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## Cystic Echinococcoses in Mongolia: Molecular Identification, Serology and Risk Factors

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# Cystic Echinococcoses in Mongolia: Molecular Identification, Serology and Risk Factors

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## Abstract

**Background:** Cystic echinococcosis (CE) is a globally distributed cestode zoonosis that causes hepatic cysts. Although *Echinococcus granulosus* sensu stricto (s.s.) is the major causative agent of CE worldwide, recent molecular epidemiological studies have revealed that *E. canadensis* is common in countries where camels are present. One such country is Mongolia.

**Methodology/Principal Findings:** Forty-three human hepatic CE cases that were confirmed histopathologically at the National Center of Pathology (NCP) in Ulaanbaatar (UB) were identified by analysis of mitochondrial *cox 1* gene as being caused by either *E. canadensis* (n = 31, 72.1%) or *E. granulosus* s.s. (n = 12, 27.9%). The majority of the *E. canadensis* cases were strain G6/7 (29/31, 93.5%). Twenty three haplotypes were identified. Sixteen of 39 CE cases with data on age, sex and province of residence were citizens of UB (41.0%), with 13 of the 16 cases from UB caused by *E. canadensis* (G6/7) (81.3%). Among these 13 cases, nine were children (69.2%). All pediatric cases (n = 18) were due to *E. canadensis* with 17 of the 18 cases (94.4%) due to strain G6/7. Serum samples were available for 31 of the 43 CE cases, with 22 (71.0%) samples positive by ELISA to recombinant Antigen B8/1 (rAgB). Nine of 10 CE cases caused by *E. granulosus* s.s. (90.0%) and 13 of 20 CE cases by *E. canadensis* (G6/7) (65.0%) were seropositive. The one CE case caused by *E. canadensis* (G10) was seronegative. CE cases caused by *E. granulosus* s.s. showed higher absorbance values (median value 1.131) than those caused by *E. canadensis* (G6/7) (median value 0.106) ( $p = 0.0137$ ).

**Conclusion/Significance:** The main species/strains in the study population were *E. canadensis* and *E. granulosus* s.s. with *E. canadensis* the predominant species identified in children. The reason why *E. canadensis* appears to be so common in children is unknown.

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† Deceased.

## Introduction

Cystic echinococcosis (CE) is a globally distributed parasitic zoonosis caused by ingestion of the eggs of *Echinococcus granulosus* sensu lato (s.l.) [1–5]. Recent molecular re-evaluation of *E. granulosus* s.l. has revealed that it consists of 5 independent species, *E. granulosus* sensu stricto (s.s.) (G1–G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G10) and *E. felidis* [4–6]. Although *E. granulosus* s.s. (G1) is the major causative agent of human CE where sheep are grazed with dogs [6–11], recent molecular studies of human CE specimens have revealed that CE cases

caused by *E. canadensis* (G6–G10) are common in some areas where camels and other livestock including cattle, pigs, and goats are distributed [4–6,10–22]. Therefore, it is important to include molecular identification of human CE cases in epidemiological studies.

In Mongolia, more than 50% of the population lives in the capital city of Ulaanbaatar (UB), with the remainder largely following the traditional nomadic lifestyle [23–28]. For many years, CE has been recognized as a common disease in Mongolia even though there is very little published data. Since the collapse of the Soviet Union in 1991, two meetings have been held in UB on

## Author Summary

Cystic echinococcosis (CE) is a parasitic zoonosis with a cosmopolitan distribution. Molecular analysis was carried out on 43 hepatic CE cysts from 43 cases confirmed histopathologically at the NCP, Mongolia. Molecular analysis revealed two species, *Echinococcus canadensis* and *Echinococcus granulosus* s.s. Twenty three haplotypes of the *cox1* gene were identified. All pediatric cases ( $n = 18$ ) were by *E. canadensis*. Sixteen of 39 CE cases with data on age, sex and province of residence were from UB (41.0%), and 13 of these 16 cases were caused by *E. canadensis* (81.3%). Among the 13 cases from UB, nine were children (69.2%). A total of 31 serum samples from these 43 cases were analyzed for antibody response to rAgB with 22 (71.0%) samples positive by ELISA to rAgB. Thirteen of 20 *E. canadensis* (G6/7) (65%) and nine of 10 *E. granulosus* s.s. (90%) were seropositive. CE cases by *E. granulosus* s.s. showed a higher absorbance value than cases by *E. canadensis* ( $p = 0.0137$ ). This is the first study to evaluate age distribution of and antibody responses to rAgB in CE cases caused by the two species in Mongolia. It remains unknown why *E. canadensis* appears to be more common in pediatric cases.

the topic of CE. The first meeting was held at the National Center of Communicable Diseases (NCCD) in May 1995 and Tsoodol, Narantuya and Goosh from Mongolia provided overviews of human cases to date [29]. During this meeting, it was reported that in 1950, 7.8% of all surgical patients were diagnosed CE, whereas this value was only 1.9% in 1990. CE was determined to be the cause of 18% of the surgical cases seen at State Central First Hospital (SCFH) in 1993 [25–28]. The second meeting was held at the Health Science University of Mongolia (HSUM) in June 2009 for the purpose of establishing a network of CE experts [27]. During this meeting, Ayushkhuu from the National Center for Maternal and Child Health (NCMCH) summarized 25 pediatric CE cases from 2008 and 2009 (19 cases in 2008 and 6 cases from Jan to May 2009). These cases consisted of 15 boys and 10 girls, including 4 children under the age of 5-years [27]. During this same meeting, Bazarragchaa from the SCFH summarized a total of 144 (63 males and 81 females) CE cases (1989–2009 June) [27]. None of the reported cases differentiate between infection with *E. granulosus* s.s. and *E. canadensis*.

There have been several sero-epidemiology studies of CE in Mongolia [30–33]. These studies all have used hydatid cyst fluid (HCF) for screening with an ELISA. However, none of the studies used a confirmatory test to verify their findings. Most surgical cases in Mongolia are diagnosed by histopathological identification of resected lesions at the National Center of Pathology (NCP) in UB [27,28]. The present study is the first to identify the primary species/strains of CE present in Mongolian patients that had been histopathologically confirmed as having CE as well as evaluate antibody responses to the recombinant Antigen B8/1 (rAgB) by ELISA [34–43].

## Methods

### Ethical statement

Molecular analysis of human specimens and serological analysis of antibody responses, and junior researchers from several institutes in UB, Mongolia, to do laboratory analysis of these all specimens were approved by the Asahikawa Medical

University (AMU) Institutional Review Board (AMU-IRB-1435).

### Patient samples

A total of 43 CE cases were evaluated, consisting of 18 pediatric cases from the NCMCH and 27 cases from the SCFH. In total, 43 hepatic CE cysts (1 cyst per patient) were obtained and confirmed to be CE histopathologically at the NCP. Pre-surgical serum samples were available for 31 of the 43 study patients (Table 1). De-identified data on patient age, sex, and province of residence were also obtained.

### DNA analysis

Genomic DNA was extracted from 43 ethanol-fixed samples from hepatic CE patients (Table 1) using a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was kept at  $-20^{\circ}\text{C}$  until further analysis could be performed. DNA obtained was used as templates for polymerase chain reaction (PCR). The complete or partial mitochondrial cytochrome c oxidase subunit I (*cox 1*) gene was amplified by PCR as reported previously [44–46]. PCR products were treated with illustra ExoStar (GE Healthcare) to remove excess primers and dNTPs, and directly sequenced with a BigDye Terminator v3.1 and a 3500 DNA sequencer (Life Technologies). Obtained sequences were edited using Geneious Pro version 7.0.4 (created by Biomatters, available from <http://www.geneious.com>), and multiple alignments of each *cox1* haplotype were made by the program MAFFT [47] with the homologous sequences of other *Echinococcus* species available in the GenBank database. A phylogenetic tree was constructed using the neighbour-joining method and Kimura's two-parameter model [48] in Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b [49]. The robustness of the phylogenetic tree was tested by bootstrapping with 1000 replicates. For tree construction, *Versteria mustelae* was used as an outgroup because it is a sister to all members of the genus *Echinococcus* [50].

### Serology

Recombinant Antigen B8/1 (rAgB) produced from *E. multilocularis* (rEmAgB) was applied for ELISA and immunoblot tests as reported previously [36]. Several specimens with optical density (OD) values around the cut-off were re-checked by immunoblot using the same antigen for confirmation (figure not shown) [36,40,41]. ELISA was carried out in flat-bottom 96-well microplates (Nunc, Maxisorp, Roskilde, Denmark) as previously described [36]. The microplates were coated with 100 ng/ml of rEmAgB diluted in phosphate-buffered saline (PBS) and incubated at  $4^{\circ}\text{C}$  overnight. Excess antigen was removed by washing with PBS. Blocking was performed with blocking solution [1% casein in 20 mM Tris-HCl (pH 7.6) containing 150 mM NaCl] and the plates were incubated at  $37^{\circ}\text{C}$  for 1 hr. The plates were washed twice with PBS containing 0.05% Tween 20 (PBST). 100  $\mu\text{l}$  of diluted sera (1/100 dilution in blocking solution) was added and plates were incubated at  $37^{\circ}\text{C}$  for 1.5 hrs. After washing five times with PBST, 100  $\mu\text{l}$  of protein G-peroxidase conjugate (1/4000 dilution in blocking solution) (Invitrogen, Camarillo, CA) was added to each well and the plates were incubated at  $37^{\circ}\text{C}$  for 1.5 hrs. Plates were washed six times with PBST and one time with PBS and incubated with 100  $\mu\text{l}$  of substrate solution [0.4 mM 2,2-azino-di (3-ethyl-benzthiazoline-6-sulfonate) (ABTS) in 0.2 M citric acid buffer (pH 4.7)] at room temperature for 30 min. The color reaction was stopped by addition of 1% SDS. The optical

**Table 1.** Molecular identification of the causative species of 43 CE cases and antibody response to rAgB in ELISA (n = 31, cut-off value: 0.055).

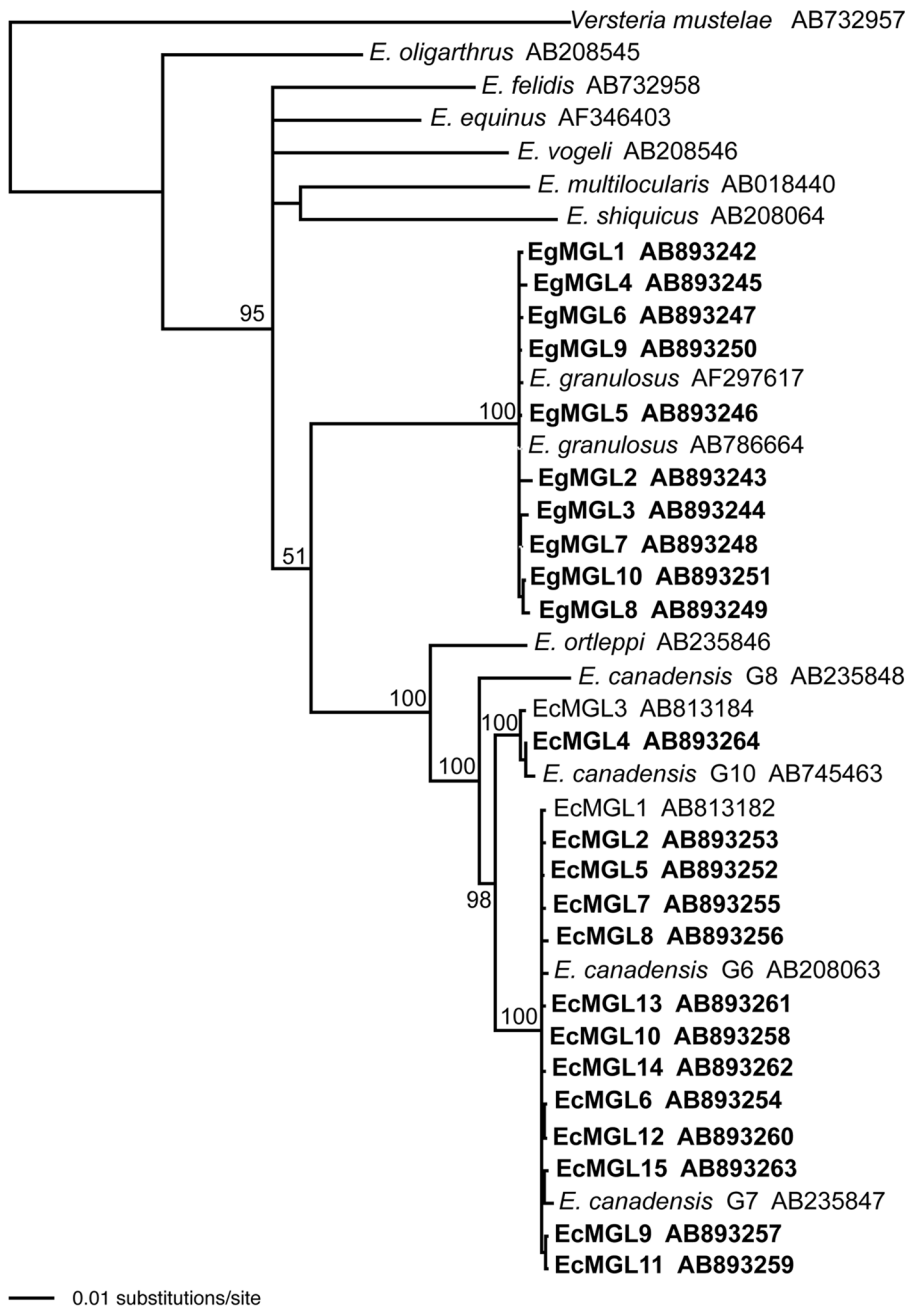
Case No.	Province	Age in years (sex)	ELISA (OD value)	PCR	Cox1 haplotype (Accession Nos.)
1	UB <sup>#</sup>	5 (F)	0.802	<i>E. canadensis</i> (G6/7)	EcMGL5 (AB893252)
2	Selenge	21 (F)	x	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
3	UB, Nalaih	4 (F)	0.011	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
4	UB	13 (F)	x	<i>E. canadensis</i> (G6/7)	EcMGL6 (AB893254)
5	x	x	0.071	<i>E. canadensis</i> (G6/7)	EcMGL7 (AB893255)
6	Umnogovi	8 (M)	0.875	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
7	Selenge	28 (M)	x	<i>E. granulosus</i> (G1)	EgMGL1 (AB893242)
8	UB	38 (F)	0.019	<i>E. canadensis</i> (G6/7)	EcMGL1* (AB813182)
9	Zavkhan	65 (F)	1.263	<i>E. granulosus</i> (G1)	EgMGL2 (AB893243)
10	Arkhangai	50 (M)	x	<i>E. granulosus</i> (G1)	EgMGL3 (AB893244)
11	x	15 (M)	x	<i>E. canadensis</i> (G10)	EcMGL4 (AB893264)
12	Selenge	62 (F)	0.051	<i>E. granulosus</i> (G1)	EgMGL4 (AB893245)
13	Dundgovi	12 (F)	0.661	<i>E. canadensis</i> (G6/7)	EcMGL8 (AB893256)
14	Tuv	56 (M)	0.023	<i>E. canadensis</i> (G10)	EcMGL4 (AB893264)
15	Dornogovi	22 (F)	0.142	<i>E. canadensis</i> (G6/7)	EcMGL9 (AB893257)
16	UB	72 (M)	1.267	<i>E. granulosus</i> (G1)	EgMGL5 (AB893246)
17	Bayan-Ulgii	48 (M)	0.273	<i>E. granulosus</i> (G1)	EgMGL6 (AB893247)
18	Selenge	68 (F)	1.472	<i>E. granulosus</i> (G1)	EgMGL7 (AB893248)
19	Umnugovi	41 (F)	0.003	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
20	UB	4 (M)	0.771	<i>E. canadensis</i> (G6/7)	EcMGL1* (AB813182)
21	UB	9 (M)	0.081	<i>E. canadensis</i> (G6/7)	EcMGL15 (AB893263)
22	UB	25 (M)	0.261	<i>E. granulosus</i> (G1)	EgMGL8 (AB893249)
23	Khuvsgul	10 (F)	0.025	<i>E. canadensis</i> (G6/7)	EcMGL10 (AB893258)
24	UB	58 (F)	0.101	<i>E. canadensis</i> (G6/7)	EcMGL10 (AB893258)
25	UB	4 (M)	x	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
26	x	13 (M)	1.386	<i>E. canadensis</i> (G6/7)	EcMGL11 (AB893259)
27	UB	5 (F)	0.011	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
28	UB	43 (F)	x	<i>E. canadensis</i> (G6/7)	EcMGL7 (AB893255)
29	UB	35 (F)	1.024	<i>E. granulosus</i> (G1)	EgMGL10 (AB893251)
30	Khovd	46 (F)	x	<i>E. canadensis</i> (G6/7)	EcMGL1* (AB813182)
31	Dornogovi	5 (M)	1.301	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
32	UB	15 (M)	x	<i>E. canadensis</i> (G6/7)	EcMGL6 (AB893254)
33	Dundgovi	16 (M)	0.036	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
34	Uvurkhangai	59 (M)	0.821	<i>E. granulosus</i> (G1)	EgMGL6 (AB893247)
35	Govi-Altai	54 (M)	x	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
36	Selenge	14 (M)	1.396	<i>E. canadensis</i> (G6/7)	EcMGL12 (AB893260)
37	Khuvsgul	38 (F)	1.387	<i>E. granulosus</i> (G1)	EgMGL9 (AB893250)
38	x	x	x	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
39	Darkhan	29 (M)	0.007	<i>E. canadensis</i> (G6/7)	EcMGL2 (AB893253)
40	UB	79 (F)	0.145	<i>E. canadensis</i> (G6/7)	EcMGL13 (AB893261)
41	Tuv	21 (F)	1.238	<i>E. granulosus</i> (G1)	EgMGL6 (AB893247)
42	UB	4 (M)	x	<i>E. canadensis</i> (G6/7)	EcMGL6 (AB893254)
43	Uvurkhangai	9 (F)	0.111	<i>E. canadensis</i> (G6/7)	EcMGL14 (AB893262)

x, not available;

\*Only partial sequence (828 bp) was obtained.

<sup>#</sup>UB: Ulaanbaatar (Capital city)

doi:10.1371/journal.pntd.0002937.t001



**Figure 1. A neighbour-joining tree of *Echinococcus* spp. constructed from the nucleotide sequences of mitochondrial *cox1* gene.** Numbers on the nodes are bootstrap values. The names of the haplotypes obtained in this study are shown in bold. doi:10.1371/journal.pntd.0002937.g001

density at 405 nm ( $OD_{405}$ ) was determined using an ELISA plate reader (Immuno Mini NJ2300, Tokyo, Japan). The cut-off point was set as the mean  $OD_{405}$  plus 3SD for 30 negative control samples.

#### Statistical analyses

ELISA OD value results and positive ratios in CE cases caused by *E. granulosus* s.s. (G1) and *E. canadensis* (G6/7) were assessed by the Wilcoxon rank sum test and Fisher's exact test, respectively. All analyses were two-tailed and a  $p$ -value < 0.05 was considered statistically significant.

#### Results

##### Molecular identification of *Echinococcus* species causing CE

Data on patient age, sex, and province of residence are shown in Table 1. Nucleotide sequences of the *cox1* gene (1608-1609 bp) were determined for 40 specimens, and consequently 23 haplotypes were obtained. Among these, 21 haplotypes (EgMGL1-10, EcMGL5-15) were newly identified in Mongolia. The nucleotide sequences of all haplotypes were deposited into DDBJ/EMBL/GenBank databases under the accession numbers AB893242-



**Figure 2. A map of Mongolia showing the distribution of 39 CE cases caused by *E. canadensis* (G6/7) (n = 26), *E. canadensis* (G10) (n = 1), and *E. granulosus* s.s. (G1) (n = 12).** Additional four cases had no record of province (see Table 1). doi:10.1371/journal.pntd.0002937.g002

AB893264. Phylogenetic analysis clearly showed that 12 specimens (10 haplotype) were *E. granulosus* s.s. (G1) and 29 (12 haplotypes) and 2 (1 haplotype) specimens were *E. canadensis* (G6/7) and (G10), respectively (Table 1, Figure 1). For three specimens (Nos. 19, 27, 45), only a partial sequence (828 bp) was determined. BLAST search revealed that the sequence was 100% identical to the *cox1* gene sequence of *E. canadensis* (G6/7) (Accession Number = AB813182). Among 43 specimens, 39 [11 *E. granulosus* s.s. (G1), 27 *E. canadensis* (G6/7), one *E. canadensis* (G10)] had data available on patient age, sex and province of residence (Table 1, Figure 2). CE cases were found from 14 provinces, including UB (Figure 2). As shown in Table 1, all pediatric CE cases (n = 18) were caused by *E. canadensis* (100%). Of the 16 CE cases from UB (16/39, 41.0%), 13 cases were caused by *E. canadensis* (G6/7) (13/16, 81.3%). Among these 13 cases caused by *E. canadensis* (G6/7), nine were children (9/13, 69.2%).

#### Antibody responses in CE cases caused by *E. granulosus* and *E. canadensis*

Among 31 serum samples examined, 22 samples (64.7%) were antibody positive to rEmAgB: 9 of 10 *E. granulosus* s.s. (G1) (90%) cases and 13 of 20 *E. canadensis* (G6/7) (65%) cases. The one *E. canadensis* (G10) case was sero-negative (Table 1). Figure 3 illustrates antibody responses of these 30 serum samples to rEmAgB by ELISA. The median absorbance ratios were 1.131 for *E. granulosus* (G1) and 0.106 for *E. canadensis* (G6/7). The *p*-values for sero-positive ratio and absorbance value between the two species were 0.2103 and 0.0137, respectively. The average ages of all CE patients were 47.6 years (range: 21 to 72 years) and 22.5 years (range: 4 to 79 years) for patients infected with *E. granulosus* s.s. (G1) and *E. canadensis*, respectively.

Of the 9 CE cases caused by *E. granulosus* s.s. (G1) with positive serology, 5 were females. Of the 12 CE cases caused by *E. canadensis* (G6/7) with positive serology, 6 were females. CE cases with the highest absorbance values (>1.200) for *E. canadensis* (G6/7) were male children, whereas cases infected with *E. granulosus* s.s. (G1) were all adults (Table 1).

#### Discussion

This is the first report on antibody responses to rAgB in patients with CE caused by *E. canadensis*. As shown in Figure 3, nine of 10 *E. granulosus* s.s. (G1) (90%) and 13 of 20 *E. canadensis* (G6/7) (65%) were antibody positive to rEmAgB. While there was no statistically significant difference in the antibody positive ratio between the two species, CE cases caused by *E. granulosus* s.s. (G1) had higher absorbance values (median ratio = 1.131) than CE cases caused by *E. canadensis* (G6/7) (median ratio = 0.106) (*p* = 0.0137). Therefore, the question remains if there is a difference in antigenicity of rAgBs produced by different species of the genus *Echinococcus* or in the antibody response to rAgB in CE patients infected with different *Echinococcus* species. The potential influences of other factors such as pathological stages of CE or differences in health conditions not examined in the present study remain unknown [1–3].

The Antigen B8/1 gene has high homology in amino acid sequences (92.6%) for *E. granulosus* s.s. (G1) (EgAgB) and *E. multilocularis* (EmAgB) and has been shown to have the same sensitivity and specificity for detecting CE cases caused by *E. granulosus* s.s. (G1) [34–43,51,52]. For example, a study in Italy [53] showed that there was no difference in sensitivity or specificity using rEgAgB versus rEmAgB to detect CE cases [36]. The merit to use rEmAgB over rEgAgB is much higher yield performance (Sako et al. unpublished). More critical comparative biochemical and molecular studies [54–61] have shown that the amino acid sequence of the epitope region of AgB is well preserved between *E. granulosus* s.s. (G1) and *E. canadensis* (G6/7), with minor difference between *E. granulosus* s.s. (G1) and *E. multilocularis*. Minor differences in amino acid sequences at the N-terminal do not result in any conformational change in the rAgB epitope itself [36], leading to the belief that rAgB prepared from any *Echinococcus* species is useful for detection of antibody response in CE. Serological studies from Australia, France, China, Jordan, Turkey, the United States, Nepal, Poland, Italy, and Iran using rEmAgB have all successfully identified cases of CE [34,36,41,53]. In retrospect, CE cases provided from different countries should have





**Accession numbers of *cox1* gene sequences of *Echinococcus granulosus* s.s. and *Echinococcus canadensis*.** The nucleotide sequences of all haplotypes reported in the present study are available in DDBJ/EMBL/GenBank database under the accession numbers AB893242-AB893264.

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## References

- Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (2001) WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. Paris:OIE1–265.
- Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 114: 1–16.
- Brunetti E, Garcia HH, Junghans T, International CE Workshop in Lima, Peru, 2009 (2011) Cystic echinococcosis: chronic, complex, and still neglected. *PLoS Negl Trop Dis* 5: e1146.
- Nakao M, McManus DP, Schantz PM, Craig PS, Ito A (2007) A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* 134: 713–722.
- Alvarez Rojas CA, Romig T, Lightowlers MW (2014) *Echinococcus granulosus* sensu lato genotypes infecting humans—review of current knowledge. *Int J Parasitol* 44: 9–18.
- Nakao M, Yanagida T, Konyaev S, Lavikainen A, Odnokurtsev VA, et al. (2013) Mitochondrial phylogeny of the genus *Echinococcus* (Cestoda: Taeniidae) with emphasis on relationships among *Echinococcus canadensis*. *Parasitology* 140: 1625–1636.
- Moro PL, Nakao M, Ito A, Schantz PM, Cavero C, et al. (2009) Molecular identification of *Echinococcus* isolates from Peru. *Parasitol Int* 58: 184–186.
- Casulli A, Interisano M, Sreter T, Chitima L, Kirkova Z, et al. (2012) Genetic variability of *Echinococcus granulosus* sensu stricto in Europe inferred by mitochondrial DNA sequences. *Infect Genetics Evol* 12: 377–383.
- Yanagida T, Mohammadzadeh T, Kamhawi S, Nakao M, Sadjjadi SM, et al. (2012) Genetic polymorphisms of *Echinococcus granulosus* sensu stricto in the Middle East. *Parasitol Int* 61: 599–603.
- Romig T, Omer RA, Zeyhle E, Hüttner M, Dinkel A, et al. (2011) Echinococcosis in sub-Saharan Africa: emerging complexity. *Vet Parasitol* 181: 43–47.
- Wahlers K, Menezes CN, Wong ML, Zeyhle E, Ahmed ME, et al. (2012) Cystic echinococcosis in sub-Saharan Africa. *Lancet Infect Dis* 12: 871–880.
- Bowles J, Blair D, McManus DP (1994) Molecular genetic characterization of the cervid strain (‘northern form’) of *Echinococcus granulosus*. *Parasitology* 109: 215–221.
- Bart JM, Abdukader M, Zhang YL, Lin RY, Wang YH, et al. (2006) Genotyping of human cystic echinococcosis in Xinjiang, PR China. *Parasitology* 133: 571–579.
- Zhang LH, Chai JJ, Jiao W, Osman Y, McManus DP (1998) Mitochondrial genomic markers confirm the presence of the camel strain (G6 genotype) of *Echinococcus granulosus* in north-western China. *Parasitology* 116: 29–33.
- Casulli A, Zeyhle E, Brunetti E, Pozio E, Meroni V, et al. (2010) Molecular evidence of the camel strain (G6 genotype) of *Echinococcus granulosus* in humans from Turkana, Kenya. *Trans R Soc Trop Med Hyg* 104: 29–32.
- Abushhewa MH, Nolan MJ, Jex AR, Campbell BE, Jabbar A, et al. (2010) Genetic classification of *Echinococcus granulosus* cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. *Mol Cell Probes* 24: 346–351.
- Jabbar A, Narankhajid M, Nolan MJ, Jex AR, Campbell BE, et al. (2011) A first insight into the genotypes of *Echinococcus granulosus* from humans in Mongolia. *Mol Cell Probes* 25: 49–54.
- Simsek S, Kaplan M, Ozercan IH (2011) A comprehensive molecular survey of *Echinococcus granulosus* in formalin-fixed paraffin-embedded tissues in human isolates in Turkey. *Parasitol Res* 109: 411–416.
- Konyaev S, Yanagida T, Ingovatova GM, Shokhet YN, Nakao M, et al. (2012) Molecular identification of human echinococcosis in the Altai region of Russia. *Parasitol Int* 61: 711–714.
- Aaty HE, Abdel-Hameed DM, Alam-Eldin YH, El-Shennawy SF, Aminou HA, et al. (2012) Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt. *Acta Trop* 121: 125–128.
- Piccoli L, Bazzocchi C, Brunetti E, Mihailescu P, Bandi C, et al. (2013) Molecular characterization of *Echinococcus granulosus* in south-eastern Romania: evidence of G1–G3 and G6–G10 complexes in humans. *Clin Microbiol Infect* 19: 578–582.
- Khademvatan S, Yousefi E, Rafiei A, Rahdar M, Saki J (2013) Molecular characterization of livestock and human isolates of *Echinococcus granulosus* from south-west Iran. *J Helminthol* 87: 240–244.
- Rausch RL (1952) Hydatid disease in boreal regions. *Arctic. J Arctic Inst North America* 5: 157–174.
- Rausch RL (1993) Biology of *Echinococcus granulosus*. In: Compendium on Cystic Echinococcosis with Special Reference to the Xinjiang Uygur Autonomous Region, The People’s Republic of China Andersen FLC, JLIU FJUT, USBrigham Young Univ Press, Provo2756
- Davaatseren N, Otogondalai A, Nyamkhuu G, Rusher AH (1995) Management of echinococcosis in Mongolia. *J Ark Med Soc* 92: 122–125.
- Ebright JR, Altantsetseg T, Oyungeler R (2003) Emerging infectious diseases in Mongolia. *Emerg Infect Dis* 9: 1509–1515.
- Gurbadam A, Nyamkhuu D, Nyamkhuu G, Tsendjav A, Sergelen O, et al. (2010) Mongolian and Japanese joint conference on ‘‘Echinococcosis: diagnosis, treatment and prevention in Mongolia’’ June 4, 2009. *Parasit Vectors* 3: 8.
- Ito A, Agvaandaram G, Bat-Ochir OE, Chuluunbaatar B, Gonchigsenghe N, et al. (2010) Histopathological, serological, and molecular confirmation of indigenous alveolar echinococcosis cases in Mongolia. *Am J Trop Med Hyg* 82: 266–269.
- Cross JH (1995) Journal of the Citizen Ambassador Program Parasitology Delegation to the People’s Republic of China and Mongolia.
- Watson-Jones DL, Craig PS, Badamochir D, Rogan MT, Wen H, et al. (1997) A pilot, serological survey for cystic echinococcosis in north-western Mongolia. *Ann Trop Med Parasitol* 91: 173–177.
- Lee DS, Chung BH, Lee NS, Nam HW, Kim JH (1999) A survey of helminthic infections in the residents of rural areas near Ulaanbaatar, Mongolia. *Korean J Parasitol* 37: 145–147.
- Huh S, Yu JR, Kim JJ, Gotov C, Janchiv R, et al. (2003) Intestinal protozoan infections and echinococcosis in the inhabitants of Dornod and Selenge, Mongolia. *Korean J Parasitol* 44: 171–174.
- Wang Y, He T, Wen X, Li T, Walli TT, et al. (2005) Human cystic echinococcosis in two Mongolian communities in Hobukesar (China) and Bulgan (Mongolia). *Trans R Soc Trop Med Hyg* 99: 692–698.
- Ito A, Ma L, Schantz PM, Gottstein B, Liu YH, et al. (1999) Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E. multilocularis* protoscolex (Em18). *Am J Trop Med Hyg* 60: 188–192.
- Mamuti W, Yamasaki H, Sako Y, Nakaya K, Nakao M, et al. (2002) Usefulness of hydatid cyst fluid of *Echinococcus granulosus* developed in mice with secondary infection for serodiagnosis of cystic echinococcosis in humans. *Clin Diagn Lab Immunol* 9: 573–576.
- Mamuti W, Yamasaki H, Sako Y, Nakao M, Xiao N, et al. (2004) An 8 kDa-subunit of Antigen B from *Echinococcus multilocularis*: molecular cloning, expression and serological evaluation. *J Clin Microbiol* 42: 1082–1088.
- Sako Y, Nakao M, Nakaya K, Yamasaki H, Gottstein B, et al. (2002) Alveolar echinococcosis: characterization of diagnostic antigen Em18 and serological evaluation of recombinant Em18. *J Clin Microbiol* 40: 2760–2765.
- Ito A, Nakao M, Sako Y (2007) Echinococcosis: serological detection of patients and molecular identification of parasites. *Future Microbiol* 2: 439–449.
- Ito A, Yanagida T, Sako Y, Nakao M, Nakaya K, et al. (2013) Chapter 23 *Echinococcus* and Echinococcosis. In: Molecular Detection of Human Parasitic Pathogens. ed Liu DCRC Press249263
- Li TY, Ito A, Chen XW, Sako Y, Qiu J, et al. (2010) Specific IgG responses to recombinant antigen B and Em18 in cystic and alveolar echinococcosis in China. *Clin Vaccine Immunol* 17: 470–475.
- Mohammadzadeh T, Sako Y, Sajjadi SM, Sarkari B, Ito A (2012) Comparison of the usefulness of hydatid cyst fluid, native antigen B and recombinant antigen B8/1 for serological detection of cystic echinococcosis. *Trans R Soc Trop Med Hyg* 106: 371–375.
- Jiang L, Zhang YG, Liu MX, Feng Z (2012) Analysis on the reactivity of five subunits of antigen B family in serodiagnosis of echinococcosis. *Exp Parasitol* 131: 85–91.

43. Barnes TS, Deplazes P, Gottstein B, Jenkins DJ, Mathis A, et al. (2012) Challenges for diagnosis and control of cystic hydatid disease. *Acta Trop* 123: 1–7.
44. Nakao M, Sako Y, Yokoyama N, Fukunaga M, Ito A (2000) Mitochondrial genetic code in cestodes. *Mol Biochem Parasitol* 111: 415–424.
45. Hüttner M, Nakao M, Wassermann T, Siefert L, Boomker JD, et al. (2008) Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. *Int J Parasitol* 38: 861–868.
46. Konyaev SV, Yanagida T, Nakao M, Ingovatova GM, Shoykhet YN, et al. (2013) Genetic diversity of *Echinococcus* spp. in Russia. *Parasitology* 140: 1637–1647.
47. Kaotoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30: 772–780.
48. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111–120.
49. Swofford DL (2002) PAUP\*: phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Massachusetts
50. Nakao M, Lavikainen A, Iwaki T, Haukisalmi V, Konyaev S, et al. (2013) Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. *Int J Parasitol* 43: 427–437.
51. Li T, Ito A, Pengcuo R, Sako Y, Chen X, et al. (2011) Post-treatment follow-up study of abdominal cystic echinococcosis in Tibetan communities of northwest Sichuan Province, China. *PLoS Negl Trop Dis* 5: e1364.
52. Li T, Chen X, Zhen R, Qiu J, Qiu D, et al. (2010) Widespread co-endemicity of human cystic and alveolar echinococcosis on the eastern Tibetan Plateau, northwest Sichuan/southeast Qinghai, China. *Acta Trop* 113: 248–256.
53. Tamarozzi F, Sako Y, Ito A, Piccoli L, Grisolia A, et al. (2013) Recombinant AgB8/1 ELISA test vs commercially available IgG ELISA test in the diagnosis of cystic echinococcosis. *Parasite Immunol.* 35:433440
54. González-Sapienza G, Cachau RE (2003) Identification of critical residues of an immunodominant region of *Echinococcus granulosus* antigen B. *J Biol Chem* 278: 20179–20184.
55. Kamenetzky L, Muzulin PM, Gutierrez AM, Angel SO, Zaha A, et al. (2005) High polymorphism in genes encoding antigen B from human infecting strains of *Echinococcus granulosus*. *Parasitology* 131: 805–815.
56. Mamuti W, Sako Y, Bart JM, Nakao M, Ma X, et al. (2007) Molecular characterization of a novel gene encoding an 8-kDa-subunit of antigen B from *Echinococcus granulosus* genotypes 1 and 6. *Parasitol Int* 56: 313–316.
57. Muzulin PM, Kamenetzky L, Gutierrez AM, Guarnera EA, Rosenzvit MC (2008) *Echinococcus granulosus* antigen B gene family: further studies of strain polymorphism at the genomic and transcriptional levels. *Exp Parasitol* 118: 156–164.
58. Bhattacharya D, Pan D, Das S, Bera AK, Bandyopadhyay S, et al. (2009) An evaluation of antigen B family of *Echinococcus granulosus*, its conformational propensity and elucidation of the agrotepe. *J Helminthol* 83: 219–224.
59. Alvarez Rojas CA, Gaudi CG, Lightowers MW (2013) Antigenic differences between the EG95-related proteins from *Echinococcus granulosus* G1 and G6 genotypes: implications for vaccination. *Parasite Immunol* 35: 99–102.
60. Mamuti W, Sako Y, Xiao N, Nakaya K, Nakao M, et al. (2006) *Echinococcus multilocularis*: developmental stage-specific expression of antigen B 8-kDa subunits. *Exp Parasitol* 113: 75–82.
61. Etebar F, Jalousian F, Hosseini Sh, Kordafshari S, Najafi A (2013) Immunoproteomics approach for EPC1 antigenic epitope prediction of G1 and G6 strains of *Echinococcus granulosus*. *Parasitol Res* 112: 3129–3135.
62. Ito A (2013) Nothing is perfect! Trouble-shooting in immunological and molecular studies of cestode infections. *Parasitology* 140: 1551–1565.
63. Ito A, Chuluunbaatar G, Yanagida T, Davaasuren A, Sumiya B, et al. (2013) *Echinococcus* species from red foxes, corsac foxes, and wolves in Mongolia. *Parasitology* 140: 1648–1654.
64. Chinchuluun B, Sako Y, Khatanbaatar I, Bayarmaa B, Lkhagvatseren S, et al. (2014) A survey of seropositivity to antigen B, an immunodiagnostic antigen for human cystic echinococcosis, in domestic animals in Mongolia. *Parasitol Int* 63: 324–326.