverse recipes. They are associated in town to Losar, the Tibetan New-Year. The boiled tubers are mixed with cooked rice, butter and sugar in order to produce the toma daisel, of which consumption brings happiness. This dish is eaten by all the family members. Boiled tubers also go with *tchang*. Tubers are collected all year round and commercialisation in urban centres is important. The consumption, by Indians of the north-western coast of the USA, of «roots» of an other sub-species of silverweed, subsp. *pacifica* (Howell) Rousi, the nutritional value of which has been studied (Kuhnlein et al., 1982; Turner et al., 1892) should be quoted. Other underground organs eaten are the roots of *Microula* cf. *sikkimensis* or pawalulu, the tubers of *Codonopsis convolvulacea* or *bala*, the roots of *Bistorta macrophylla* or *membou*. *Bala* is eaten raw in September and November. Some other plants have been quoted during surveys but not yet collected such as the roots of *polomolo*, etc.

Some flowers are eaten or sucked, mainly by children, notably those of *Codonopsis thalictrifolia* or *souпка mèto*, and of *Caragana jubata*.

The receptacle of the inflorescence of *Jurinaea dolomiaea*, local names *purge numa*, *tora*, *warokyongma*, is eaten raw (Plate 5.22). It is well known and appreciated.

In conclusion the diversity of will edible plants is behind fifty plants and offers a panel of food able to increase the chemical components of the diet.

Table 5.11. Some wild edible species in T.A.R.

Family	Species	Voucher (L & M)*	Organ eaten	Use	Transcription of Tibetan names (Tournadre, Dorje, 2003)
Asparagaceae	<i>Asparagus curillus</i> Buch.-Ham.	402	stem, fruit	vegetable	nagkatampel
Asteraceae	<i>Jurinaea dolomiaea</i> Boiss.	342, 442	receptacle	raw, starch	purge numa, warokyongwa
Berberidaceae	<i>Berberis dictyophylla</i> Franch		fruit	eaten raw	
Berberidaceae	<i>Podophyllum hexandrum</i> Royle	410	fruit	eaten raw	momsussu, omsussu
Boraginaceae	<i>Microula</i> cf. <i>sikkimensis</i> (C.B. Clarke) Hemsl.	366, 481	root	starch	pawalulu, atuputo
Campanulaceae	<i>Codonopsis convolvulacea</i> Kurz	M 15346	tuber	starch	bala, myewa
Fabaceae	<i>Caragana jubata</i> Poir		flower	eaten raw	
Grossulariaceae	<i>Ribes</i> sp. 1		fruit		
Grossulariaceae	<i>Ribes</i> sp. 2		fruit		
Polygonaceae	<i>Bistorta macrophylla</i> (D. Don) Soják.	506	root	boiled, starch	membou
Rosaceae	<i>Potentilla anserina</i> L. subsp. <i>anserina</i>	335	tuber	toma daisel preparation	troma, toma
Rosaceae	<i>Rosa omeiensis</i> Rolfe	433	fruit pulp	eaten raw	
Scrophulariaceae	<i>Incarvillea</i> cf. <i>youngusbandii</i> Sprague	M 15784	root	starch	pupsta

* Collection L & M = Leteinturier & Malaisse; M = Malaisse.



© Malaisse François

Plate 5.21. Some edible fruits. A. *Rosa omeiensis* or *sindou*: flowering twig – B. & C. *Rosa omeiensis* or *sindou*: fruiting twig – D. & E. Rosehips offered for sale in town – F. Idem, detail – G. *Podophyllum hexandrum* or *momsussu*: habit – H. *Podophyllum hexandrum* or *momsussu*: mature fruits – I. *Podophyllum hexandrum* or *momsussu*: dried fruits for winter consumption – J. *Ribes* sp. 1: fruiting twig – K. *Ribes* sp. 2: fruiting twig – L. *Asparagus curillus*: fruiting phenophase.



Plate 5.22. Some Tibetan plants with edible underground organs. A. *Potentilla anserina* subsp. *anserina* or *toma*: habit – B. Idem, tubers sale in town – C. *Codonopsis convolvulacea* – D. *Polygonum polystachyon* – E. *Bistorta macrophylla*: habit – F. *Bistorta macrophylla*: inflorescence – G. *Jurinaea dolomiaea*: edible receptacle – H. *Jurinaea dolomiaea*: flowering phenophase.

5.4

Protinet

François Malaisse, Françoise Mathieu, Georges Lognay, Emile Delcarte, Bernard Wathelet, William Claus, Philippe Goyens, Lopsang Rinchen, Lakpa Wangdu, Rinzen Wangla

Introduction

Previous studies have established the poor and very little diversified diet of most of Tibetan peasants in several rural areas. Also from latter matters developed in the present book (Chap. 5.3.), it appears easily that nettles are a valuable potherb, and even a fine alternative food. Therefore, to encourage eating nettles in a more routine way could easily contribute to improve the nutritional status of people and more specifically children. As a consequence, it could have an implication on Kashin-Beck disease prevention!

A new programme called PROTINET (Programme on Tibetan nettles) is born. Its objectives are to increase interest of local populations regarding their ancient knowledge and tradition of nettle consumption, as well as an establishment of nettle fields in villages.

Programmes and actions that have to be undertaken in South Central Tibet are listed and commented in this chapter. It includes both a better knowledge regarding diversity of nettles in T.A.R. as well as regarding the methods of cultivation. These aspects will be shortly discussed. A better knowledge of the various aspects of local sensibility for this diet is also of interest.

5.4.1. Nettle as diet in T.A.R.

Nettle has been considered since a long time as a food, a fodder, and a medicinal plant. The consumption of stinging nettle as a vegetable dates back to antique times. In Ancient Greece, it was known and mentioned by Hippocrates (460-377 B.C.) and Theophrastus (372-285 B.C.). Its use as food is praised and advised in *Materia Medica* by Dioscorides and *Naturalis Historia* by Plinius, both works dating back to the first century (Randall, 2003). The remedial properties of the plant



© Wangla Rinzen

© Malaise François

Plate 5.23. Nettle as food in South Central Tibet. A. *Urtica dioica* subsp. *dioica* in the wild – B. *Urtica cannabina* – C. *Urtica triangularis* in the wild – D. A plot of *Urtica* sp. – E. Drying nettles or *sapo* at home – F. Basket of nettle leaves – G. Nettle soup or *suptuk* – H. *Inachis io*: third instar caterpillar – I. *Inachis io*: fourth instar caterpillar – J. *Inachis io*: chrysalis.

are also listed (Wetherilt, 2003). In T.A.R. also the plant is listed in diverse books of medicinal plants (Tsarong, 1994; Dga' ba'i rdo rje, 1995).

The leaves of *Urtica* species have been reported to be excellent sources of some important minerals and vitamins (Booth et al., 1963; Trofimova, 1977; Adamski et al., 1980) and to have a higher level and a better quality of protein when compared with many other green leafy vegetables (Adamski et al., 1984; Ullrich et al., 1984; Wetherilt, 2003; Bertrand 2005).

Most of our programme's villages have access to nettles. Even better, numerous peasants know nettle clumps in a distance of less than 300 m from their houses. Nettles are found in several sites, notably in rocky places, along water channels and around cesspools.

All the villagers in our programme's areas eat nettles, but there are big differences on the quantity of consumption between villages to villages and families to families (Plate 5.23). It seemed that the poorer families eat larger quantity and more frequently nettles than the rich ones. Also family traditions of eating nettles are different from one family to another. There are families eating fresh and dried nettles all year long, but some other families eat only few times (1-4) a year. Many families have also dried nettles to use for their domestic animals.

There are some typical reasons of eating nettles:

- many people eat nettles as subsidiary food for part time of the year or all year along. It is true mainly for farmers;
- many people also eat nettles during Sagadawa, as respect to be vegetarian for two weeks (Sagadawa is from the first to the 15th of the 4th month of Tibetan lunar calendar; the 15th is the date of the birth and death of Buddha; usually it's the month of June in the universal calendar);
- people eat fresh nettles also as it is the first fresh food of the year, on springtime; therefore eating one or few times nettles for auspicious gesture is fine;
- also some people, especially monks or nuns eat nettles for meditation and honoured to Milarepa (a famous Tibetan poet monk in the 13th century who survived eating only nettles and eventually his body became green).

On the other hand, there are many stories or rumours preventing eating too much nettles. It would provoke high blood pressure, pain to knees, headaches, etc.

There are only two recipes of nettles:

- sauce: to smash fresh or dried nettles and add little water and spices;
- soup: nettles, fat, water, salt and add some *tsampa* (barley powder) to obtain a thicker soup.

Both are eaten with *tsampa* breads as accompany.

There are nettles seen in the Lhasa Barkhor market to sell. The price of 300 g (the measure of an empty can) is around 5-6 yuan during Sagadawa, but after Sagadawa the price comes down to around 2.00 yuan per 300 g. Definitely nettles are one of vegetables people preferred to eat during this period.

The villagers in Lhoca prefecture told us that they do not eat the nettles grown in cesspools but they sell them in the market.

According to the villager's knowledge two or three kinds of nettles are recognized. They are *sanak* near houses, *sakar* on the summit of mountains, and *sakaro* near streams. Some villagers make a distinction between white and dark nettles. They consider that the white nettle grows under shade, in between gaps of rocks, under other big plants such as bushes, meaning that the white nettles are not exposed to sunshine therefore they are less dark. The villagers say that the taste of the white nettles is more delicious than the dark ones. Several locators prefer white nettles that occur in high mountains to black nettles growing near the houses.

Locally, nettle fruits or *sapoi pukku* are eaten in September-October after boiling them in hot water. Old nettles are given to livestock.

5.4.2. Nettles diversity in T.A.R.

Within the Urticaceae of China, nettles are plants armed with stinging hairs and opposite leaves. Moreover they have female flowers without staminodes; the perianth lobes of female flowers are free, the lateral outer pair being much smaller than the dorsiventral pair.

According to Chen et al. (2003; 2004) in the Flora of China, the genus *Urtica* L. amounts about 30 species of which 14 species occur in P.R. China; three of them being endemic (Plate 5.24).

Table 5.12. lists these species, presenting some information on distribution. It indicates that 7 taxa occur in T.A.R. During our surveys we collected *Urtica triangularis*, *Urtica hyperborea* and *Urtica dioica* subsp. *dioica*.

5.4.3. Phytotechny and cultivation of nettles in South Central Tibet

We have no information about cultivation of nettles in South Central Tibet, nor on its local phytotechny. Therefore there is an urgent necessity to carry out



Plate 5.24. Diversity of nettles in China. A. *Urtica hyperborea* at Chak pass – B. *Urtica dioica* var. *dioica*: leaf – C. *Urtica hyperborea*: habit – D. *Urtica dioica* var. *dioica*: infrutescence – E. *Urtica mairei* – F. *Urtica cannabina* – G. *Urtica fissa* – H. *Urtica triangularis* – I. *Urtica urens* – J. *Urtica angustifolia* – K. *Urtica ardens*.

Table 5.12. Diversity and distribution of *Urtica* species in P.R. China (Xizang = T.A.R.).

Taxa	Distribution	Altitudinal distribution (m)
<i>Urtica atrichocaulis</i> (Hand.-Mazz.) C.J.Chen	Guizhou, Sichuan, Yunnan	300-2,600
<i>Urtica taiwaniana</i> S.S.Ying	Taiwan	3,400-3,600
<i>Urtica urens</i> L.	Liaoning, Xinjiang, Xizang	500-2,900
<i>Urtica triangularis</i> Hand.-Mazz. subsp. <i>triangularis</i>		2,500-3,700
<i>Urtica triangularis</i> Hand.-Mazz. subsp. <i>pinnatifida</i> (Hand.-Mazz.) C.J.Chen	Gansu, Qinghai, Sichuan, Yunnan	3,400-4,100
<i>Urtica triangularis</i> Hand.-Mazz. subsp. <i>trichocarpa</i> C.J.Chen	Gansu, Qinghai, Sichuan	2,200-3,000
<i>Urtica cannabina</i> L.	Gansu, Hebei, Heilongjiang, Jilin, Liaoning, Nei Mongol, Ninxia, Qinghai, Shaanxi, Shanxi, Sichuan	800-2,800
<i>Urtica hyperborea</i> Jacq. ex Wedd.	Gansu, Qinghai, Sichuan, Xinjiang, Xizang	3,000-5,200
<i>Urtica laetevirens</i> Maxim.	Anhui, Gansu, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jilin, Liaoning, Nei Mongol, Qinghai, Shaanxi, Shandong, Shanxi, Sichuan, Xizang , Yunnan	100-3,500
<i>Urtica angustifolia</i> Fisch. ex Hornem.	Hebei, Heilongjiang, Jilin, Liaoning, Nei Mongol, Shandong, Shanxi	800-2,200
<i>Urtica dioica</i> L. subsp. <i>dioica</i>	Qinghai, Xinjiang, Xizang	3,200-4,800
<i>Urtica thunbergiana</i> Siebold & Zucc.	Taiwan, Yunnan	1,200-2,500
<i>Urtica fissa</i> E.Pritz.	Anhui, Fujian, Gansu, Guangxi, Guizhou, Henan, Hubei, Hunan, Shaanxi, Yunnan	100-2,000
<i>Urtica mairei</i> H.Lév.	Xinjiang, Xizang , Yunnan	1,500-3,400
<i>Urtica ardens</i> Link	Guangxi, Xizang , Yunnan	2,400-2,700
<i>Urtica parviflora</i> Roxb.	Guangxi, Xizang , Yunnan	1,500-2,400

observations on ecology and phenology, as well as on herbivory of the species present in the area of concern. Herbivory has been observed in several villages. Caterpillars of *Inachis io* or peacock, belonging to the Nymphalidae family, appears to be not rare.

The villager's motivation of planting nettles in the gardens is not very high. They say they have nettles near their houses. Other reasons given to avoid plantation are: «too much eating is bad for health», etc. As a result, most of the experimenting gardens took place in the village leader's houses (Plate 5.25). But the combination of planting other vegetables such as cabbage and radish motivates some families to grow nettles.

Some nettle gardens are already started in Sangri county (Lhoca prefecture), Nanang and Medrokongkar counties (Lhasa prefecture). Cabbage and radish seeds are provided to those families who have planted nettles.

If we could provide new nettle recipes (Figure 5.6), it will motivate farmers to increase their nettle intakes, especially for their children.

All in total, 90 families are scheduled to plant the experimenting nettle gardens in three methods: soil with water, soil with urine, and soil with manure.

Results will be available after the first year trials.



Figure 5.6. Nettle omelette.



Plate 5.25. Protinet in action. A. Plot preparation – B. Checking nettle garden – C. Collecting seeds of *Urtica hyperborea* – D. Nettle garden – E. & F. Councils of KBD team on peasants about nettle's garden – G. & H. Successful nettle cultivation.

References

- Adamski R., Bieganska J., 1980. Studies of chemical substances present in *Urtica dioica* L. leaves. Part 1. Trace elements. *Herba Pol.*, **26**(3), 177-180.
- Adamski R., Bieganska J., 1984. Studies on substances present in *Urtica dioica* L. leaves. Analysis for protein, amino acids and nitrogen containing nonprotein substances. *Herba Pol.*, **30**(1), 17-26.
- Adhikari M.K., Devkota S., Twari R.D., 2005. Ethnomycological knowledge on uses of wild mushrooms in Western and Central Nepal. *Our Nature*, **3**, 13-19.
- Adhikari M.K., Durrieu G., 1996. Ethnomycologie népalaise. *Bull. Soc. Mycol. Fr.*, **112**, 31-41.
- Allander E., 1987. The epidemiology of Kashin-Beck disease. Some reflections on possibilities for international cooperation. In: Levander, O.A. (ed.). *Nutrition'87. Proceedings of the AIN (American Institute of Nutrition) Symposium. Washington, DC, March 29-April 2 1987.* p. 72-75.
- Allander E., 1990. *Kashin-Beck Disease. A WHO report on the present situation. Conclusions and recommendations.* Stockholm: Huddinge University Hospital, Department of Social Medicine.
- Allander E., 1991. *Kashin-Beck disease (KBD). An update on present situation and possibilities to continue a program for prevention. Report on consultancy of Kashin-Beck disease.* Manila, P.R. China: WHO/(WP)NCD/CHN/NCD/002-A.
- Allander E., 1994. Kashin-Beck Disease. An analysis of research and public health activities based on a bibliography 1849-1992. *Scand. J. Rheumatol.*, **23** (Suppl. 99), 1-36.
- Anonymous, 1989. *Working Criteria of Prevention and Treatment of Kashin-Beck Disease (KBD).* Approved in National Conference on KBD surveillance. Translated by Drs Xue Shaomin and Xu Xiong.
- Anonymous, 1994. *Diagnostic Criteria of Kashin-Beck Disease.* National Conference on KBD surveillance, 1-14. Translators: Drs Xue Shaomin and Xu Xiong.
- AOCS, 1998. *Official methods and recommended practises of the American Oil Chemist's Society.* 5th ed.
- Bai F.Y., Chen Y.D., Chen Q.T., 1990. Investigation on the contamination fungi of grains in Tian-Shui Kashin-Beck areas. *Chin. J. Control Endem. Dis.*, **5**(1), 33-34.
- Beall C.M., 1981. Growth in a population of Tibetan origin at high altitude. *Ann. Hum. Biol.*, **8**, 31-38.
- Beall C.M., Goldstein M.C., 1987. Hemoglobin concentration of pastoral nomads permanently resident at 4,850-5,450 meters in Tibet. *Am. J. Phys. Anthropol.*, **73**, 433-438.
- Beck E.V., 1906. To the problem of disforming endemic osteoarthritis in the Baikal area. *Russ. Vrach. (Russian physician)*, **3**, 74-75.
- Beck E.V., 1908. Ueber Osteoarthritis deformans endemica im Transbaikalgebiet. *Arch. Klin. Chir.*, **86**, 662-685.
- Bell C.A., 1928. *The People of Tibet.* Oxford, U.K.: Oxford Clarendon Press.
- Bergère M.-C., 1989. *La république populaire de Chine de 1949 à nos jours.* Paris : Armand Colin.
- Berlin B., 1992. *Ethnobiological Classification: Principles of Categorization of Plants and Animals in traditional Societies.* Princeton, New Jersey, USA: Princeton Univ. Press.
- Bertrand B., 2005. *Les secrets de l'Ortie.* 9^e éd. Aspet, France : Editions de Terran.
- Bhandary H.R., 1985. 33.- Mushrooms. In: Majupuria T.C. (Ed.). *Nepal Nature's Paradise.* Bangkok: White Lotus Co., 235-246.
- Bhandary H.R., 1991. Some edible and medicinal fungi from Dumre to Manag, Mustang and Pokhara. *J. Nat. Hist. Mus.*, **12**(1-4), 47-60.
- Bhattacharjee S.K., 2000. *Handbook of aromatic plants.* Jaipur, India: Pointer Publisher.
- Billiet F., Léonard J., 1986. *Voyage botanique au Cachemire et au Ladhakh (Himalaya occidentale).* Meise, Belgique : Jardin Botanique National Belgique.
- Booth V.H., Bradford M.P., 1963. Tocopherol contents of vegetables and fruits. *Br. J. Nutr.*, **17**, 575-581.
- Bresinsky A., Besl H., 1985. *Giftpilze. Ein Handbuch für Apotheker, Ärzte und Biologen.* Stuttgart: Wissenschaftliche Verlagsgesellschaft.
- Brummit R.K., Powell C.E., 1992. *Authors of Plant Names.* Kew, UK: Royal Botanical Gardens.
- Bui Dai Thu, Schultink W., Dillon D., Gross R., Dhevita Leswara N., Ha Hui Khoi, 1999. Effect of daily and weekly micronutrient supplementation on micronutrient deficiencies and growth in young Vietnamese children. *Am. J. Clin. Nutr.*, **69**, 80-86.

- Chamberlain M., 1996. Ethnomycological experiences in South West China. *Mycologist*, **10**(4), 173-176.
- Chang D.H.S., 1981. The vegetation zonation of the Tibetan plateau. *Mt. Res. Dev.*, **1**, 29-48.
- Chang D.H.S., 1983. The Tibetan plateau in relation to the vegetation of China. *Ann. Missouri Bot. Gard.*, **70**, 564-570.
- Chasseur C., Suetens C., Haubruge E., Mathieu F., Begaux F., Tenzin T., Nolard N., 1996. Grain and flour storage conditions in rural Tibetan villages affected by Kashin-Beck disease in Lhasa prefecture, Tibetan Autonomous Region: environmental approach. In: Miller P.A. (ed). *Proceedings of the International Congress of Bacteriology and Applied Microbiology Division, Jerusalem, Israël, August, 18-23*, 245.
- Chasseur C., Suetens C., Nolard N., Begaux F., Haubruge E., 1997. Fungal contamination in barley and Kashin-Beck disease in Tibet. *Lancet*, **350**, 1074.
- Chasseur C., Suetens C., Michel V., Mathieu F., Begaux F., Nolard N., Haubruge E., 2001. A 4 year study of the mycological aspects of Kashin-Beck disease in Tibet. *Int. Orthop.*, **25**(3), 154-158.
- Chasseur S., 2008. *Analyse du phénomène d'exode rural des femmes tibétaines*. Mémoire de licence en Sciences psychologiques : Université de Mons-Hainaut (Belgique).
- Chen J.R., Friis I., Wilmot-Dear C.M., 2003. Urticaceae. In: Wu Z.Y., Raven P.H. (eds). *Flora of China. Vol. 5. (Ulmaceae - Basellaceae)*. Beijing: Science Press; St Louis, Miss., USA: Missouri Botanical Garden Press. 78-84.
- Chen J.R., Lin Q., Friis I., Wilmot-Dear C.M., Monro A.K., 2004. Urticaceae. Illustrations. In: Wu Z.Y., Raven P.H. (eds). *Flora of China. Vol. 5. (Ulmaceae - Basellaceae)*. Beijing: Science Press; St Louis, Miss., USA: Missouri Botanical Garden Press. 84-86.
- Chevallier D., Langlois C., Pujol R., 1988. A propos d'ethnozoologie : entretien avec R. Pujol. *Terrain*, **10**, 108-112.
- Christensen H., 2002. *Ethnobotany of the Iban & the Kelabit*. Kuching, Malaysia: Sarawak Forest Department; Aarhus, Denmark: NEPcon and University of Aarhus.
- Couplan F., 1998. *The Encyclopedia of edible plants of North America*. New York, USA: Mc Graw-Hill.
- Cunningham A.B., 2001. *Applied ethnobotany. People, wild plants use and conservation*. London: Earthscan Publications.
- Daniggelis E., 1995. *Preliminary anthropological assessment of the determinants of malnutrition among children in Tibet. Report*. Santa Cruz, CA, USA: Tibet Child Nutrition and Collaborative Health Project.
- David-Néel A., 1993. *Gargantua au pays des neiges*. Saint Michel en l'Herm, France : Edition Dharma.
- Deng J., 1990. The geographical distribution characteristics of Kashin-Beck disease in Sichuan province. In: *Chinese Academy of Preventive Medicine (CAPM). Proceedings of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, p. 27-30.
- Dga' ba'i rdo rje, 1995. *Khrungs dpe dri med shel gyi me long*. Lhasa, P.R. China: Mi rigs dpe skrun khang.
- di Castri F., 1981. L'écologie : naissance d'une science de l'homme et de la nature. *Courr. UNESCO*, **34**, 6-11.
- Dobremez J-F., Vigny F., 1998. Flore, étages de végétation et domaines biogéographiques en Himalaya. *Ecologie*, **29**(1-2), 1-440.
- Egashira Y., 1990. An Outline of History of Research on Kashin-Beck Disease in Japan. In: *Chinese Academy of Preventive Medicine (CAPM). Proceedings of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 149-151.
- Eggum B.O., 1987. Protein quality of cassava leaf. *Br. J. Nutr.*, **24**, 761-768.
- Fan Yun-qi, 1980. Chemical characteristics of the lakes in Xizang. In: *Proceedings of the symposium on Qinghai-Xizang (Tibet) plateau*. Abstract. Beijing: Academia Sinica, 244-245.
- FAO/WHO 1989. Report of the joint expert committee on Food additives. Beijing: WHO, n°776.
- FAO/WHO 1990. Report of the joint expert consultation on protein quality evaluation. FAO, Rome 4-8 December 1989.
- Feng-qin L., Takumi Y., 2000. Alternaria Mycotoxins in Weathered Wheat from China. *J. Agric. Food Chem.*, **48**(7), 2920-2924.
- Folch J., Lees M., Sloane S.G.H., 1957. A simple method for the isolation and purification of totals lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.
- Foster H.D., Zhang L., 1995. Longevity and selenium deficiency: evidence from the People's Republic of China. *Sci. Total Environ.*, **170**(1-2), 133-139.
- Friis I., 1989. Urticaceae. In: Polhill R.M. (Ed.). *Flora of Tropical East Africa*. Rotterdam, The Netherlands: A.A. Balkema.
- Garbyal S.S., Aggarwal K.K., Babu C.R., 2004. Impact of *Cordyceps sinensis* in the rural economy of interior villages of Dharchula sub-division of Kumaon Himalayas and its implications in the society. *Indian J. Tradit. Knowl.*, **3**(2), 182-186.
- Gaud R.D., Semwal J.K., 1983. Some little known wild edibles of Garhwal Himalaya. *Man Environ.*, **7**, 161-165.
- Ge K., Yang G., 1993. The epidemiology of selenium deficiency in the etiological study of endemic diseases in China. *Am. J. Clin. Nutr.*, **57**(2 Suppl.), 259S-263S.
- Girisham S., Rao G.V., Reddy S.M., 1985. Mycotoxin producing fungi associated pearl millet. *Natl Acad. Sci. Lett.*, **8**, 333-335.
- Goetghebeur F., 1992. Le pays, son histoire, ses habitants et sa culture. *La voix du Tibet*, **2-3** (Numéro spécial), 31-45.
- Gragson T.L., Blount B.G., 1999. *Ethnoecology. Knowledge, Resources, and Rights*. Athens, Georgia, USA: The University Georgia Press.
- Grenard F., 1904. *Le Tibet ; le pays et ses habitants*. Paris: Armand Colin.

- Greulich W.W., Pyle S.I., 1959. *Radiographic atlas of skeletal development of hand and wrist*. 2nd ed. Palo Alto, CA, USA: Stanford Univ. Press.
- Haines S.S., Klemperer S.L., Brown L., Jingru. G., Mechie J., Meissner R., Ross A., Wenjin Z., 2003. INDEPTH III seismic data: from surface observations to deep crustal processes in Tibet. *Tectonics*, **22**(1), 1001.
- Harris N.B.W., Ronghua X., Lewis C.L., Chengwei J., 1988. Plutonic Rocks of the 1985 Tibet Geotraverse, Lhasa to Golmud. *Philos. Trans. R. Soc. London. Ser. A*, **327**, 145-168.
- Harris N.S., Crawford P.B., Yangzom Y., Pinzo L., Gyaltsen P., Hudes M., 2001. Nutritional and health status of Tibetan children living at high altitudes. *New Engl. J. Med.*, **344**, 341-347.
- Harris N.S., Yangzom Y., Pinzo L., Gyaltsen P., Crawford P.B., 1996. Effects of age, community location, and illness on nutritional status of high altitude Tibetan children, 0-7 years. *Int. Child Health: a digest of current information*, **7**, 99-114.
- Hartmann H., 1972. Über die Vegetation des Karakorum. II Teil: Rasen- und Strauchgesellschaften im Bereich des alpinen und der höheren subalpinen Stufe des Zentral-Karakorum. *Vegetatio*, **24**, 91-157.
- Hartmann H., 1987. Pflanzengesellschaften trockener Standorte aus der subalpinen und alpinen Stufe im südlichen und östlichen Ladakh. *Candollea*, **42**, 277-326.
- Hartmann H., 1990. Pflanzengesellschaften aus der alpinen Stufe des westlichen, südlichen und östlichen Ladakh mit besonderer Berücksichtigung der rasenbildenden Gesellschaften. *Candollea*, **45**, 525-574.
- Hartmann H., 1995. Beitrag zur Kenntnis der subalpinen Wüsten-Vegetation im Einzugsgebiet des Indus von Ladakh (Indien). *Candollea*, **50**, 367-410.
- Hartmann H., 1997. Zur Flora und Vegetation der Halbwüsten, Steppen und Rasengesellschaften im südöstlichen Ladakh (Indien). *Jahrb. Ver. Schutz Bergwelt (Munich)*, **62**, 129-188.
- Haubruege E., Chasseur C., Debouck C., Begaux F., Suetens C., Mathieu F., Michel V., Gaspar C., Rooze M., Hinsenkamp M., Gillet P., Nolard N., Lognay G., 2001. The prevalence of mycotoxins in Kashin-Beck disease. *Intern. Orthop.*, **25**(3), 159-161.
- Hawksworth D.L., Kirk P.M., Sutton B.C., Pegler, D.N., 1995. *Ainsworth & Bisby's dictionary of the fungi*. 8th ed. Wallingford, Oxon, UK: CAB International.
- Haynes J.S., Walser M.M., 1986. Ultrastructure of *Fusarium*-induced tibial dyschondroplasia in chickens: a sequential study. *Vet. Pathol.*, **23**(4), 499-505.
- Hinsenkamp M., Rippens F., Begaux F., Mathieu F., De Maertelaer V., Lepere M., Rooze M., Haubruege E., Stallenberg B., 2001. Anatomic distribution of radiographic abnormalities in Kashin-Beck disease. *Int. Orthop.*, **25**(3), 142-146.
- Holmgren P.K., Holmgren N.H., Barnett L.C., 1990. *Index Herbariorum. Part I: The Herbaria of the World*. 8th ed. New York: New York Botanical Garden.
- Irvine F.R., 1952. Food plants of West Africa. *Lejeunia*, **16**, 27-51.
- Irvine F.R., 1956. The edible cultivated and semi-cultivated leaves of West Africa. *Mater. Veg.*, **2**, 35-42.
- Ishii K., Kobayashi J., Ueno Y., Ichinoe M., 1986. Occurrence of trichothecin in wheat. *Appl. Environ. Microbiol.*, **52**, 331-333.
- Jest C., 1972. Plantes sauvages utilisées comme aliments à Dolpo, Haute vallée himalayenne du Népal. In: Thomas J.M.C., Bernot L. (eds). *Langues et Techniques. Vol. 2*. Paris: Nature et Société, 669-676.
- Jingwei Z., 1982. *The Alpine plants of China*. Beijing: Science Press; New York, USA: Gordon and Breach.
- Joshi K., 1991. The uses of wild plants by local communities on the mountainous region of Nepal. *Mt Environ. Dev. (J. of Environmental Management Action Group, Nepal)*, **1**(2), 43-52.
- Kletter C., Kriechbaum M. (Eds.), 2001. *Tibetan Medicinal Plants*. Stuttgart, Germany: Medpharm Scientific Publishers.
- Kolsteren P., 1992. Kashin-Beck disease. *Ann. Soc. Belg. Med. Trop.*, **72**(2), 81-91.
- Kolsteren P., Atkinson S., Maskall K., 1995. Une enquête nutritionnelle au Tibet. *Cah. Santé*, **5**, 247-252.
- Kravchenko L.F., 1959. The main principles and tasks of conservative treatment of the Urov disease patients (Kashin-Beck). In the collection: *The second Scientific Conference of the Urov Scientific Research Station*, Sretensky, Russia.
- Krogh P., Christensen D.H., Hald B., Harlou B., Larsen C., Pedersen E.J., Thrane U., 1989. Natural occurrence of the mycotoxin fusarochromanone, a metabolite of *Fusarium equiseti*, in cereal feed associated with tibial dyschondroplasia. *Appl. Environ. Microbiol.*, **55**, 3184-3188.
- Kuhnlein H.V., Turner N.J., Kluckner P.D., 1982. Nutritional significance of two important «root» foods (springbank clover and Pacific silverweed). *Ecol. Food Nutr.*, **12**, 89-95.
- La Grange M., Mathieu F., Begaux F., Durand M-C., 2001. Kashin-Beck disease and drinking water in Central Tibet. *Int. Orthop.*, **25**(3), 167-169.
- Larsen A., 2001. *Ombidi: a pilot study in northern Namibia*. <http://www.larsen-twins.dk/ombidi/100ombidi.html> (june 2008).
- Le Thi Top, Berger J., 2005. Multiple micronutrient supplementation improves anemia, micronutrient nutrient status, and growth of Vietnamese infants: double-blind, randomized, placebo-controlled trial. *J. Nutr.*, **135**, 660S-665S.
- Lee Y.W., Mirocha C.J., Shroeder D.J., Walser M.M., 1985. TDP-1, a toxic component causing tibial dyschondroplasia in broiler chickens, and trichothecenes from *Fusarium roseum* 'Graminearum'. *Appl. Environ. Microbiol.*, **50**, 102-107.
- Levander O.A., 1987. Etiological Hypotheses Concerning Kashin-Beck Disease. O.A. Nutrition'87. AIN symposium proceedings. In: Levander, O.A. (ed.).

- Nutrition'87. Proceedings of the AIN (American Institute of Nutrition) Symposium. Washington, DC, March 29-April 2 1987*, p. 67-71.
- Lewis O.A.M., 1996. A short ion-exchange column method for the estimation of cystine and methionine. *Nature*, **209**, 1239-1241.
- Li B., 1980. A preliminary study of the subnival vegetation of Xizang. In *Proceedings of the symposium on Qinghai-Xizang (Tibet) plateau (abstract)*. Beijing: Academia Sinica, 284.
- Li C.Z., 1979. The effect of sodium selenite and vitamin E on 224 cases of patients with Kashin-Beck disease based upon X-ray examination and discussion about the etiology of the disease. *Chin. Med. J. (Chung Hua I Hsueh Tsa Chih)*, **59**(3),169.
- Li C.Z., Huang J.R., Li C.X., 1987. Sodium selenite as a preventive measure for Kashin-Beck disease as evaluated in X-ray studies. In: Combs G.F.Jr., Levander O.A., Spallholz J.E., Oldfield J.E. (eds). *Selenium in Biology and Medicine. Proceedings of the Third International Symposium on Selenium in Biology and Medicine, held May 27-June 1, 1984, Xiangshan, P.R. China*. New York: Van Nostrand Reinhold, p. 934-937.
- Li J., Ren S., Chang D., Wan X., Liu W., 1990. Selenium in Environment and its Relation with Kashin-Beck Disease. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases. WHO, Beijing*, p. 79-86.
- Liang S., 1990. The Prophylactic and Therapeutic Effects of Selenium on Kashin-Beck Disease. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 102-109.
- Liang S.T., Mu S.Z., Zhang J.C., Zhang F.J., Shang X., 1987. Effects of selenium supplementation in prevention and treatment of Kaschin-Beck disease. In: Combs G.F.Jr., Levander O.A., Spallholz J.E., Oldfield J.E. (eds). *Selenium in Biology and Medicine. Proceedings of the Third International Symposium on Selenium in Biology and Medicine, held May 27-June 1, 1984, Xiangshan, P.R. China*. New York: Van Nostrand Reinhold, p. 938-946,.
- Liu D.S., 1981. Geological and ecological studies of Qinghai-Xizang plateau. In *Proceedings of the Symposium on Qinghai-Xizang (Tibet) plateau. Vol. 2, Environmental and ecology of Qinghai-Xizang plateau, Beijing*.
- Liu F.D., Wang Z.L., Hinsenkamp M., 1998. Osteotomy at the knee for advanced cases of Kashin-Beck disease. *Int. Orthop.*, **22**, 87-91.
- Lopez A.D., Mathers C.D., Ezzati M., Jamison D.T., Murray C.J.L., 2006. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet*, **367**, 1747-1757.
- Luo Y., Yoshizama T., Yang J.S., Zhang S.Y., Zhang B.J., 1992. A survey of occurrence of Fusarium mycotoxins in corn and wheat samples from Shaanxi and Shanxi Provinces, China. *Mycotoxin Res.*, **8**, 85-91.
- Ma Dequan, 2002. *Genetic resources of Tibetan Barley in China*. Beijing: China Agriculture Press.
- Ma T., Guo J., Wang F., 1993. The epidemiology of iodine-deficiency disease in China. *Am. J. Clin. Nutr.*, **57**(suppl), 264S-266S.
- Mabey R., 1997. *Flora Britannica*. London: Chatto & Windus.
- Malaisse F., 1997a. L'ethno-écologie, source de développement en sociétés tropicales. In *Séance d'ouverture de l'année académique 1997-1998. Leçon inaugurale*. Gembloux, Belgium: Faculté universitaire des Sciences agronomiques.
- Malaisse F., 1997b. *Se nourrir en forêt claire africaine. Approche écologique et nutritionnelle*. Gembloux, Belgium: Presses Agronomiques de Gembloux; Wageningen, The Netherlands: C.T.A.
- Malaisse F., 2001. Lessons from the past for a better future: Ethnoecology, a promising link between tradition and science regarding biodiversity management. In: *Science and Tradition: Roots and Wings for Development*. Brussels: Royal Academy of Overseas Sciences; UNESCO, 89-106.
- Malaisse F., Haubruge E., Mathieu F., Begaux F., 2001. Ethno-agricultural approach to the rural environment in the prevention of Kashin-Beck disease. *Int. Orthop.*, **25**(3), 170-174.
- Malaisse F., Begaux F., Chasseur C., Dolkar P., Leteinturier B., Lognay G., Mathieu F., Rinchen L., Wathélet B., Haubruge E., 2003. Ethno-ecological approach of Tibet South-Central rural environment knowledge as a support to Kashin-Beck disease's etiology. *Bull. Séanc. Acad. R. Sci. Outre-Mer*, **48**(2002-2003), 319-341.
- Malinovskii N.N., 1957. *N.I. Kashin (1825-1872)*. Moscow: State Publishing House for Medical Literature.
- Manandhar N.P., 1989. *Useful wild plants of Nepal*. Stuttgart, Germany: Franz Steiner Verlag.
- Manandhar N.P., 2002. *Plants and people of Nepal*. Portland, U.S.A.: Timber Press.
- Marchenay P., 1975. L'enquête régionale ethnoécologique. In: *L'homme et l'animal, Premier colloque d'Ethnozoologie*. Paris: Institut International d'Ethnoscience, 127-136.
- Martin G.J., 1995. *Ethnobotany. A methods manual*. London: Chapman & Hall.
- Martin G.J., 2001. Ethnobotany and Ethnoecology. In: *Encyclopedia of Biodiversity 2*. London: Academic Press, p. 609-621.
- Mathieu F., Begaux F., Lan Z.Y., Suetens C., Hinsenkamp M., 1997. Clinical Manifestations of Kashin-Beck Disease in Nyemo Valley, Tibet. *Int. Orthop.*, **21**(3), 151-156.
- Mathieu F., Begaux F., Suetens C., De Maertelaer V., Hinsenkamp M., 2001a. Anthropometry and Clinical Features of Kashin-Beck Disease in Central Tibet. *Int. Orthop.*, **25**(3), 138-141.
- Mathieu F., Suetens C., Begaux F., De Maertelaer V., Hinsenkamp M., 2001b. Effects of Physical Therapy on Patients with Kashin-Beck Disease. *Int. Orthop.*, **25**(3), 191-193.

- Maundu P.M., Ngugi G.W., Kabuye C.H.S., 1999. *Traditional food plants of Kenya*. Nairobi: National Museums of Kenya.
- Merialdi M., Caulfield L.E., Figueroa A.F., Costigan K.A., Dominici F., Dipietro J.A., 2004. Randomized controlled trial of prenatal zinc supplementation and fetal bone growth. *Am. J. Clin. Nutr.*, **79**, 826-30.
- Miehe G., 1997. Alpine vegetation types of the central Himalaya. Polar and alpine tundra. *Ecosyst. World*, **3**, 161-184.
- Mo D.X., 1987. Pathology and Selenium Deficiency in Kashin-Beck Disease. In: Combs G.F.Jr., Levander O.A., Spallholz J.E., Oldfield J.E. (eds). *Selenium in Biology and Medicine. Proceedings of the Third International Symposium on Selenium in Biology and Medicine, held May 27-June 1, 1984, Xiangshan, P.R. China*. New York: Van Nostrand Reinhold, p. 924-933.
- Mo D.X., 1990. Advances in the Pathology of Kashin-Beck Disease and its Relationship with Selenium and Other Elements. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 42-55.
- Moore S., Spackman D., Stein W., 1958. Chromatography of amino acids on polystyrene sulfonate resins. *Anal. Chem.*, **30**, 1186-1206.
- Moreno-Reyes R., Suetens C., Mathieu F., Begaux F., Zhu D., Rivera M.T., Boelaert M., Neve J., Perlmutter N., Vanderpas J., 1998. Kashin-Beck Osteoarthropathy in Rural Tibet in Relation to Selenium and Iodine Status. *New Engl. J. Med.*, **339**(16), 1112-1120.
- Moreno-Reyes R., Mathieu F., Boelaert M., Begaux F., Suetens C., Rivera M.T., Nève J., Perlmutter N., Vanderpas J., 2003. Selenium and iodine supplementation of rural Tibetan children affected by Kashin-Beck osteoarthropathy. *Am. J. Clin. Nutr.*, **78**(1), 137-44.
- Munday R., 1985. Studies on the mechanism of toxicity of the mycotoxin sporidesmin. IV. Inhibition by copper-chelating agents of the generation of superoxide radical by sporidesmin. *J. Appl. Toxicol.*, **5**(2), 69.
- Nazarea V.D., 1999. *Ethnoecology. Situated Knowledge/ Located lives*. Tucson, GA, USA: The University of Arizona Press.
- Nazarea V.D., 2006. A view from a point: Ethnoecology as situated knowledge. In: Haenn N., Wilk R. (Eds.). *The environment in Anthropology: A reader in Ecology, Culture, and Sustainable living*. New York, USA: New York University Press, 34-39.
- Negi K.S., Tiwari J.K., Gaur R.D., Pant K.C., 1993. Notes on Ethnobotany of Five Districts of Garhwal Himalaya, Uttar Pradesh, India. *Ethnobotany*, **5**(1-2), 73-81.
- Németh E., 1999. *Caraway: the genus Carum* (Medicinal and aromatic plants industrial profiles. Vol. 7). New York: Harwood Academic Publishers.
- Nesterov A.I., 1964. The clinical course of Kashin-Beck disease. *Arthritis Rheum.*, **7**(1), 29-40.
- Norman J., 2002. *Herb and spice*. London: Dorling Kindersley.
- Osmaston H.A., 1998. Agriculture in the main Lhasa valley. In: Clarcke G.E. (ed.). *Development, Society and Environment in Tibet. Proceedings of the 7th Seminar of the International Association for Tibetan Studies, Graz 1995. Vol. 5*. Vienna: Verlag der Osterreichischen Akademie der Wissenschaften, p. 121-151.
- Paoletti M.G., Dreon A.L., Lorenzoni G.G., 1995. Pistic, traditional food from Western Friuli, N.E. Italy. *J. Econ. Bot.*, **49**(1), 26-30.
- Pasteels J.L., Liu F.D., Hinsenkamp M., Rooze M., Mathieu F., Perlmutter N., 2001. Histology of Kashin-Beck lesions. *Int. Orthop.*, **25**(3), 151-153.
- Pearce J.A., Houjun M., 1988. Volcanic Rocks of the 1985 Tibet Geotraverse: Lhasa to Golmud. *Philos. Trans. R. Soc. London. Ser. A*, **327**, 169-201.
- Pegler D.N., Yao Y-J., Li Y., 1994. The Chinese caterpillar fungus. *Mycologist*, **8**, 3-5.
- Peng A., Bei N., Tang C., Xu L., Wang W., Rui H., Wang Y., 1990. Studies on the Relationship Between Selenium and Humic Acid Extracted from Kashin-Beck Disease regions. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases. WHO, Beijing*, 87-92.
- Peng A., Wang W.H., Wang C.X., Wang Z., Rui H.F., Wang W., Yang Z.W., 1999. The role of humic substances in drinking water in Kashin-Beck disease in China. *Environ. Health Perspect.*, **107**, 293-296.
- Peng A., Wang Y., Wang W., Xu L., Yang C., 1987. Inhibitory effect on the toxicity of *Fusarium* toxin by selenium. *Acta Sci. Circumstantiae*, **7**(2), 237-239.
- Peng A., Wang Z., Wang W., Yang C., 1991. A new hypothesis for the etiology of Kashin-Beck disease. *J. Environ. Sci. (China)*, **3**(4), 5-14.
- Peng A., Yang C., Rui H., Li H., 1992. Study on the pathogenic factors of Kashin-Beck disease. *J. Toxicol. Environ. Health*, **35**(2), 79-90.
- Peng A., Yang C.L., 1985. Determination of Free Radicals in Drinking Water from some Kashin-Beck Diseased regions. *Acta Sci. Circumstantiae*, **5**(1), 15.
- Pitt J.I., Hocking A.D., Samson R.A., King A.D., 1992. Recommended methods for mycological examination of foods. In: Samson R.A., Hocking A.D., Pitt J.I., King A.D. (eds). *Modern Methods in Food Mycology*. Amsterdam, The Netherlands: Elsevier, p. 365-368.
- Pohle P., 1990. *Useful plants of Manang District (A contribution to the ethnobotany of the Nepal Himalaya)*. Nepal Research Centre Publ. **16**. Stuttgart, Germany: Franz Steiner Verlag, 65 p.
- Polunin O., Staiton A., 1997. *Flowers of the Himalaya*. Oxford, UK: Oxford University Press.
- Pujol R., 1975. Définition d'un écosystème avec deux exemples: étude ethnozoobotanique des cardères (*Dipsacus*) et inter-relations Homme-Animal-Truffe. In: *L'homme et l'animal, Premier Colloque d'Ethnozologie*. Paris: Institut international d'ethnoscience, 91-144.

- Randall C. 2003. Historical and modern uses of *Urtica*. In: Kavalali G.M. (Ed.). *Urtica. Therapeutic and Nutritional Aspects of Stinging Nettles*. London: Taylor & Francis, p. 12-24.
- Ren S., Zhang X., Li W., Wang W., Wang J., Zhang Y., 1991. Humic acid and free radical in environment of Kashin-Beck disease areas. *J. Environ. Sci. (China)*, **3**(4), 95-101.
- Rosin I.V., Butko V.S., Kalabukhov E.P., 1982. Some biochemical and biophysical aspects of the pathogenesis of Kashin-Beck disease. *Ter. Arkh.*, **54**(2), 80-82.
- Sacherer J., 1979. The high altitude ethnobotany of the Rolwaling Sherpas, a contribution to Nepalese studies. *Contrib. Nepalese Stud.*, **6**, 46-64.
- Sasaki S., Iwata H., Ishiguro N., Habuchi O., Miura T., 1994. Low-selenium diet, bone, and articular cartilage in rats. *Nutrition*, **10**(6), 538-543.
- Schaller G.B., 1998. *Wildlife of the Tibetan steppe*. Chicago, USA: The University of Chicago Press.
- Schweinfurth U., 1956. Über klimatische Trockentäler im Himalaya. *Erdkunde*, **10**, 297-302.
- Schweinfurth U., 1957. *Die horizontale und vertikale Verbreitung des Vegetation im Himalaya*. Bonn, Germany: Ferd. Dümmler.
- Schweinfurth U., 1984. The Himalaya: complexity of a mountain system manifested by its vegetation. *Mt. Res. Devel.*, **4**, 339-344.
- Sharma S.D. 2000. Food for life. *The Tribune*, India. <http://www.tribuneindia.com/2000/20000130/spectrum/main8.htm> (june 2008)
- Shrestha P., 1988. Contribution to the ethnobotany of the Tamangs of Katmandu valley. *Contrib. to Nepalese Stud.*, **15**(2), 147-266.
- Smuts C.M., Lombard C.J., Spinnler Benadé A.J., Dhansay M.A., Berger J., Le Thi Hop, López de Romaña G., Untoro J., Karyadi E., Erhardt J., Gross R., 2005. Efficacy of a foodlet-based multiple micronutrient supplement for preventing growth faltering, anemia and micronutrient deficiency of infants: the four country IRIS trial pooled data analysis. *J. Nutr.*, **135**, 631S-638S.
- Sokoloff L., 1987. Kashin-Beck disease: Historical and Pathological Perspectives. In: Levander, O.A. (ed.). *Nutrition'87. Proceedings of the AIN (American Institute of Nutrition) Symposium. Washington, DC, March 29-April 2 1987*, 61-63.
- Sokoloff L., 1988. Kashin-Beck disease: current status. *Nutr. Rev.*, **46**(3), 113-119.
- Sokoloff L., 1989. The history of Kashin-Beck disease. *N.Y. State J. Med.*, **89**(6), 343-351.
- Stein R.A., 1962. *La civilisation tibétaine*. Paris : Dunod.
- Sudre P., Mathieu F., 2001. Kashin-Beck disease: From etiology to prevention or from prevention to etiology? *Int. Orthop.*, **25**(3), 175-179.
- Suetens C., Moreno-Reyes R., Chasseur C., Mathieu F., Begaux F., Haubruge E., Durand M.C., Nève J., Vanderpas J., 2001. Epidemiological support for a multifactorial aetiology of Kashin-Beck disease in Tibet. *Int. Orthop.*, **25**(3), 180-187.
- Sundriyal M., Sundriyal R.C., 2001. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. *Econ. Bot.*, **55**(3), 377-390.
- Takamori T., 1968. *Kashin-Beck's Disease*. Gifu, Japan: Gifu University School of Medicine, Professor Tokio Takamori Foundation, 2nd Department of Internal Medicine.
- Tan J. (ed.), 1989. *The Atlas of Endemic Disease and their Environments in the People's Republic of China*. Beijing: Sciences Press.
- Tan J., Zhu W., Li R., Zheng D., Hou S., Zhu Z., Wang W., 1990. Geographic Distribution of Kashin-Beck Disease in China and the Relation of Ecological Chemico-Geography to its Occurrence. In: *Chinese Academy of Preventive Medicine (CAPM). Proceedings of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 12-26.
- Toffin G., 1985. Contribution à l'ethnozoologie des Tamang du massif du Ganesh Himal (Népal central). *J. Agric. Trad. Bot. Appl.*, **32**, 83-126.
- Toffin G., Wiart J., 1985. Recherches sur l'ethnobotanique des Tamang du massif du Ganesh Himal (Népal central) : Les plantes non cultivées. *J. Agric. Trad. Bot. Appl.*, **32**, 127-175.
- Toffin G., Jest C., Blamont D., 1986. Les populations de la région Ankhu Khola – Trisuli. In : Dobremez J.-F. (ed.) : *Les collines du Népal central. écosystèmes, structures sociales et systèmes agraires*. Paris : I.N.R.A., 79-118.
- Toledo V.M., 1992. What is Ethnoecology? Origins, scope and implications of a rising discipline. *Ethnoecologia*, **1**(1), 5-21.
- Tournadre N., Dorje S., 2003. *Manuel de tibétain standard, langue et civilisation. Langues et Monde*. Paris : L'Asiathèque.
- Trofimova E.P., 1977. Some wild food plants of Tadzhikistan as sources of vitamins. *Izv. Akad. Nauk Tadzh SSR, Otd. Biol. Nauk*, **1**, 43-48.
- Troll C., 1939. Das Pflanzenkleid des Nanga Parbat. Begleitworte zur Vegetationskarte der Nanga-Parbat-Gruppe (NW-Himalaya), 1:50.000. *Wiss. Veröff. Dtsch Mus. Ldkd, Leipzig*, N.F. **7**, 151-180.
- Troll C., 1972. The three-dimensional zonation of the Himalaya system. In Troll C. (ed.). *Geocology of the High Mountain Regions of Eurasia*. Stuttgart, Germany: Franz Steiner Verlag, Erdwissenschaften Forschung **4**, 264-275.
- Tsarong T.J., 1994. *Tibetan Medicinal Plants*. Kalimpong, India: Tibetan Medical Publications.
- Tuormaa T.E., 1996. The adverse effects of manganese deficiency on reproduction and health. *J. Orthomolecular Med.*, **11**(3), 69-79.
- Turner N.J., Kuhnlein H.V., 1982. Two important «root» foods of the Northwest Coast Indians: Springbank clover (*Trifolium wormskioldii*) and Pacific silverweed (*Potentilla anserina* ssp. *pacifica*). *Econ. Bot.*, **36**(4), 411-432.
- Ullrich I., Jahn-Deesbach W., 1984. Proteingehalt und Proteininzusammensetzung verschiedener Unkrautarten. *Angew. Bot.*, **58**(3-4), 255-266.

- Vavilov I., 1926. Centres of origin of cultivated plants. *Bull. Appl. Bot. Genet. Plant Breed.*, **16**, 1-248.
- Viard M., 2004. *Les plantes à épices*. Ingersheim, France : S.A.E.P.
- von Wissmann H., 1961. Stufen und Gürtel der Vegetation und des Klimas in Hochasien und seinen Randgebieten. B. Thermische Raumgliederung (1. Teil) und Frostboden. *Erdkunde*, **15**, 19-44.
- Voshchenko A.V., Ivanov V.N., 1990. Kashin-Beck Disease in the USSR. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 152-196.
- Walser M.M., Morris V.C., Levander O.A., 1988. Effect of dietary selenium on the development of Fusarium-induced tibial dyschondroplasia in broiler chickens. *Avian Dis.*, **32**(1), 84-88.
- Wang A., Yang M., Liu J., 2005. Molecular phylogeny, recent radiation and evolution of gross morphology of the rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trnL-F* sequences. *Ann. Bot.*, **96**(3), 489-498.
- Wang J.T., 1980. Basic characteristics of steppe vegetation in the Xizang plateau. In *Proceedings of the symposium on Qinghai-Xizang (Tibet) plateau (abstract)*. Beijing: Academia Sinica, 277.
- Wang J.T., 1988a. The steppes and deserts of Xizang plateau (Tibet). *Vegetatio*, **75**, 135-142.
- Wang J.T., 1988b. A preliminary study on alpine vegetation of the Qinghai-Xizang (Tibet) plateau. *Acta Phytoecol. Geobot. Sinica*, **12**, 81-90.
- Wang X., Fosmire G.J., Gay C.V., Leach R.M.Jr., 2002. Short-term zinc deficiency inhibits chondrocytes proliferation and induces cell apoptosis in the epiphyseal growth plate of young chickens. *J. Nutr.*, **132**(4), 665-673
- Wang Y., Yang Z., Gilula L.A., Zhu C., 1996. Kashin-Beck disease: radiographic appearance in the hands and wrists. *Radiology*, **201**(1), 265-270.
- Wang Z., 1990. Studies on the prevalence of Kashin-Beck disease in Heilongjiang province. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and Non-communicable diseases, WHO, Beijing*, 31-35.
- Wang Z., Lu S., Jing D., Li J., Ren S., Chen D., 1990. Observation by X-ray studies on the effects of selenite supplemented salt on the prevention and treatment of Kashin-Beck Disease. In: Tan, J. et al (eds). *Environmental life elements and health*. Beijing: Sciences Press, 356-357.
- Wang Z., Xu L., Li H., Peng B., Rui H., Wang W., Peng A., 1991. Role of interaction between selenium and organic matters on cause of Kashin-Beck disease. *J. Environ. Sci. (China)*, **3**(4), 15-28.
- Ward F.K., 1935. A sketch of geography and botany of Tibet, being materials for a flora of that country. *J. Linn. Soc. Bot.*, **50**, 239-265.
- Wasson W.P., Wasson R.G., 1957. *Mushrooms, Russia and History*. Vol.I. New York: Pantheon Books.
- Wei X., Wright G.C., Sokoloff L., 1986. The effect of sodium selenite on chondrocytes in monolayer culture. *Arthritis Rheum.*, **29**(5), 660-664.
- Wetherilt H., 2003. Nutritional evaluation of *Urtica* species. In: Kavalali G.M. (Ed.). *Urtica. Therapeutic and Nutritional Aspects of Stinging Nettles*. London: Taylor & Francis, 56-64.
- WHO, 1996. *Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes*. Geneva: World Health Organization.
- Woodland D.W., 1982. Biosystematics of the perennial North American taxa of *Urtica*. II Taxonomy. *Syst. Bot.*, **7**(3), 282-290.
- Wu S., Feng J., 1992. Characteristics, exploitation and protection of biological resources in the Tibetan Plateau. In: China Society of the Qinghai-Xizang Plateau Research. (ed.). *Proceedings of the First Symposium of the Qinghai-Tibet Plateau*. Beijing: Science Press, 29-89.
- Wu S., Yang Y.P., Fei Y., Wu S.G., 1995. On the flora of the alpine region in the Qinghai-Xizang (Tibet) plateau, China. *Acta Bot. Yunnanica*, **17**, 233-250.
- Wu W., Cook M.E., Chu Q., Smaller E.B., 1993. Tibial dyschondroplasia of chickens induced by fusarochromanone, a mycotoxin. *Avian Dis.*, **37**(2), 302-309.
- Wu Z.Y., Tang Y.C., Li X.W., Wu S.G., Li H., 1981. Dissertations upon the origin, development and regionalization of Xizang flora through the floristic analysis. In: *Symposium on Qinghai-Xizang (Tibet) Plateau (1981). Geological and ecological studies of the Qinghai-Xizang (Tibet) Plateau: Proceedings of the Symposium on Qinghai-Xizang (Tibet) Plateau held May 25-June 1, 1980, Beijing, P.R. China*. Beijing: Science Press.
- Wylie T., 1959. A standard system of Tibetan transcription. *Harvard J. Asiat. Stud.*, **22**, 261-267.
- Yamamuro T., 2001. A historical overview of studies on Kashin-Beck disease performed by Japanese researchers. *Int. Orthop.*, **25**(3), 134-137.
- Yang C., Wolf E., Roser K., Delling G., Muller P.K., 1993a. Selenium deficiency and fulvic acid supplementation induces fibrosis of cartilage and disturbs subchondral ossification in knee joints of mice: an animal model study of Kashin-Beck disease. *Virchows Arch. A Pathol. Anat. Histopathol.*, **423**(6), 483-491.
- Yang C., Niu C., Bodo M., Gabriel E., Notbohm H., Wolf E., Muller P.K., 1993b. Fulvic acid supplementation and selenium deficiency disturb the structural integrity of mouse skeletal tissue. An animal model to study the molecular defects of Kashin-Beck disease. *Biochem. J.*, **289**(3), 829-835.
- Yang J.B., 1995. Research report on the etiology of Kashin-Beck disease (KBD). *Chin. J.*, **14**, 201-204.
- Yang J.B., 1997. Study on Chinese strategy for Kashin-Beck disease control. *China Endemo-Epidemic Dis. Mag.*, **16**(3), 129-131.
- Yang J.B., 1998. *Research on the cause of Kashin-Beck disease*. Heilongjiang, P.R. China: Heilongjiang Sciences and Technology Press House.

- Yang JB, Sun D, Wang Z., 1995. Determination of T-2 toxin in the staple food from the sick families in Kashin-Beck disease (KBD) areas. *Chin. J.*, **14**, 146-149.
- Yang JB., 1990. Epidemiological Studies on the Causes of Kashin-Beck Disease. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 63-78.
- Yin J., Xu J., Liu C. Li H., 1988. The Tibetan Plateau: Regional stratigraphic context and previous work, *Philos. Trans. R. Soc. London. Ser. A*, **327**, 145-168.
- Yin P., 1990. Clinical Investigation of Kashin-Beck Disease and its Diagnosis. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and non-communicable diseases, WHO, Beijing*, 36-41.
- Yin P., Guo X., Zhang S. Bai C., 1990. Studies on the Preventive and Therapeutic Effects of Comprehensive Method and Se-Fortified Wheat on Kashin-Beck Disease. In: *Chinese Academy of Preventive Medicine (CAPM). Proceedings of the international workshop on Kashin-Beck disease and non-communicable diseases. WHO, Beijing*, 110-115.
- Ying M., Wang Y., Chu Q., Fu G., Jin L., 1990. Radiology and Pathology of Kashin-Beck Disease. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and Non-communicable diseases, WHO, Beijing*, 56-62.
- Yu W, Wang Y, Jiang Y, Cheng X, Wang L, Genant K., 2002. Kashin-Beck disease in children: radiographic findings in the wrist. *Skeletal Radiol.*, **31**(4), 222-225.
- Zhai S., 1990. Investigation on the Relationship Between Kashin-Beck Disease and Drinking Water. In: *Chinese Academy of Preventive Medicine (CAPM). Proceeding of the international workshop on Kashin-Beck disease and Non-communicable diseases, WHO, Beijing*, 96-101.
- Zhai S.S., Zhao Z., Shao G., Yao J., Cheng W., Zheng F., Li S., Zhang H., 1980. The investigation on the relationship between KBD and drinking water or food-stuff in Qian An plain and prairie. *The collection of Papers on Endemic Disease Published by the Second Endemic Diseases Control Institute in Jilin Province*, **2**, 22-46.
- Zhang B.P., Chen X.D., Li B.L., Yao Y.H., 2002. Biodiversity and conservation in the Tibetan Plateau. *J. Geogr. Sci.*, **12**(2), 135-143.
- Zhang F., Wang K., Xu S., 1991. The damage effects of oxy free radicals and fulvic acid on chondrocytes. *J. Environ. Sci. (China)*, **3**, 48-54.
- Zhang J., 1988. An observation on the seasonal change of urinary excretion of glycosaminoglycan and hair selenium content in the children with Kashin-Beck disease at an early stage. *Chin. J. Endemiol.*, **7**(4), 211-214.
- Zhang Q., Yang G.P., Dai X., Sun J.Z., 1994. A comparative analysis of genetic polymorphism in wild and cultivated barley from Tibet using isozyme and ribosomal DNA markers. *Genome*, **37**, 631-638.
- Zhang X., Wang Z., Li S., Ren S., Wang W., 1982. Study on the content of humic acid in KBD regions. In: *Proceedings of the Second Songhua River Symposium on Environmental Sciences*. Changchun, P.R. China: Jilin People Press.
- Zhang X.Z., Zheng D., Yang Q.Y. 1982. Physical geography of Xizang (Tibet). In: *The series of the scientific expedition to the Qinghai-Xizang plateau*. Beijing: Science Press.
- Zhang Y., Cheng Y., Hong Y., Li S., 2003. Zinc deficiency on pathological changes of femur epiphyseal growth plate in rats. *Wei Sheng Yan Jiu*, **32**(1), 16-19.
- Zhao K., Wang D., Song H., 1980. Marshes of Xizang plateau. In: *Proceedings of the symposium on Qinghai-Xizang (Tibet) plateau (abstract)*. Beijing: Academia Sinica, 284-285.
- Zheng D., 1983. Untersuchungen zur floristisch-plantengeographischen Differenzierung des Xizang-Plateaus (Tibet), China. *Erdkunde*, **37**, 34-47.
- Zheng D., Zhang Y.Z., Yang Q.Y., 1979. On the natural zonation in the Qinghai-Xizang Plateau. *Acta Geogr. Sinica*, **34**, 1-11.

Appendix I

Appendix 1. Villages' network investigated during the ethno-environmental surveys in the study area of Tibet Autonomous Region.

Prefecture	County	Community	Village and sites	Altitude (m)	Latitude North	Longitude East	Population	Population survey	Families	Families survey	Preference survey	
Lhoca	Dranang	Dache	Tsondu		29°15'18"	091°24'81"		4		2	2	
	Ghongkar	Chatang	Sema		29°17'11"	090°38'58"		1		1	1	
	Ghongkar	Kampa	Kampatonde		29°15'30"	090°37'22"		1		1	1	
	Ghongkar	Kampa	Kampa		29°14'47"	090°37'35"		1		1	1	
	Netong	Kyipa	Damtchu		29°23'04"	091°50'04"	115	2		1	1	
	Netong	Kyipa	Klepa		20°21'55"	091°49'51"	266	1		1	1	
	Netong	Kyipa	Lhakang		29°22'45"	091°49'42"	250	9		6	7	
	Netong	Kyipa	Namcatchakju		29°21'58"	91°49'54"	178	2	42	1	1	
	Netong	Kyipa	Nangga	3625	29°21'47"	091°50'15"	160	8	39	3	6	
	Netong	Kyipa	Namong		29°23'53"	091°50'49"	160	2	42	1	2	
	Netong	Kyipa	Tchuding	3810	29°23'20"	091°51'20"	180	4	39	1	2	
	Zangri	Shupa	Biba I		29°17'22"	092°02'20"	130	2	23	1	2	
	Zangri	Shupa	Biba II		29°19'05"	092°02'45"	130	2	23	1	2	
	Zangri	Shupa	Biba III		29°21'06"	092°02'04"	130	2	23	1	2	
	Zangri	Tsingshe	Tchamel		29°24'10"	092°19'40"	165	2		1	1	
	Zangri	Tsingshe	Tsingshe		29°25'55"	092°21'10"	160	1		1	1	
			Djamey		29°24'09"	092°19'36"	155	2	30	1	1	
			Gyabra					3		1	3	
	Shikatse	Rimpung	Dekiling	Rimpung		29°11'50"	089°53'00"		2		1	2
		Rimpung	Dekiling	Saka	3940	29°09'41"	089°53'58"	98	5	14	3	3
Rimpung		Dekiling	Targye	3960	29°08'15"	089°53'42"	167	2	27	1	2	
Rimpung		Dekiling	Youngda		29°10'41"	089°53'26"	61	4	7	2	4	
Shätonge-mön		Danakpu	Lhaka		29°39'59"	088°37'38"	310	2	54	1	2	
Shätonge-mön		Rinchentse	Demaku		29°30'32"	088°32'00"	120	3	24	3	3	
Shätongemön		Rinchentse	Lunduptse		29°34'33"	088°34'48"	500	4	100	2	2	
Lhasa		Dakste	Dichen	Lamotse	3790	29°39'05"	091°22'54"	154	3	30	2	2
		Dakste	Dichen	Poronkang	3786	29°38'37"	091°24'17"	216	4	41	2	2
		Dakste	Lamo	Lhaamo		30°02'00"	091°12'30"	216	4	41	2	2
		Damxung	Namtso Xiang	Namco		30°48'43"	091°08'12"		2		1	2
		Damxung	Namtso Xiang	Namtsocham		30°45'07"	091°05'59"		2		1	2
	Lhüntrup	Nanang	Barchak	3971	30°04'58"	091°33'37"	155	2		1	2	
	Lhüntrup	Nanang	Burukang	3993	30°34'34"	091°34'59"	321	3		1	2	
	Lhüntrup	Nanang	Chora	3996	30°05'23"	091°33'02"	104	2		1	1	
	Lhüntrup	Nanang	Karchung		30°09'45"	091°30'20"	160	2		1	2	
	Lhüntrup	Nanang	Karteu	4061	30°07'07"	091°26'32"	52	2		1	2	
Lhüntrup	Nanang	Katgau		30°08'40"	091°32'10"		3		2	2		

Prefecture	County	Community	Village and sites	Altitude (m)	Latitude North	Longitude East	Population	Population survey	Families	Families survey	Preference survey
	Lhüntrup	Nanang	Nanang com.		30°11'40"	091°25'00"		3		1	2
	Lhüntrup	Nanang	Nearme	3992	30°05'52"	091°30'31"	291	3		1	3
	Lhüntrup	Nanang	Pongo-Chala km 6.5		30°17'43"	091°33'30"		2		1	2
	Lhüntrup	Nanang	Tchake	4035	30°08'44"	091°22'24"		2		1	1
	Lhüntrup	Nanang	Tha na Kang	3957	30°04'09"	091°38'17"	226	2		1	0
	Lhüntrup	Nanang	Rukum	3963	30°03'59"	091°40'08"	337	2		1	2
	Lhüntrup	Nanang	Unakang	3965	30°04'29"	091°37'33"	228	3		2	2
	Lhüntrup	Nanang	Wapuk	3993	30°05'37"	091°32'02"	270	2		1	2
	Lhüntrup	Tanggo	laza		30°17'18"	091°30'02"	186	2		1	2
	Lhüntrup	Tanggo	Pongo-Zingda km 32		30°17'43"	091°33'30"		1		1	1
	Lhüntrup	Tanggo	Pongo-Zingda km 45		30°18'47"	091°33'35"	48	2		1	1
	Lhüntrup	Tanggo	Pongo-Zingda km 49	4169	30°19'26"	091°36'04"	225	3		1	2
	Lhüntrup	Tanggo	Sebu		30°19'17"	091°31'52"	40	1		1	1
	Lhüntrup	Tanggo	Sedukang		30°18'46"	091°31'16"	150	2		1	2
	Lhüntrup	Tanggo	Tanggo	4149	30°18'36"	091°31'28"	214	2		1	2
	Lhüntrup	Tanggo	Tartong		30°18'20"	091°30'57"	40	2		1	2
	Lhüntrup	Tanggo	Yubu		30°16'42"	091°24'46"	83	2		1	2
	Lhüntrup	Tanggo	Yurak	4134	30°18'24"	091°29'37"	240	4		2	2
	Lhüntrup	Tanggo	Zingda	4210	30°19'19"	091°38'19"	40	3		1	2
	Mältro Gunkar	Mumba	Baraka	4125	30°02'56"	092°08'07"		2		1	2
	Mältro Gunkar	Mumba	Gontchuting	4063	30°01'38"	092°04'38"	265	2	40	1	2
	Mältro Gunkar	Mumba	Lawa	4103	30°02'38"	092°06'52"	170	1		1	1
	Mältro Gunkar	Niema Jingra	Balok	4049	29°56'33"	091°57'42"	76	2		1	2
	Mältro Gunkar	Niema Jingra	Barakang	4032	29°57'26"	091°57'07"	180	2		1	1
	Mältro Gunkar	Niema Jingra	Mara	4023	29°58'02"	091°56'39"	185	1		1	1
	Mältro Gunkar	Niema Jingra	Yarikang	3937	29°59'51"	091°59'28"	901	3		1	2
	Mältro Gunkar	Tashigang	Kargya		29°26'00"	091°55'00"		4	?	1	2
	Nyemo	Nyemo	Foupa	3940	29°30'12"	090°05'47"	170	2		1	2
	Nyemo	Nyemo	Sholo	3890	29°28'06"	090°07'50"		2		1	2
	Nyemo	Shume	Chuti	3915	29°29'35"	090°15'43"	84	3		1	2
	Nyemo	Shume	Geding	4167	29°34'09"	090°16'07"	141	1		1	1
	Nyemo	Shume	Nyshu Seni	3961	29°31'08"	090°15'44"	140	2		1	2
	Nyemo	Shume	Pachen	3932	29°31'14"	090°15'58"	113	2		1	2
	Nyemo	Shume	Shangkang		29°34'10"	090°16'07"	100	2		1	2
	Nyemo	Shume	Sheu	4065	29°32'58"	090°15'56"		3		1	2
	Nyemo	Shume	Sheulba		29°32'57"	090°16'02"	170	1		1	1
	Nyemo	Shume	Toshong	3903	29°27'47"	090°15'31"	150	2		1	2
	Nyemo	Shume	Zongsue	3919	29°29'32"	090°15'49"	287	4		2	2

Prefecture	County	Community	Village and sites	Altitude (m)	Latitude North	Longitude East	Population	Population survey	Families	Families survey	Preference survey
	Nyemo	Tarong	Conxiam		29°24'47"	090°11'12"	311	2		1	2
	Nyemo	Tarong	Gonsham		29°24'03"	090°10'06"		2		1	2
	Nyemo	Tarong	Lume		29°24'35"	090°11'23"	342	14	59	9	8
	Nyemo	Tarong	Ludukang		29°24'29"	090°11'05"	231	3		1	2
	Nyemo	Tarong	Luteu		29°24'39"	090°12'21"	245	7	42	6	5
	Tölung	Mar	Dham		29°55'11"	90°42'49"	778	1	158	1	1
	Tölung	Dechen	Tshupsang		30°00'35"	90°44'40"	820	2	139	2	2
TOTAL 3	12	22	26				215	215	>1070	116	164

The survey was conducted in three prefectures, 12 counties, 22 communities, 75 villages and 7 sites. It involved 116 families and some 215 peoples and resulted in 164 questionnaires.

Population = effective of population in the village concerned; pop. survey = number of individuals (locators) interviewed; families = number of families in the village concerned; families survey = number of families approached for the survey in the village of concern; preference survey = number of individuals having given a response on the ranking questionnaires.

Appendix II

Appendix II. Methods of chemical analysis of fungi and plant materials

The fresh plant material (edible part) has been dried on sun. Dry matter was determined from grinded material after drying until constant weight at 110°C. The majority of the major and minor mineral elements have been determined by Atomic Absorption Spectrometry after acid digestion, excepted SiO₂ and chloride which were measured by spectrophotometry and thiocyanate titration respectively. Fluorides have been determined after sodium hydroxide fusion with a selective ion electrode.

Nitrogen (N) content was measured according to Kjeldahl using Tecator System 20 digester and auto 1030 Kjeltac apparatus. Amino acid composition was established according to Moore et al. (1958) on an Alpha plus Pharmacia LKB system. Most amino acids were determined after hydrolysis with 6N hydrochloric acid with 0.1% phenol at 110°C for 24 hours under nitrogen. To avoid the loss of cystine and methionine during acid hydrolysis, performic acid oxidation of cystine to cysteic acid and methionine to methionine sulphone was first carried out according to Lewis (1966).

The protein quality was evaluated by calculating the chemical score (or amino acid score). It characterises the balance of essential amino acid and is a useful guide to appreciate protein quality in food (Eggum, 1987). The chemical score is the analytically determined level of the first-limiting amino acid expressed as a percentage of the level of the same amino acid recommended in the Provisional Reference Pattern.

$$\text{Amino acid score} = \frac{\text{FLAAP (mg/g N)}}{\text{FLAAR (mg/g N)}} \times 100$$

FLAAP: amount of the first-limiting amino acid present

FLAAR: amount of the first-limiting amino acid recommended

In the present study, we used the FAO/WHO (1990) Provisional Reference Pattern. Chemical index allowing comparisons with other vegetal protein sources that have been established according to FAO/WHO (1989).

After grinding, total lipids were extracted with 2: 1 v/v chloroform-methanol mixture (Folch et al., 1957). The extracts were concentrated under reduced pressure at 35°C and weighted. Fatty acid methyl esters (FAME) were prepared from crude lipids by boron-trifluoride catalysed trans-esterification according to AOCS (1998) method and analysed by gas chromatography on a Agilent HP6890 apparatus fitted with a cold-on column injector and a FID detector maintained at 250°C. The operating conditions were fixed as follows: 25 m x 0.25 mm CPWAX-FFAP 58 CB Varian column (df = 0.2 μm); temperature programme: from 55°C to 150°C at 30°C/min and from 150°C to 230°C at 5°C/min. Helium at 1 ml/min⁻¹ was used as carrier gas. FAME were identified on the basis of their retention data and by co-injection of pure references. The identifications were confirmed by gas chromatography coupled to mass spectrometry (GCMS) on a Hewlett-Packard HP5971 Mass spectrometer coupled to a HP 5890 Series II gas chromatograph. The chromatographic conditions were the same as above and the MS data were obtained in the EI mode at 70eV (Source T°: 250°C, scanned mass range: 35 to 500 amu). The recorded spectra were compared with those of the WILEY275.L computed database and carefully interpreted.

The Editors

François Malaisse

François Malaisse, born in Antwerp (Belgium) in 1934, is Agricultural Engineer (Water and Forests) of Gembloux Agricultural University (1956), Master in Science (Botany) of Brussels Free University (1960) and obtains a Ph. D. in Botany at the Official University of Congo, Lubumbashi (1968). He was Professor of several Universities in D.R. Congo, Belgium (Gembloux Agricultural University, Brussels Free University, Mons-Hainaut University, Tropical Medicine Institute Prince Leopold) and Italy (Pollenzo University of Gastronomic Sciences). He is a member of the Royal Academy for Overseas Sciences. Its research concerns ecology, plant systematic and ethnoecology mostly in overseas countries of Africa. He carried out 8 surveys in Tibet Autonomous Region since 1998. He has published or edited a tenth of books and more than 300 papers and parts of books.

Françoise Mathieu

Françoise Mathieu, born in Belgium in 1960, is Bachelor in Physical Therapy (1982) and holds a Ph.D. in Physical Therapy and Rehabilitation (2001) from Brussels Free University (ULB). The subject of her thesis was «Kashin-Beck Disease (KBD): Clinical Features, X-rays Aspects and Clinical Trial of a Physical Therapy Treatment in Central Tibet». She studied also Tropical Medicine (1988) at the Tropical Medicine Institute Prince Leopold, Antwerp (Belgium); Methodology and Practice in Epidemiology (2003) and Medical Statistics (2005) at the University of Bordeaux – France.

She joined Médecins Sans Frontières/Doctors Without Borders (MSF) in 1986 and worked in several countries and contexts until 2002. The last 10 years (1992 – 2002), Dr Mathieu managed the KBD projects for MSF. Within this context, she was living permanently in Tibet Autonomous Region from 1992 till 1998.

In 2002, together with 3 colleagues, she created the non-profit organization «Kashin-Beck Disease asbl-vzw» which is registered in Belgium. She was president of the board of directors for the last 6 years. At present, she is still directing the different projects that the organization is implementing in Tibet Autonomous Region (P.R. China). Every year, she is paying several visits to Tibet Autonomous Region.

She had published several papers about the Kashin-Beck disease.



**Kashin-Beck Disease
Foundation**



Centre for Disease Control
and Prevention of Tibet
Autonomous Region

Summary

Kashin-Beck disease is probably one of the most neglected diseases. It occurs in only a very limited part of the world, in a crescent shape area situated from South East Siberia to North West China. It affects mainly the rural population of these remote regions and more specifically the children. They are suffering from a lot of joint pain and lose their mobility. They stop to grow properly and they became severely handicapped.

Since more than 15 years, an international team together with his partner, the Centre for Disease Control and Prevention of Tibet Autonomous Region, are studying this disease with, sometimes, very limited means but always in a marvellous environment. Their daily workmates are the lovely children, the yaks and other animals of the Himalaya region as well as the unique variety of flowers and plants, under the beautiful shadow of some of the highest peaks of the world.

Their unique multidisciplinary approach of the complex issue of the Kashin-Beck disease is done with a constant worry of a better knowledge of the environment and its population.

Through a lot of beautiful pictures and some more scientific data, the authors, with an immoderate enthusiasm, will drive you along the valleys of the high Tibetan plateau and will accompany you for a visit from one village to another to meet these patients and understand the difficulties of their daily life.

Page-setting: Verniers Dominique
Printing: Bietlot at Gilly, Belgium

National Lottery
creates chances 

ISBN 978-2-87016-093-0



9 782870 160930