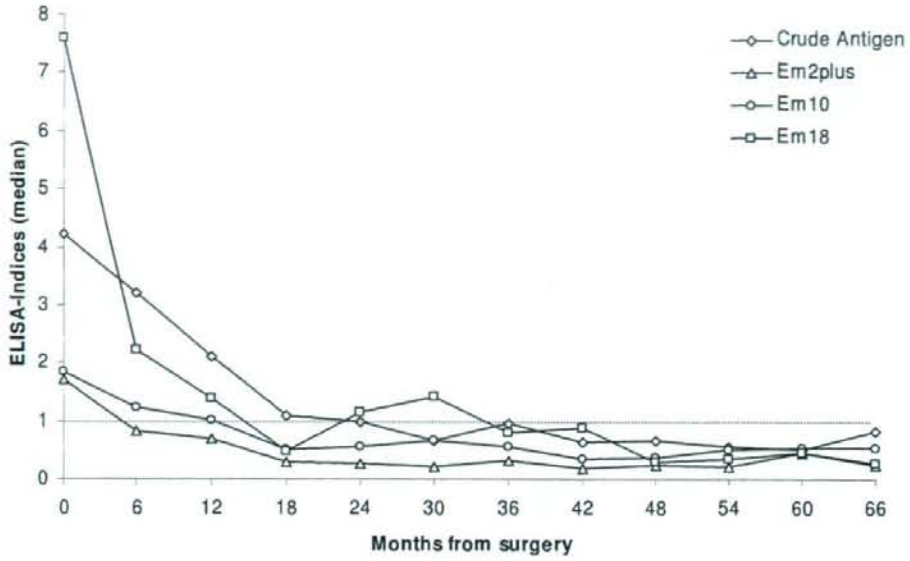
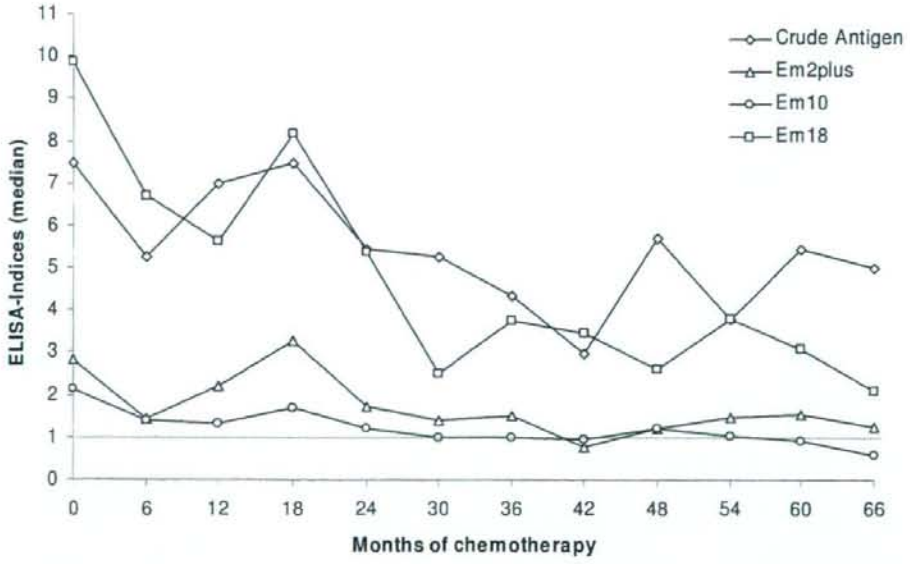
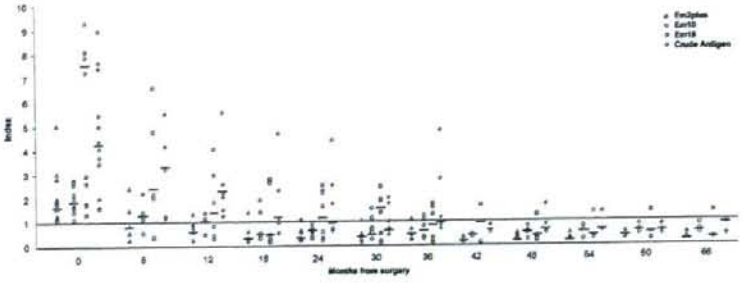
Figure 2

A

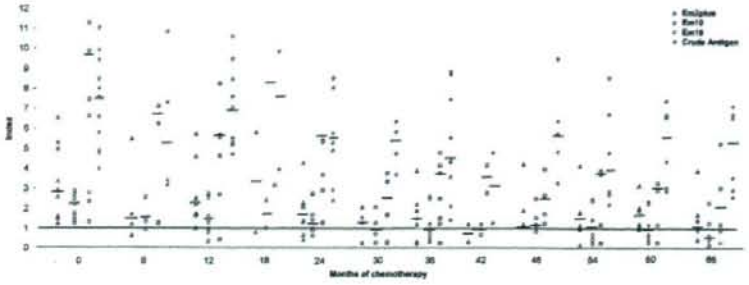

Crude Antigen
Em2plus
Em10
Em18
ELISA-Indices (median)
Months from surgery
0
1
2
3
4
5
6
7
8
0
6
12
18
24
30
36
42
48
54
60
66

B

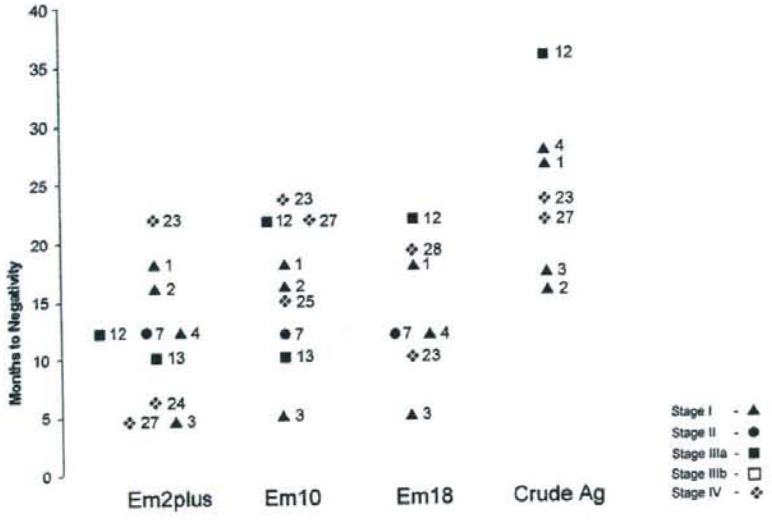

Crude Antigen
Em2plus
Em10
Em18
ELISA-Indices (median)
Months of chemotherapy
0
1
2
3
4
5
6
7
8
9
10
11
0
6
12
18
24
30
36
42
48
54
60
66



372x147mm (300 x 300 DPI)



368x143mm (300 x 300 DPI)



119x82mm (300 x 300 DPI)

Tables

Table 1. Characteristics of patients with alveolar echinococcosis included in the study

Patient Number	Stage	PNM* Code	Status*	Age**	Sex	Follow-up Duration
1	I	P1N0M0	curative resection	62 yrs	F	5.5 yrs
2	I	P1N0M0	curative resection	24 yrs	F	5 yrs
3	I	P1N0M0	curative resection	22 yrs	F	4 yrs
4	I	P1N0M0	curative resection	33 yrs	F	2 yrs
5	I	P1N0M0	apparently dead, fully calcified lesion	58 yrs	M	4 yrs
6	I	P1N0M0	unresectable, stable disease	61 yrs	M	3.5 yrs
7	II	P2N0M0	curative resection	38 yrs	F	3 yrs
8	II	P2N0M0	unresectable, stable disease	71 yrs	M	6 yrs
9	II	P2N0M0	unresectable,	68 yrs	F	5.5 yrs

			stable disease			
10	II	P2N0M0	unresectable, stable disease	59 yrs	F	6.5 yrs
11	II	P2N0M0	unresectable, stable disease	60 yrs	F	6 yrs
12	IIIa	P3N0M0	curative resection	25 yrs	F	4.5 yrs
13	IIIa	P3N0M0	curative resection	62 yrs	M	5.5 yrs
14	IIIa	P3N0M0	recurrence after resection	17 yrs	F	3.5 yrs
15	IIIa	P3N0M0	unresectable, stable disease	69 yrs	F	6 yrs
16	IIIa	P3N0M0	unresectable, stable disease	39 yrs	F	4 yrs
17	IIIa	P3N0M0	apparently dead, fully calcified lesion	57 yrs	F	6 yrs

2

18	IIIb	P3N1M0	progression after palliative resection	32 yrs	M	6 yrs
19	IIIb	P4N0M0	unresectable, stable disease	60 yrs	F	5 yrs
20	IIIb	P3N1M0	unresectable, stable disease	49 yrs	F	5.5 yrs
21	IIIb	P2N1M0	recurrence after resection	50 yrs	M	5 yrs
22	IIIb	P3N1M0	unresectable, stable disease	74 yrs	F	1.5 yrs
23	IV	P4N1M0	curative resection	56 yrs	F	6.5 yrs
24	IV	P4N1M0	curative resection	30 yrs	M	3 yrs
25	IV	P4N1M0	curative resection	72 yrs	F	3 yrs
26	IV	P4N1M0	unresectable, stable disease	71 yrs	F	5.5 yrs

27	IV	P4N1M0	curative resection	52 yrs	M	2.5 yrs
28	IV	P4N1M0	curative resection	63 yrs	F	1.5 yrs

*) as assessed by imaging (apparently dead lesion, progressive disease and stable disease) or imaging and histology (curative resection)

**) age at first blood sample drawn

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009, p. 252–254
0095-1137/09/$08.00+0 doi:10.1128/JCM.01476-08

Vol. 47, No. 1

Development of an Immunochromatographic Test To Detect Antibodies against Recombinant Em18 for Diagnosis of Alveolar Echinococcosis▽

Yasuhito Sako,[1]* Kenta Fukuda,[2] Yukuharu Kobayashi,[2] and Akira Ito[1]

Department of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa, 078-8510 Hokkaido, Japan,[1] and Division of Research and Development, Adtec Co., Ltd., Yokkaichi 1693-6, Usa, 879-0471 Oita, Japan[2]

Received 1 August 2008/Returned for modification 17 September 2008/Accepted 29 October 2008

An immunochromatographic test (ICT) for the rapid detection of antibodies to *Echinococcus multilocularis* was developed. The ICT showed a sensitivity of 94% and a specificity of 95.4%. High degrees of agreement were observed between the ICT and an enzyme-linked immunosorbent assay ($\kappa = 0.93$) and between the ICT and immunoblot analysis ($\kappa = 0.97$). It is expected that the ICT developed in this study will be useful for the serodiagnosis of alveolar echinococcosis.

Alveolar echinococcosis (AE), caused by the larval stage of *Echinococcus multilocularis*, is a serious parasitic disease of humans in countries of the higher latitudes of Northern Hemisphere. In the previous decade, a lot of new data have been published on prevalence of *E. multilocularis* in final and intermediate hosts in areas where it had previously not been recorded (5). Humans are accidentally infected with *E. multilocularis* by ingestion of eggs excreted with the feces of carnivores harboring adult tapeworm of this species. It is thought that humans become exposed to *E. multilocularis* by handling of infected definitive hosts or by ingestion of food contaminated with eggs. Oncospheres hatched from eggs in the small intestine of humans migrate via the portal system into various organs, mainly the liver, and differentiate and develop into the metacestode stage. The metacestodes propagate asexually like a tumor, leading to organ dysfunction. Since clinical symptoms usually do not become evident until 10 or more years after initial parasite infection, early diagnosis and treatment especially during asymptomatic period are important for reduction of morbidity and mortality (14). About a third of patients have cholestatic jaundice, and about a third of patients have epigastric pain. In the remaining patients, *E. multilocularis* infections are incidentally detected during medical examination for symptoms such as fatigue, weight loss, and hepatomegaly (15). At present, diagnosis of AE is primarily based on imaging techniques including echography, computed tomography, magnetic resonance imaging, and positron emission tomography with [^{18}F]fluoro-deoxyglucose (3). However, these imaging techniques are sometime limited by the small size of visualized lesions and atypical images, which are difficult to distinguish from abscesses or neoplasms. Moreover, these imaging techniques are unsuitable for diagnosis in isolated communities. Therefore, immunological tests have been considered important methods to confirm clinical findings, to give diagnostic help by providing information on the parasite in case of unclear images, or to survey in areas of endemicity where imaging techniques are not readily available (4, 9, 11). Previously, we have reported an enzyme-linked immunosorbent assay (ELISA) and an immunoblot analysis (IB) by using recombinant *E. multilocularis* 18-kDa antigen (Em18), the breakdown product of ezrin-radixin-moesin-like protein (2) that is also known as EM10 (8), EM II/3 (7), or EM4 (10) by the cysteine peptidase, and demonstrated that these two tests have a high potential for differentially diagnosing AE (1, 12, 16, 18). However, these two methods are time-consuming and require special materials and equipments, which make them not suitable for clinical applications. In contrast, an immunochromatographic test (ICT) is a simple, rapid, and reliable method for detection of specific antibodies to infectious agents. In the present study, we developed an ICT with rEm18 antigen for diagnosis of AE and compared ICT with ELISA and IB.

The rEm18 was expressed in a bacteria system as described previously (16) with some modifications. Briefly, a DNA fragment encoding the Em18 was amplified by PCR with the primers 5'-GGGAATTCAAGGAGTCTGACTTAGCGGA T-3' and 5'-TTGGATCCTAGGGCTTCACTTTCATCATCC TG-3'. The PCR products were digested with EcoRI and BamHI and cloned into bacterial expression vector pTWIN-1 (New England Biolabs, Beverly, MA) for producing a fusion protein with chitin binding domain/mini-inteins. The cloned plasmid was transfected into *Escherichia coli* ER2566 strain and expression of the recombinant protein was induced by the addition of 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) to the culture. The expressed rEm18 was purified by using a chitin column (New England Biolabs) according to the manufacturer's instructions. The purified rEm18 did not have the fusion partner, because rEm18 was released by intein activity of the fusion partner itself during purifications (6). The purified rEm18 (1 mg/ml) and anti-goat immunoglobulin G (IgG) antibody (1 mg/ml) were sprayed onto a nitrocellulose membrane in a 1-mm-wide line as test and control lines, respectively. The nitrocellulose membrane with rEm18 and anti-goat IgG antibody, absorbent pad, and substrate reservoir pad were assembled on a laminated membrane card, and the assembled sheet was cut into strips 5 mm in width. The strip was placed into a plastic assay device (Mitsubishi Chemical Medience, Tokyo, Japan) with windows for applying a serum sample and

* Corresponding author. Mailing address: Department of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1, Asahikawa, 078-8510 Hokkaido, Japan. Phone: 81-166-68-2422. Fax: 81-166-68-2429. E-mail: yasusako@asahikawa-med.ac.jp.

▽ Published ahead of print on 5 November 2008.

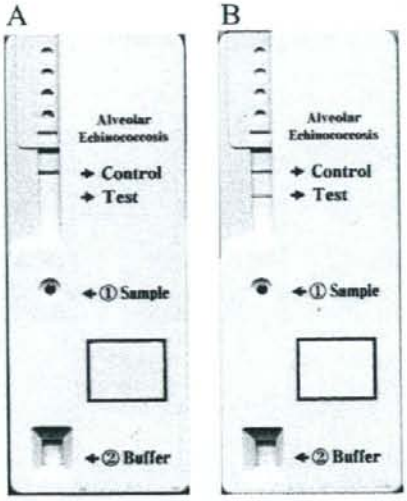


FIG. 1. Examples of ICT tests with negative and positive sera. (A) Result with a negative serum (one blue band at control line in the result window); (B) result with a positive serum (two blue bands at control and test lines in the result window). The inscriptions "Sample" and "Buffer" represents the positions for loading of sample and of substrate solution, respectively.

a substrate solution. For assay, first, 10 μl of serum sample was mixed 20 μl of a serum dilution buffer containing 0.1 mg of alkaline phosphatase-conjugated anti-human IgG antibody (Dako, Tokyo, Japan)/ml in a tube, and the mixed serum sample was applied into the sample window of the plastic device. Soon after application of the serum sample (within 30 s), 200 μl of the substrate solution was loaded onto the substrate reservoir pad, and the result was evaluated after 20 min. BCIP (5-bromo-4-chloro-3-indolylphosphate) was used for color development. As shown in Fig. 1, a sample was considered positive if two color lines corresponding to rEm18 and anti-goat IgG antibody appeared in the result window, and a sample was considered as negative if one color line corresponding to anti-goat IgG antibody appeared in the result window. In cases where there was no appearance of a colored anti-goat IgG antibody line, the assay was invalid even if a colored rEm18 line appeared. ELISA and IB were performed as described previously (16), except using the rEm18 prepared in the present study.

A total of 94 serum samples, including 50 serum samples from AE patients, 24 serum samples from cystic echinococcosis (CE) patients, and 20 serum samples from healthy persons, were examined by ICT, ELISA, and IB. Each diagnosis of AE and CE had been carried out by imaging techniques, clinical findings, histological observations (if feasible), and/or serology of IB with recombinant Em18 (16) or EmAgB8/1 (13). As shown in Table 1, 47 AE and 2 CE patient sera were determined to be positive by ICT, and none of sera from healthy persons showed positive reactions; thus, the sensitivity and specificity of ICT were 94.0 and 95.4%, respectively. There were no significant differences in sensitivity and specificity among ICT, ELISA, and IB ($P > 0.1$, Pearson chi-square test).

TABLE 1. Results of ICT, ELISA, and IB with sera from AE patients, CE patients, and healthy persons

Serum sample source	Total no. of samples examined	Samples examined by:					
		ICT		ELISA		IB	
		No. positive	%	No. positive	%	No. positive	%
AE patient	50	47	94.0	47	94.0	47	94.0
CE patient	24	2	8.3	1	4.2	3	12.5
Healthy subject	20	0	0	0	0	0	0

Two CE patient sera, determined to be positive by ELISA and/or IB, were also positive by ICT. This is not an incomprehensible result, because it is known that a few CE patient sera react to rEm18 even though rEm18 is highly specific antigen for AE (11, 12, 16, 18). These results indicated that the ICT is a sensitive and specific method for the diagnosis of *E. multilocularis* infection.

The results obtained by ICT were compared to those of previously established ELISA and IB with rEm18 (Table 2). All ELISA-positive samples, except one from AE patient, were ICT positive. Two ELISA-negative samples with the optical density values 0.068 and 0.079 close to the cutoff optical density value of 0.093 at 405 nm were ICT positive, and both were also positive by IB (data not shown). All IB-positive samples, except for one from a CE patient, were ICT positive, and none of the IB-negative samples was ICT positive. The degrees of agreement between ICT and ELISA and between ICT and IB were estimated by kappa analysis (17). A kappa statistics value of >0.75, 0.40 to 0.75, or <0.4 represents excellent agreement, good to fair agreement, and poor agreement, respectively. High degrees of agreement were observed between ICT and ELISA ($\kappa = 0.93$) and between ICT and IB ($\kappa = 0.97$), which indicated that ICT is reliable.

In conclusion, we developed a rapid, simple, sensitive, and specific ICT with rEm18 for detection of specific antibodies to *E. multilocularis* infection. Although ICT, ELISA and IB with rEm18 show similarities to each other with regard to both sensitivity and specificity, ICT has the following advantages: (i) expertise, experience, and special equipment are not required; (ii) 20-min incubation is enough to detect specific antibodies; and (iii) it is more economical than ELISA and IB. These advantages suggest a high diagnostic potential for the ICT in clinical practice in providing immediate and proper treatments and in mass-screening programs in areas of endemicity as a

TABLE 2. Comparison of ICT with ELISA and IB[a]

Samples examined by ICT	Samples examined by:					
	ELISA			IB		
	No. positive	No. negative	Total	No. positive	No. negative	Total
Positive	47	2	49	49	0	49
Negative	1	44	45	1	44	45
Total	48	46	94	50	44	94

[a] Results with a total 94 sera shown in Table 1 were used for comparisons.

primary screening tool. Further analysis on stability of ICT and a large-scale evaluation might be necessary.

This study was financially supported in part by Hokkaido Translational Research Project from the Ministry of Education of Japan on the development of the rapid test for echinococcosis (from 2007 onward) to A.I.

REFERENCES

1. **Bart, J. M., M. Piarroux, Y. Sako, F. Grenouillet, S. Bresson-Hadni, R. Piarroux, and A. Ito.** 2007. Comparison of several commercial serologic kits and Em18 serology for detection of human alveolar echinococcosis. Diagn. Microbiol. Infect. Dis. **59**:93–95.
2. **Brehm, K., K. Jensen, P. Frosch, and M. Frosch.** 1999. Characterization of the genomic locus expressing the ERM-like protein of *Echinococcus multilocularis*. Mol. Biochem. Parasitol. **100**:147–152.
3. **Bresson-Hadni, S., E. Delabrousse, O. Blagosklonov, B. Bartholomot, S. Koch, J. P. Miguet, G. A. Mantion, and D. A. Vuitton.** 2006. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. Parasitol. Int. **55**(Suppl.):S267–S272.
4. **Carmena, D., A. Benito, and E. Eraso.** 2007. The immunodiagnosis of *Echinococcus multilocularis* infection. Clin. Microbiol. Infect. **13**:460–475.
5. **Eckert, J., F. J. Conraths, and K. Tackmann.** 2000. Echinococcosis: an emerging or re-emerging zoonosis? Int. J. Parasitol. **30**:1283–1294.
6. **Evans, T. C., Jr., J. Benner, and M. Q. Xu.** 1999. The *in vitro* ligation of bacterially expressed proteins using an intein from *Methanobacterium thermoautotrophicum*. J. Biol. Chem. **274**:3923–3926.
7. **Felleisen, R., and B. Gottstein.** 1994. Comparative analysis of full-length antigen II/3 from *Echinococcus multilocularis* and *E. granulosus*. Parasitology **109**:223–232.
8. **Frosch, P. M., M. Frosch, T. Pfister, V. Schaad, and D. Bitter-Suermann.** 1991. Cloning and characterisation of an immunodominant major surface antigen of *Echinococcus multilocularis*. Mol. Biochem. Parasitol. **48**:121–130.
9. **Gottstein, B.** 1992. Molecular and immunological diagnosis of echinococcosis. Clin. Microbiol. Rev. **5**:248–261.
10. **Hemmings, L., and D. P. McManus.** 1991. The diagnostic value and molecular characterisation of an *Echinococcus multilocularis* antigen gene clone. Mol. Biochem. Parasitol. **44**:53–61.
11. **Ito, A., M. Nakao, and Y. Sako.** 2007. Echinococcosis: serological detection of patients and molecular identification of parasites. Future Microbiol. **2**:439–449.
12. **Ito, A., N. Xiao, M. Liance, M. O. Sato, Y. Sako, W. Mamuti, Y. Ishikawa, M. Nakao, H. Yamasaki, K. Nakaya, K. Bardonnet, S. Bresson-Hadni, and D. A. Vuitton.** 2002. Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for differential diagnosis of alveolar echinococcosis: results of a blind test. J. Clin. Microbiol. **40**:4161–4165.
13. **Mamuti, W., H. Yamasaki, Y. Sako, M. Nakao, N. Xiao, K. Nakaya, N. Sato, D. A. Vuitton, R. Piarroux, M. W. Lightowlers, P. S. Craig, and A. Ito.** 2004. Molecular cloning, expression, and serological evaluation of an 8-kilodalton subunit of antigen B from *Echinococcus multilocularis*. J. Clin. Microbiol. **42**:1082–1088.
14. **McManus, D. P., W. Zhang, J. Li, and P. B. Bartley.** 2003. Echinococcosis. Lancet **362**:1295–1304.
15. **Pawlowski, Z. S., J. Eckert, D. A. Vuitton, R. W. Ammann, P. Kern, P. S. Craig, K. F. Far, F. De Rosa, C. Filice, B. Gottstein, F. Grimm, C. N. L. Macpherson, N. Sato, T. Todorov, J. Uchino, W. von Sinner, and H. Wen.** 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment, p. 20–71. *In* J. Eckert, M. A. Gemmell, F.-X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health, Paris, France.
16. **Sako, Y., M. Nakao, K. Nakaya, H. Yamasaki, B. Gottstein, M. W. Lightowlers, P. M. Schantz, and A. Ito.** 2002. Alveolar echinococcosis: characterization of diagnostic antigen Em18 and serological evaluation of recombinant Em18. J. Clin. Microbiol. **40**:2760–2765.
17. **Thomas, E. E., M. L. Puterman, E. Kawano, and M. Curran.** 1988. Evaluation of seven immunoassays for detection of rotavirus in pediatric stool samples. J. Clin. Microbiol. **26**:1189–1193.
18. **Xiao, N., W. Mamuti, H. Yamasaki, Y. Sako, M. Nakao, K. Nakaya, B. Gottstein, P. M. Schantz, M. W. Lightowlers, P. S. Craig, and A. Ito.** 2003. Evaluation of use of recombinant Em18 and affinity-purified Em18 for serological differentiation of alveolar echinococcosis from cystic echinococcosis and other parasitic infections. J. Clin. Microbiol. **41**:3351–3353.

2. Copley CG, Egglestone SI. Auxotyping of *Neisseria gonorrhoeae* isolated in the United Kingdom. J Med Microbiol. 1983;16:295–302.
3. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 1979;7:1513–23. DOI: 10.1093/nar/7.6.1513
4. Papadimos TJ, Escamilla J, Batchelor RA, Lane EW, Biddle JW. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from a military population in San Diego. Sex Transm Dis. 1988;15:196–9.
5. Edwards U, Rogall T, Böcker H, Emde M, Böttger E. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal DNA. Nucleic Acids Res. 1989;17:7843–53. DOI: 10.1093/nar/17.19.7843
6. Henderson G, Ritchie WT. Gonococcal meningitis. Neurol Psychiatr. 1909;7:57–87.
7. Smith D. Gonococcal meningitis. Lancet. 1922;1:1217. DOI: 10.1016/S0140-6736(00)55116-2
8. Burgis JT, Nawaz H III. Disseminated gonococcal infection in pregnancy presenting as meningitis and dermatitis. Obstet Gynecol. 2006;108:798–801.
9. Ross JDC. Systemic gonococcal infection. Genitourin Med. 1996;72:404–7.
10. Knapp JS, Holmes KK. Disseminated gonococcal infection caused by *Neisseria gonorrhoeae* with unique nutritional requirements. J Infect Dis. 1975;132:204–8.

Address for correspondence: Fernando Vázquez, Hospital Monte Naranco, Avda, Dres, Fernandez Vega 9, 33012 Oviedo, Spain; email: fvazquez@uniovi.es

Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Echinococcoses and Tibetan Communities

To the Editor: The People's Republic of China accounts for >500,000 cases of echinococcosis and more disability-associated life years (DALYs) lost because of this disease than any other world region (*1*,*2*). Hydatid cysts of *Echinococcus granulosus* (cystic echinococcosis [CE]), or the more pathogenic lesions with multiple vesicles caused by *E. multilocularis* infection (alveolar echinococcosis [AE]), usually grow slowly in the liver, so that severe illness and death may eventually occur in a high proportion of those with untreated infections (*3*,*4*). Apart from surgery, long-term anthelminthic therapy (>6 months) with the benzimidazole compound albendazole, although parasitostatic only, has a beneficial outcome in >50% of cases (*5*). To control the transmission of this zoonosis, veterinary public health measures must be emphasized (*6*).

In 2004 the Chinese Ministry of Health (MoH) undertook a nationwide assessment of 8 parasitic diseases, including malaria, schistosomiasis, and echinococcosis. To identify echinococcosis, 7 provincial MoHs carried out a mass abdominal screening of 34,500 persons using portable ultrasound scanners. The overall prevalence (2.5%) was highest in Tibetan communities in the Tibet Autonomous Region and in northwestern Sichuan and Qinghai Provinces (these latter regions form part of the eastern Tibetan Plateau). Collaborative studies involving the Sichuan Center for Disease Control and Prevention (based in Chengdu) and an international consortium of research institutes partly funded by the US National Institutes of Health (Bethesda, MD, USA) have shown an increasingly serious public health problem at the village, township, and county levels. In Shiqu County of Ganze Tibetan Autonomous Prefecture, 414 (12.9%) of nearly 3,199 persons surveyed by ultrasound (with serologic confirmation) exhibited CE or AE, including 19% in this category (*7*). The effects of human echinococcosis are substantial, with >50,000 DALYs lost in a population of 63,000 in Shiqu County (*8*).

Despite increased urbanization in China, >70% of Tibetans still live as seminomadic pastoralists on the high grasslands at an altitude >3,500 m. Most Tibetan herdsmen families keep at least 1 dog, and large numbers of ownerless stray dogs are tolerated by pastoralists and Buddhist monks. Risk factors for human echinococcosis (both CE and AE) in Tibetan communities usually include occupation, age (older persons are at higher risk), gender (higher risk for female), environment (pastoral landscapes), livestock ownership, and a history of dog ownership, as well as indicators of low socioeconomic status, including poor water quality and illiteracy (*7*,*9*) The prevalence levels of human AE in Ganze Tibetan Autonomous Prefecture (Sichuan Province) are among the highest recorded anywhere in the world. This situation presents a formidable challenge for early diagnosis, optimal affordable treatment, and prevention and control. Markham Hospital in Aba Tibetan Autonomous Prefecture (Sichuan) performed 1,200 operations for echinococcosis from 1992 through 2005, 20% for AE disease. For remote, high-altitude, pastoral Tibetan communities, however, long-term albendazole therapy is the only realistic treatment option, but regular follow-up of patients is difficult in these poorly accessible communities.

To address the public health concerns and consider options for controlling hydatidosis/echinococcosis in the eastern Tibetan Plateau, an International Workshop on Treatment, Prevention and Control of Echinococcosis was held in Chengdu in May 2006

with support from the Sichuan Center for Disease Control and Prevention, MoH Beijing, the New Zealand International Aid and Development Agency [NZIAD]), Fogarty-National Institutes of Health (USA), Xinjiang Medical University, and the Boulder-Lhasa Sister City Project. Recommendations stressed the following public health needs: improved treatment centers within the known disease-endemic counties or prefectures for long-term follow-up of patients after surgery and chemotherapy for both CE and AE disease, a better understanding of the epidemiology and ecology of transmission, and planning for pilot control interventions against both CE and AE transmission. NZAID made a detailed report of the implementation and effects of a pilot echinococcosis control program (2000–2006) in Datangma County, Ganze Tibetan Autonomous Prefecture (Sichuan Province). Problems occurred chiefly because of poor intersectoral cooperation, difficult logistics, cultural antagonism, lack of participatory planning, difficult access, treatment of dogs (with praziquantel), vaccination of livestock with the new EG95 vaccine (*6*), and lack of adequate surveillance of dog and livestock infection levels. The report indicated how many of these difficulties could be overcome. Consequently, the People's Republic of China MoH and provincial disease control networks approved funding in July 2006 to initiate pilot intervention programs against echinococcosis in 17 Tibetan autonomous counties of northwest Sichuan. Control options initially focused on regular supervised dosing of owned dogs and stray dogs (with praziquantel) by local operatives from district disease control centers and on improving health education at primary healthcare levels. Surveillance relies on measuring regularly the degree of *Echinococcus* infection in dogs by using a coproantigen test. Also, a specific age cohort of schoolchildren is monitored by ultrasound and serologic testing each year to determine changes in the prevalence of the 2 diseases. Albendazole is provided free, and the cost of surgery for hydatid disease is also subsidized through the new National Rural Cooperative Medical Insurance System.

In addition to the major public health problem now being recognized for echinococcosis in Tibetan communities, their general health indices are low (higher prevalence of tuberculosis, bone diseases such as arthritis, and poorer health in general) because of living and working at altitudes >4,000 m, compared with those in most other areas of China. Access and outreach should be improved (in conjunction with animal health initiatives) (*10*) for effective delivery of treatment, vaccination, and health education packages to these largely scattered and marginalized pastoral communities.

Philip S. Craig, Tiaoying Li, Jiamin Qiu, Ren Zhen, Qian Wang, Patrick Giraudoux, Akira Ito, David Heath, Bill Warnock, Peter Schantz, and Wen Yang

Author affiliations: University of Salford, Salford, UK (P.S. Craig); Sichuan Center for Disease Control and Prevention, Chengdu, People's Republic of China (T. Li, J. Qui, Q. Wang, W. Yang); Aba Army Hospital, Markang, People's Republic of China (R. Zhen); Universite de Franche-Comte, Besancon, France (P. Girardoux); Asahikawa Medical College, Asahikawa, Japan (A. Ito); Agresearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand (D. Heath); Boulder-Lhasa Sister City Project, Boulder, Colorado, USA (B. Warnock); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (P. Schantz)

DOI: 10.3201/eid1410.071636

References

1. Ito A, Urbani C, Qui J, Vuitton DA, Dongchuan Q, Heath DD, et al. Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. Acta Trop. 2003;86:3–17. DOI: 10.1016/S0001-706X(02)00269-3
2. Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. Emerg Infect Dis. 2006;12:296–303.
3. McManus DP, Zhang W, Li J, Bartley PB. Echinococcosis. Lancet. 2003;362: 1295–304. DOI: 10.1016/S0140-6736(03) 14573-4
4. Craig P. *Echinococcus multilocularis*. Curr Opin Infect Dis. 2003;16:437–44. DOI: 10.1097/00001432-200310000-00010
5. Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. World Health Organization/World Organisation for Animal Health manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health; 2001.
6. Craig PS, McManus DP, Lightowlers MW, Chabalgoity JA, Garcia HH, Gavidia CM, et al. Prevention and control of cystic echinococcosis. Lancet Infect Dis. 2007;7:385–94. DOI: 10.1016/S1473-3099(07)70134-2
7. Li T, Qiu J, Yang W, Craig PS, Chen X, Xiao N, et al. Echinococcosis in Tibetan populations, western Sichuan Province, China. Emerg Infect Dis. 2005;11:1866–73.
8. Budke CM, Qiu J, Zinsstag J, Qian W, Torgerson PR. Use of disability adjusted life years in the estimation of the disease burden of echinococcosis for a high endemic region of the Tibetan Plateau. Am J Trop Med Hyg. 2004;71:56–64.
9. Wang Q, Qiu J, Yang W, Schantz PM, Raoul F, Craig PS, et al. Socioeconomic and behavior risk factors of human alveolar echinococcosis in Tibetan communities in Sichuan, People's Republic of China. Am J Trop Med Hyg. 2006;74:856–62.
10. Zinsstag J, Ould Taleb M, Craig PS. Health of nomadic pastoralists: new approaches towards equity effectiveness [editorial]. Trop Med Int Health. 2006;11:565–8. DOI: 10.1111/j.1365-3156.2006.01615.x

Address for correspondence: Philip S. Craig, Biomedical Sciences Research Institute, School of Environment and Life Sciences, University of Salford, Greater Manchester M54 WT, UK; email: p.s.craig@salford.ac.uk

Mammalia 72 (2008): 309–319 © 2008 by Walter de Gruyter • Berlin • New York. DOI 10.1515/MAMM.2008.048

Small mammal assemblages and habitat distribution in the northern Junggar Basin, Xinjiang, China: a pilot survey

Patrick Giraudoux[1,*], Hongxia Zhou[2,*], Jean-Pierre Quéré[3], Francis Raoul[1], Pierre Delattre[3], Vitaly Volobouev[4], Thomas Déforêt[1], Akira Ito[5], Wulamu Mamuti[6], Renaud Scheifler[1] and Philip Simon Craig[7]

[1] Department of Chrono-environment UMR CNRS 6249 usc INRA, University of Franche-Comté, 1 Place Leclerc, 25030 Besancon cedex, France, e-mail: patrick.giraudoux@univ-fcomte.fr
[2] Department of Epidemiology, Public Health School, Guangxi Medical University, Nanning, Guanxi, China, e-mail: juliazhou86@yahoo.com
[3] CBGP-UMR 1062 INRA, Campus International du Baillarguet, CS 30016 34988 Montferrier sur Lez, France
[4] National Museum of Natural History, 16 rue Buffon, 75005, Paris, France
[5] Department of Parasitology, Asahikawa Medical College, Asahikawa, Japan
[6] Department of Parasitology, Xinjiang Medical University, 83000 Urumqi, Xinjiang, China
[7] Biomedical Sciences Research Institute, School of Environment and Life Sciences, University of Salford, Manchester M5 4WT, UK

*Corresponding authors

Abstract

Small mammal assemblages were surveyed in five areas of the northern Junggar basin, Xinjiang, China, using standard trapping methods and index transects. In total, 23 species were recorded. The relationships between habitats and the distribution of the main species were described at local scale in Baihaba, Altai mountains. Three types of assemblages linked to (i) forest (*Myodes rufocanus*, *Microtus agrestis*, *Myodes rutilus*, *Apodemus peninsulae*, *Sorex isodon*), (ii) transitional areas and farmland (mixed pool of species including *Apodemus uralensis*), and (iii) grassland (*Ellobius tancrei*, *Microtus obscurus*, *Cricetulus migratorius*) were identified. Additional species, such as *Microtus oeconomus*, could be found along small streams. At a broader scale, species composition was estimated in Kokehada, Narenhebuke and Fuhai areas and compared. Although ecological gradients from mountain grasslands to cold semi-desert partly explain differences in assemblage composition on a regional scale, similar habitats at the same altitude may, however, harbour different assemblages. For example, *Spermophilus erythrogenys* was the dominant species of the Kokehada grassland and *Microtus obscurus* and *Ellobius tancrei* were dominant in Baihaba grassland. In conclusion, the need for multi-scale standard descriptions of small mammal assemblage distribution in the northern Junggar basin of China is indicated.

Keywords: community; grassland; mountain; scale; semi-desert.

Introduction

Small mammals exist in virtually every terrestrial habitat. They are generally the main food resource for predator communities (carnivores, birds of prey, etc.), and some species can be an agricultural pest. They can also be reservoir hosts for pathogen agents impacting public health and thus may play a critical role in disease transmission (Gratz 1994, Giraudoux et al. 2006). If the presence of species over large continental areas may be considered as the result of biogeography and evolution, population dynamics and distribution patterns at finer time-space resolution are generally considered as a response to ecological factors, including the spatial arrangement of optimal habitats in a landscape and the result of individual dispersion and community processes, such as predation (Hansson and Henttonen 1988, Bjornstad et al. 1999, Lidicker 2000, Huitu et al. 2004).

A minimum of 170 species of small mammals has been recorded in continental China. Atlases of distribution, species lists and identification keys generally document the distribution of small mammals at a continental or regional scale (Wang and Yang 1983, Ma et al. 1987, Lu and Yan 1989, Zhu and Chen 1993, Zhang 1997, Shenbrot and Krasnov 2005), but species distribution at a more local scale has been sparsely documented (e.g., sub-regional and habitat range). Serious economic losses and public health problems have been reported to be associated with small mammals in China since the 1950s (Zhao 1996). Rodent outbreaks were frequently recorded in deforestation areas of north-eastern China at the end of the 1950s (Xia 1996) and involved the genus *Myodes* (formerly *Clethrionomys*). Indication of population outbreaks as a consequence of anthropogenic modification of habitats was also found for *Microtus limnophilus* Büchner and *Cricetulus longicaudatus* Milne-Edwards in deforestation areas of south Gansu, for *Lasiopodomys brandtii* Radde in the Inner Mongolia Autonomous Region, for *Mus musculus* and *Microtus obscurus* in the extensive farmland areas of Xinjiang Uygur Autonomous Region and for *Microtus obscurus* and *Spermophilus erythrogenys* in the pastures of northern Xinjiang (Wang and Yang 1983, Zhu and Chen 1993, Giraudoux et al. 1998).

More than 85 species of small mammals have been reported in Xinjiang Uygur Autonomous Region, the largest (1.7 million km²) administrative region of China (Wang and Yang 1983), with contrasted natural conditions rang-

ing from deserts to alpine ecosystems. Investigations of fauna and flora have been conducted for years and mostly aimed to establish species lists and identification criteria (Ma 1981, Wang and Yang 1983, Ma et al. 1987, Halik et al. 1999). However, quantification of small mammal distribution amongst habitat patches on a local range (some square kilometres), and turnover of assemblages between ecosystems or regions over larger range (some ten thousands of square kilometres) are poorly documented for China.

The northern Junggar basin lies between the Tarbatagai mountains to the northwest and the Altai mountains to the northeast. It is a remote, poorly accessible area with low human population density, and the paucity of small mammal studies reported from this region reflects difficulties of working in this isolated region. In the current study, we hypothesise that standard sampling methods in relation to land cover and habitat patches can provide a rapid assessment of small mammal communities in such a remote area. The objective was to provide a first assessment of small mammal communities and to determine at which scales further studies of small mammal communities should be undertaken in northern Xinjiang.

Study sites and methods

The results presented here have been obtained during the course of a research programme on the epidemiology and transmission ecology of the zoonotic cestode *Echinococcus multilocularis* Leuckart. Thus, field selection and the duration of each stay were to a degree preselected, including field accessibility, time available and logistics for ecological and medical teams (Wang et al. 2001). Three main geographical areas were monitored: (1) Baihaba area in northern Altai; (2) Kokehada and Narenhebuke area in the Tarbatagai mountains; and (3) Fuhai area in the northern Junggar basin (Figure 1).

Baihaba area

Baihaba village (86.78 E, 48.69 N) is located 30 km from the Kanas National Nature Reserve (87.07 E, 49.26 N) close to the Kazakstan border. In the Kanas National Nature Reserve meteorological station, the mean annual precipitation is 1000 mm, mean temperature is -16°C in January and 15.9°C in July (Halik et al. 1999). The altitude of the study area around the village ranges between 1210 and 1780 m above sea level and the area is typical of the middle alpine belt of the Xinjiang Altai mountains. Six types of habitats were identified: (1) dense forest dominated by *Larix sibirica*, *Picea obovata*, *Pinus sibirica*, *Betula pendula* and *Populus tremula*; (2) open forest where the dominant species were the same but with grass cover on the ground and undergrowth of *Spiraea chamaedryfolia*, *Lonicera altaica*, *L. hispida*, *Rosa alberti*, *Ribes* sp., *Cotoneaster* sp., *Spiraea hypericifolia*, *Alchemilla vulgaris*, *Salix* sp., *Caragana jubata*; (3) shrubland

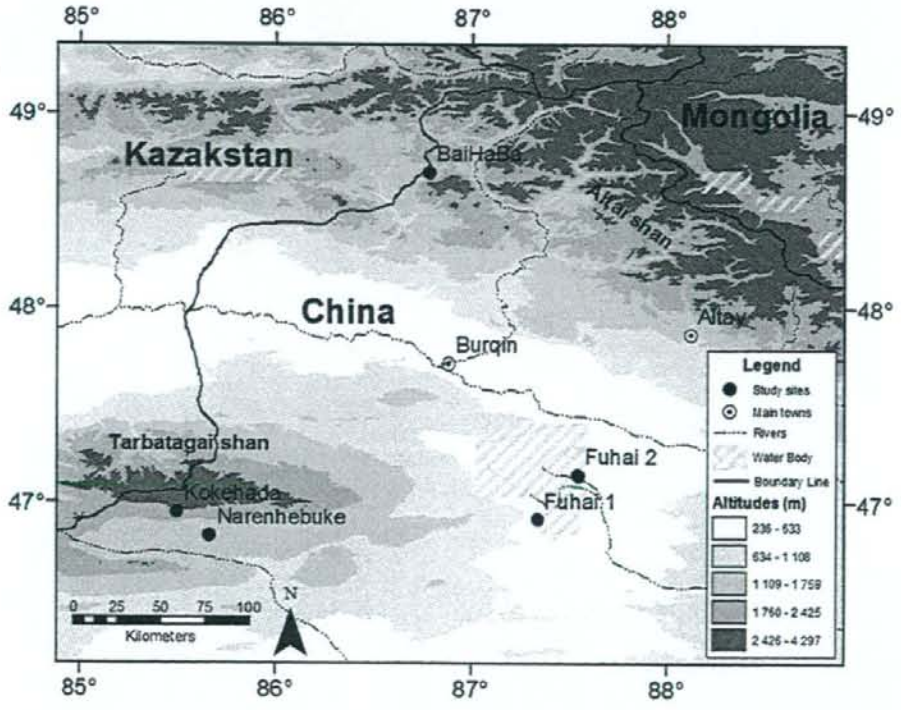


Figure 1 Northeast Xinjiang (China): location of the study sites (digital elevation model 1 km from the US National Oceanic and Atmospheric Administration, altitude classes computed by the Jenks' method, water bodies from ESRI World Basemap Data).

with dominant species *Rosa alberti*, *Spiraea hypericifolia*, *Hamamelis mollis oliver* and large grass cover, including *Alchemilla vulgaris*; (4) grassland, mainly extensive herbaceous pastures with *Deschampsia caespitosa*, *Carex pediformis*, *Dactylis glomerata*; (5) village garden, mainly potatoes and green Chinese onion bordered with grass strips and woodlots; and (6) farmland, mosaic of fields and *Carex* grass cropped for hay.

Small mammals were sampled in September 1998 with INRA (Institut National de Recherche Agronomique) live traps (5×5×15 cm) (Aubry 1950), small break-back traps (SBBT, snapping bar 4.5×4.5 cm) and big break-back traps (BBBT, snapping bar 9×12 cm). Traps were baited with dough consisting of a mixture of flour, peanut butter and water. Additionally, a small number of tongue-traps and Sherman traps (10×10×25 cm) were used for trapping strictly subterranean species and to link species to surface indices (e.g., runways, earth hills, faeces, etc.).

A total of 57 trap lines were set up. Each line consisted of 25 traps of the same type, 3 m distant from each other (Giraudoux et al. 1998, Raoul et al. 2006). Traps were checked every morning, re-baited and reset, for 3 consecutive nights. Such lines were considered "standard" lines. Standard lines were paired, i.e., each INRA line corresponded to a SBBT line in the same habitat. Traps were occasionally stolen during the field survey; others were set for only 2 nights for logistical reasons. Those lines were not used for statistical analysis.

Small mammals were humanely killed, weighed and dissected for determination of sex and reproductive status, and parasitological examination. Heads (or the whole body for some specimens of every species) were preserved in 5% formalin solution. Skull and skin were prepared and identified at the Centre de Biologie et Gestion des populations, Montferrier (France), using the following references: Allen (1940), Corbet (1978), Hoffmann (1987), Gromov and Erbajeva (1995) and Musser et al. (1996). Tissue samples of the genus *Microtus* and *Apodemus* were preserved for later karyotyping and DNA cytochrome *b* sequencing at the Museum National d'Histoire Naturelle of Paris and at the Centre de Biologie et Gestion des populations, Montferrier. Nomenclature followed Wilson and Reeder (2005), except for the genus *Arvicola* (Wust-Saucy 1998) and the complex *Microtus arvalis/obscurus* where preliminary results of karyotype and DNA analysis indicate that *M. obscurus* can be distinguished (Tougard et al. in preparation).

The relative density of small mammals is expressed as the number of animals caught per trap×night number (further termed trap nights). Landscape transects were performed to establish a general linkage between open habitats and small mammal indices over larger areas. Along each transect, the presence-absence of rodent indices (burrow, corridor, faeces, earth tumulus, etc.) and the habitat types were noted within every 10 paces (approximately 8–9 m) intervals. Indices have all been confirmed by non-standard trapping. *Ellobius tancrei* leaves a conspicuous fine grain earth tumulus (10–30 cm diameter, 5–10 cm high), sometimes with a middle or side hole from which a breach is maintained on one side of the hill. Those tumuli are quite different from those of *Arvicola terrestris* and *Talpa* sp. (see Giraudoux et al. 1995 for full description). *Microtus obscurus* generally does not build earth tumuli and leaves long ramified runways in grassland, on which characteristic dark olive shape faeces of 3 mm length can be found. However, these indices are not specific (e.g., other *Microtus* and the genus *Cricetulus* have quite similar droppings), thus we grouped them under the term "*Microtus* size species". A small mammal density index was defined as the ratio of intervals where one or more indices were recorded to the total number of intervals sampled (Giraudoux et al. 1995, Quéré et al. 2000).

Kokehada and Narenhebuke area

Kokehada (85.5 E, 46.95 N) is a 'summer pasture' used by Mongol herdsmen for sheep and cattle grazing (Wang et al. 2001). The altitude ranges from 1700 to 2500 m above sea level. The area consists of Alpine overgrazed grassland and grassy steppe progressively blending into a semi-desert at lower altitude. The closest forest is more than 200 km distant. Vegetation consists of *Carex* sp., *Cobresia* sp., with crawling scrub bushes of *Juniperus* sp. and *Sabina* sp. Large grassy banks of Alpine cushions were present in the valley bottoms along streams. Small mammals were sampled in July 1996 in alpine grassland (trap line types: 2 INRA, 2 SBBT, 1 BBBT for 3 nights), bottom valley stream banks (2 SBBT, 1 INRA for 1 night) and in a steppe area (1 BBBT for 1 night).

The surroundings of Narenhebuke village (85.67 E, 46.83 N; altitude 1290 m) are used as 'winter pastures' by Mongol herdsmen and consist of a typical semi-desert steppe. Trap lines were set up in May 1995: (1) in a steppe dominated by *Caragana soongorica*, *Artemisia* sp., *Stipa gobica* and *Festuca* sp. (2 BBBT, 1 night), and (2) in the alpine cushions of grassy river banks surrounding the village (1 SBBT, 2 nights).

Additionally, *Spermophilus erythrogenys* were caught with cage traps (CT, grid cages of 25×10×10 cm) in Kokehada pasture.

Landscape transects were performed to establish a general linkage between habitats and small mammal indices over large areas (see description above).

Fuhai area

Fuhai County is located near Fuhai Lake in the western Junggar Basin. Annual rainfall ranges between 100 and 150 mm (Lu and Yan 1989). Two sampling sites were selected: (1) site 1 (87.34 E, 46.92 N; altitude 470 m) was situated in a semi-desert at the southern edge of Fuhai Lake. Sparse bushes of *Ammoniptanthus nanus*, *Anabasis salsa* and *Caragana* sp. were dominant in this area. (2) Site 2 (87.55 E, 47.14 N; altitude 480 m) was situated at 30 km to the north of Fuhai town, in a cultivated oasis. The dominant plant species there included *Achnatherrum splendens*, *Artemisia* sp. and *Calligonum* sp. The species *Blaeagnus oxycarpa* was also present at lower density.

Three lines of BBBT and three lines of SBBT were set in both sites for 1 night in September 1998.

Statistics

Considering sample sizes, robust non-parametric statistics were used: the Wilcoxon and Mann-Whitney U-test

or Kruskal-Wallis test for k>2 comparisons followed by a multiple comparison according to the Siegel and Castellan (1988) method when indicated. The Friedman test was used for longitudinal data (e.g., repeated measurements over time), and the χ^2-test was used for contingency tables. To represent the main relationships between species and habitats, a factorial correspondence analysis was computed on the trap lines×species table where indicated. Computations were carried out using R 2.5.1 (R Development Core Team 2007), the packages ade4 1.4-3 (Chessel et al. 2004) and pgirmess 1.3.3 (Giraudoux 2007).

Results

Baihaba

A total of 282 small mammals representing 14 taxa were trapped (3735 trap nights) (Table 1). *Microtus oeconomus* identifications were confirmed by karyotyping, *Microtus obscurus* by karyotyping and DNA sequencing, and *Apodemus peninsulae* by DNA sequencing.

Trapping efficiency

Comparison of trapping efficiency was achieved for INRA and SBBT traps only, due to small sample sizes for other types of trap lines and because no animals were caught using the BBBT traps. The average number of animals captured was 5.8 animals per 100 trap nights for INRA traps, and 6.8 for SBBT traps. Differences were not significant (Mann-Whitney U-test, p=0.93). Moreover, small mammal species did not show differential response to trap types. In all habitats except farmland, there was a significant decrease in the number of captures per 100 trap nights according to the number of trapping nights (Friedman test, p=0.04) (Figure 2). This means that the sampling effort had a significant effect on local population density of small mammals. Figure 3 shows that the cumulative number of species trapped reached an asymptote after the second night in all habitats. Thus, the trapping effort was probably enough to sample most of the trappable species in each habitat. These results suggest that 3 nights trapping was sufficient for relevant comparisons amongst habitats.

Table 1 Species and number of animals (n) trapped in Baihaba.

Code	Name	n
Appe	*Apodemus peninsulae* Thomas	16
Apsp	*Apodemus* species	1
Apur	*Apodemus uralensis* Thomas	48
Myrf	*Myodes rufocanus* Sundevall	12
Myru	*Myodes rutilus* Pallas	67
Crmi	*Cricetulus migratorius* Pallas	16
Elta	*Ellobius tancrei* Blasius	9
Miag	*Microtus agrestis* Linnaeus	7
Miob	*Microtus obscurus* Eversmann	82
Mioe	*Microtus oeconomus* Pallas	8
Sina	*Sicista napea* Hollister	3
Sois	*Sorex isodon* Turov	3
Sosp	*Sorex* species	5
Sotu	*Sorex tundrensis* Merriam	2

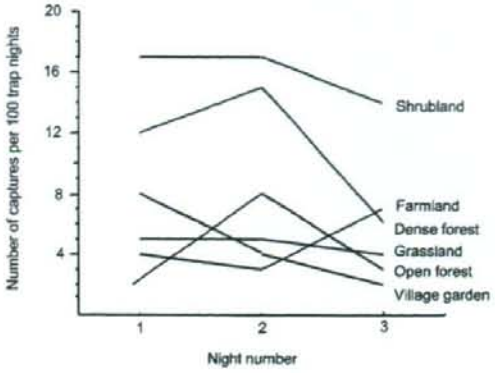


Figure 2 Evaluation of the trapping effort on trapping success in the six habitats of the Baihaba area.

Standard sampling

Population relative densities of *Apodemus peninsulae*, *Apodemus uralensis* and *Myodes rutilus* were higher in forests, and shrubland for the two species of *Apodemus*, with intrusions in village gardens, and no captures occurred in grassland (Table 2). *Microtus agrestis* and *Sorex isodon* were found in forest exclusively. By contrast, *Cricetulus migratorius* relative densities were higher in grassland, farmland and shrubs, and *Microtus obscurus* was found in all habitats except dense forest (Table 2). The trapping of the eight *Microtus oeconomus* in open forest and garden was actually linked to the vicinity of small streams. A correspondence analysis was carried out with the species having shown differences in relative densities between habitats, except *M. oeconomus* whose distribution was linked to streams. A factorial map illustrates how species and habitats were ordered (Figure

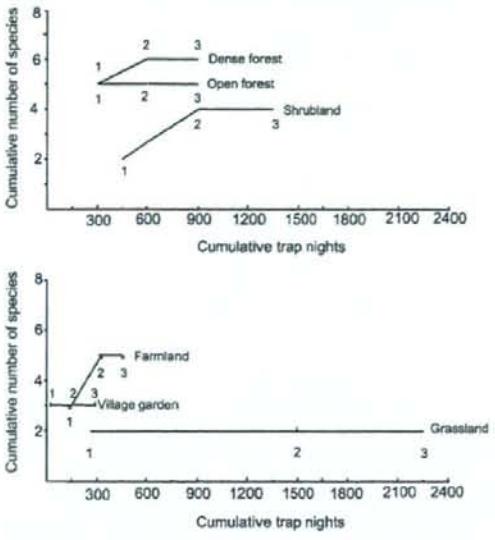


Figure 3 Evaluation of the trapping effort on species richness estimate in the six habitats of the Baihaba area.

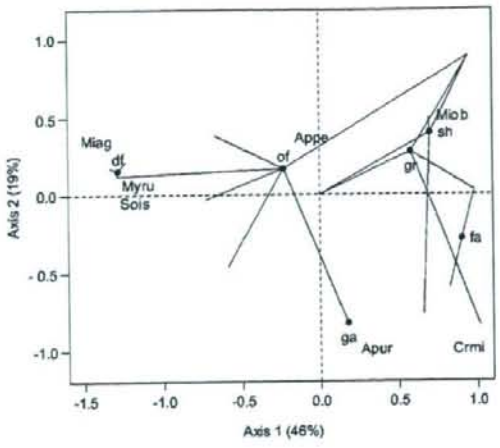


Figure 4 Factorial map of the correspondence analysis of the matrix trap line×species. Trap lines belonging to the same habitat are linked to the barycentre of the habitat by segments. df, dense forest; of, open forest; ga, village garden; gr, grassland; sh, shrubland; fa, farmland. Species codes are given in Table 1.

4). The two factorial axes (65% of the total inertia) show a species gradient from dense forest to grassland, farmland and shrubland, with open forest and village garden habitats in an intermediate position.

Index transects

A total of 2835 intervals of 10 paces were walked (approximately 25 km). Figure 5 shows the index distribution of *Ellobius tancrei* and of smaller small mammals (*Myodes*, *Microtus* and *Cricetulus* faeces could not be differentiated). Under the assumption of independency between sample units, the density index of *E. tancrei* in grassland and shrubland was significantly higher than in other habitats (χ^2=89.4, df=1, p<0.00001). The density index of smaller species of small mammals in grassland, shrubs and forest was higher than in forest pastures (χ^2=6.5, df=1, p=0.01).

Kokehada and Narenhebuke area

Considering the small number of trap lines and the differences in the number of night traps amongst habitats, relative densities could not be compared and were not computed here. Thus, the results are expressed as the number of trapped animals. Evidence of species presence will be discussed on this basis.

In total, 33 animals (561 trap nights) representing six species were trapped in the Kokehada area (Table 3). *Apodemus uralensis*, *Cricetulus eversmani*, *C. migratorius*, *Meriones meridianus* and *Spermophilus erythrogenys* were caught in pastures, while *Microtus gregalis* was trapped in the Alpine cushions of stream banks.

In total, 15 rodents representing four species were trapped in the semi-desert around the Narenhebuke village (60 trap nights). *Allactaga sibirica*, *Stylodipus telum* and *Spermophilus erythrogenys* were caught in the steppe areas around the village, and *Microtus gregalis* in the Alpine cushions of a stream bank.

Figures 6 and 7 show the distribution of small mammal indices along transects walked in Kokehada and Narenhebuke, respectively. In Kohehada, significantly different frequencies between habitats were observed for *Spermophilus erythrogenys* indices (χ^2=28.2, df=1, p< 0.0001) with larger numbers in slope grassland and shrub areas. No significant differences between habitats were recorded for smaller size species and the larger species *Marmota bobak*.

In Narenhebuke, no differences between habitats were found. Furthermore, *Spermophilus* indices were more than six times lower than in the Kokehada summer pastures.

Fuhai area

In the semi-desert, five rodents representing four species were trapped (150 trap nights): *Allactaga sibirica*, *Meriones meridianus*, *Stylodipus telum* and *Mus musculus* (Table 3). In the oasis, a total of 41 rodents representing five species were trapped (150 trap nights): *Apodemus uralensis*, *Cricetulus migratorius*, *Meriones tamariscinus*, *Microtus oeconomus* and *Mus musculus*.

Discussion

The logistics of small mammal studies in the northern Junggar basin are reflected by the paucity of studies reported from this region. To our knowledge, only lists of trapped species in large regions of Xinjiang have been published (see Ma et al. 1987), which did not report the sampling design nor the stratification of small mammal species per habitat. Here, we present for the first time results of small mammal surveys carried out in northeast Xinjiang in relation to land cover with a quantitative analysis at the community level. Obviously, a community assessment would be more complete if temporal variation was incorporated (e.g., several species trapped in the present study belong to genera which include cyclic species, such as *Microtus*, see below). However, we are aware of unavoidable biases and incompleteness that must lead to considering results cautiously and a call for more comprehensive studies. Nevertheless, we think that using standard methods in small mammal community assessment may be a first step towards better habitat and community definition of small mammal species of China and community comparisons (Giraudoux et al. 1998, Raoul et al. 2006).

Limitations of the study

In one study site (Baihaba), 3-night standard trapping could be carried out and the effect of sampling on population estimated. This was not the case for the other areas where trapping was carried out over a shorter duration and with a smaller number of trap lines due to circumstantial constraints of a larger programme aiming at screening human populations for echinococcoses (Wang et al. 2001). However, we think that in this case, occasional trapping could be targeted to a first assessment

Table 2 Trapping results in the Baihaba area expressed as the number of animals trapped per 100 trap nights.

Line code	Habitat	Species											
		Appe	Apsp	Apur	Myrf	Myru	Crmi	Miag	Miob	Mioe	Sina	Sois	Sosp	Sotu
01	Dense-forest (a)					5.3		1.3				1.3	1.3	
JA		1.3			2.7	13.3		2.7				1.3		
02						1.3								
JB						12.0								
03	Open-forest (b)								1.3					1.3
JC		1.3				1.3								
04									5.3					
JD				4.0		1.3			1.3					
10		1.3		4.0		5.3						1.3	2.7	
JJ				1.3		6.7			1.3					
11		2.7				2.7								
JK					5.3	12.0				2.7				
12	Shrubland (c)													
JL									4.0					
13									4.0					
JM									1.3					
16		5.3		4.0					20.0					
JP		1.3		12.0			4.0		8.0					
14	Village garden (d)			8.0		2.7			2.7	5.3				
22	Farmland (e)		1.3	2.7			2.7		4.0		4.0			
JV							1.3		2.7					
07	Grassland (f)													
JG														
08									2.7					
09														
21									1.3					
JU							2.7		1.3					
23							1.3		2.7					
JW									4.0					
05														
06									2.7					
Type of test		MW (a–c/d–f)		MW (a–e/f)		KW	KW	KW	KW	KW		MW (a,b/c–f)		
Probability of Ho		0.03	ns	0.05	ns	0.0004	0.04	0.01	0.05	0.005	ns	0.03	ns	ns
Multiple comparison ($p<0.05$)		–	–	–	–	a/f	ns	ns	ns	ns	–	–	–	–

Species code is given in Table 1. MW: Mann-Whitney U-test (letters show which contrasts have been used, e.g., a–c/d–f indicates habitats a, b, c pooled compared to d, e, f pooled); KW: Kruskal-Wallis test (factor levels are habitats). Where multiple comparisons were indicated: letters show the habitats that were significantly different; ns: no significant difference.

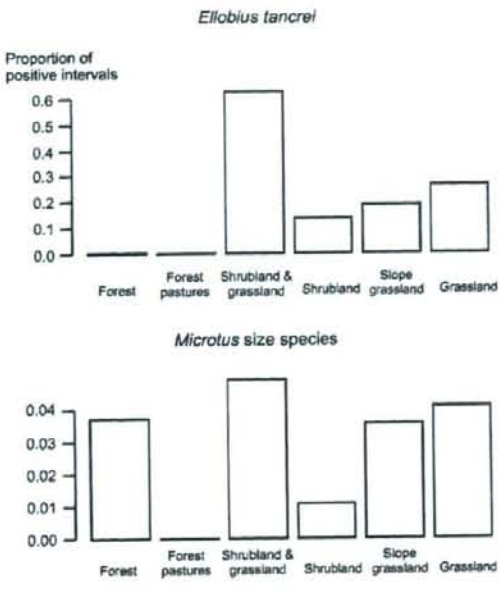


Figure 5 Index distribution of *Ellobius tancrei* and *Microtus* size species along transects in the open habitats of the Balhaba area.

of species present on a comparative basis (see below). Although little is known about species sensitivity to trap types, it is received wisdom that species are not equally responsive to trapping (for instance ground squirrels are difficult to catch with BBBT traps set up in a standard line). Subsequently, relative densities computed as number of animals per 100 trap nights do not allow direct comparisons between species, but permit comparisons between habitats on an ordinal scale for each trappable species. Methods grounded on surface indices allow monitoring at a smaller scale (over large areas). This would not be possible with standard trapping (Giraudoux et al. 1995, Quéré et al. 2000). Furthermore, some species are difficult to catch with surface traps (e.g., the subterraneous *Ellobius tancrei*); moreover, SBBT and INRA traps do not capture species over 70 g and BBBT traps over 200 g. These species can, however, be easily detected from their indices; *Ellobius tancrei* leaves conspicuous earth hills, different from those of *Arvicola terrestris* Linnaeus, *Talpa* sp., *Marmota* sp. and *Spermophilus* dens are characteristic. Many studies have been carried out with these methods and results obtained are reasonably consistent if the limits of index specificity are known and taken into account (see e.g., Giraudoux et al. 1998, Delattre et al. 1999, Duhamel et al. 2000, Hansson 2002, Michelat and Giraudoux 2006, Raoul et al. 2006).

Assemblages of small mammals and habitats

Species sampled in the present study are in general conformity with regional and national species lists of China (Ma 1981, Zhang 1997).

Balhaba area

The Balhaba area was the only place where standard trapping could be achieved allowing reasonable comparisons of small mammal species relative densities between habitats. Based on results obtained here, we propose a first scheme for the northern Junggar Basin, recording small mammal species assemblages at local scale according to a gradient from forest to grassland (Figure 8). Three assemblages could be recognised corresponding to three main habitat classes: (I) forest habitats, characterised by *Myodes rufocanus*, *Microtus agrestis*, *Myodes rutilus*, *Apodemus peninsulae* and *Sorex isodon*; (II) grassland areas, characterised by *Ellobius tancrei*, *Microtus obscurus*, *Cricetulus migratorius*; and (III) transitional areas and farmland, which includes additional species, such as *Apodemus uralensis*. Additionally, the presence of *Microtus oeconomus* was recorded in the vicinity of small streams in forest as well as in gardens. Species richness was higher in forest and in transitional areas and farmland, i.e., assemblages I and III (9 and 8 species, respectively) and lower in grasslands (3 species). Sampling pressure was comparable amongst the three assemblages (12, 9 and 10 trap lines, respectively). This is likely to limit sampling-related bias in the comparison of richness among communities (Gotelli and Colwell 2001).

However, this scheme has several biases. Firstly, commensal small mammals (rats, mice) and large rodent species (e.g., *Marmota bobak* Müller) have not been sampled. Secondly, a representation has been obtained from the very short time span of a 2-week survey, which

Table 3 Species and number of animals trapped in Fuhai, Narenhebuke and Kokehada.

	Kokehada	Narenhebuke	Fuhai 1 (semi-desert)	Fuhai 2 (oasis)
Allactaga sibirica Forster		10	1	
Apodemus uralensis Thomas	3			3
Cricetulus eversmani Brandt	2			
Cricetulus migratorius Pallas	3			20
Meriones meridianus Pallas	1		1	
Meriones tamariscinus Pallas				14
Microtus gregalis Pallas	7	1		
Microtus oeconomus Eversmann				2
Mus musculus Linnaeus			1	2
Stylodipus telum Lichtenstein		1	2	
Spermophilus erythrogenys Brandt	17	3		