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The Status of Human Echinococcosis in Western Mongolia

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Objective: The purpose of this study was to identify cases of human echinococcosis in the Bayan-Ulgii, Khovd provinces, to determine their WHO classification by ultrasound and to identify the haplotype in surgically cysts removed. **Methods:** A total of 1063 people participated in ultrasound screening. Patients who had a positive echinococcosis by ultrasound underwent serologic testing for detection of antibodies for echinococcosis. **Results:** Ninety people were positive for the liver cystic lesions by ultrasound screening of which 12 cases were newly diagnosed cystic echinococcosis. Four were cystic echinococcosis stages 1-2, five were stage 3, while three were cystic echinococcosis stage 4. Thirty-three patients with previously known echinococcosis stage 2 and alveolar echinococcosis stage 4 were also examined. Out of 29 cystic echinococcosis cases, 24 underwent treatment. Of 24 who underwent treatment, only one was cured. The haplotypes *E. canadensis* G6/7 and *E. granulossus* s.s. G1 were confirmed by evaluating the *cytochrome c oxidase subunit* I gene of mitochondrial DNA in two patients in Bayan-Ulgii. **Conclusion:** Ninety people were positive for cystic echinococcosis in western Mongolia of which 12 cases were newly diagnosed cases. Of the 12 new cystic echinococcosis cases, 4 were stages 1-2, 5 were stage 3 and 3 were stage 4 by WHO classification. The haplotype in Bayan-Ulgii population were *E. canadensis* and s.s.

Keywords: Echinococcosis, Ultrasound Screening, Serology Test, Mongolia.

Introduction

Cystic echinococcosis (CE) and alveolar echinococcosis (AE) are chronic and potentially fatal diseases that are caused by the larval stage of *E. granulossus* and *E. multilocularis*, respectively. The definitive hosts of *E. granulossus* and *E. multilocularis* are domestic dogs and wild canids infected through the ingestion of livestock offal containing hydatid cysts with viable protoscoleces[1,2]. Transmission of CE and AE to intermediate hosts such as livestock and rodents is by unintentional consumption of parasite eggs excreted in the feces of the definitive hosts. Humans are considered as an aberrant intermediate host: larvae released from the eggs can invade and develop, forming fluid-filled cysts or hydatids, which are mostly (80%) located in the liver, but may occur in any other organs[3].

Human echinococcosis widely distributed all over the world[4]. At least 270 million people (58% of the total population) are at the risk of Echinococcosis in Central Asia, including Mongolia[5]. There has been little research on human echinococcosis epidemiology, especially in western regions of Mongolia[6]. Previous studies have shown that in the neighboring provinces of Khovd and Bayan-Ulgii serological prevalence of antibodies against *E. granulossus* antigen B among semi-nomadic pastoralist is 5.2%[7]. For the first time, cysts of *E. multilocularis* have been found in the liver of infected vole collected at Khar Us Lake of Khovd Province[8]. Hydatid cysts representing larval stages of *E. multilocularis* were identified and the DNA sequenced from people who were evidently infected several years earlier and all were AE cases confirmed from the western regions of Mongolia[9]. The latest retrospective study involved the review of patients who had an operation for CE in three tertiary level hospitals in Ulaanbaatar. That study recommended creating a system at all levels of the health care system to triage and stage patients with CE into the four WHO treatment modalities[10].

Western Mongolian population is engaged in agriculture, semi-nomadic pastoralism, and its environmental conditions and nomadic lifestyle put it at high risk for zoonotic parasitic disease, yet virtually no little information exists on echinococcosis in this remote area. The induction of morbidity depends on the number, size and developmental status of the cysts (active or inactive), the involved organ, localization of the cysts within the organ, pressure of the cysts on the surrounding tissues and structures and defense mechanisms of the infected individual. Clinical signs of parasitization may occur after a highly variable incubation period of several months or years. Specially, clinical manifestations of human echinococcosis vary from asymptomatic infection to severe morbidity, and even mortality depending complications and the health status of those parasitized. Our study aimed to screen for human echinococcosis in two provinces which are considered to have the highest prevalence of parasitization in western Mongolia and then to determine the stage of their infection using the WHO-IWGE classification, and to identify the haplotype in cysts surgically removed from patients.

Materials and Methods

Study area

We conducted a cross-sectional study of echinococcosis in Bayan-Ulgii and Khovd provinces, which are both located in the western regions of Mongolia. A total of 18 soums (subdivision of province) including 11 from Khovd, 7 from Bayan-Ulgii were involved in our study and they bordered China (XUAR) and Russia (Figure 1).

Sampling method

Bayan-Ulgii and Khovd were selected based on the high prevalence identified in previous studies[6,7]. A simple random sampling method was used for choosing soums to study within provinces. According to the EpiInfo website, StatCalc-Sample Size and Power we estimated sample size in term of population survey or descriptive study. The population size of two provinces were 184,658 (According to the 2016 National Statistical Office data) with acceptable margin of error 0.1% and 99.9% Confidence Interval[11,12]. The required sample size was 1075 (design effect=1, cluster number=2). The actual sample size of people screened was 1063 people with a response rate of 98.9%.

Ultrasound screening

A screening abdominal ultrasound was performed on 1063 people, using a portable ultrasound scanner (model 3.5MG67N-35F2.4; Aloka, Tokyo, Japan). Sonograms were recorded using a thermal printer (Sony, Tokyo, Japan). The CE cases were recorded using the WHO Informal Working Group classification of ultrasonographic images[13]. The patients with newly diagnosed CE were advised by the researchers to seek a treatment at The

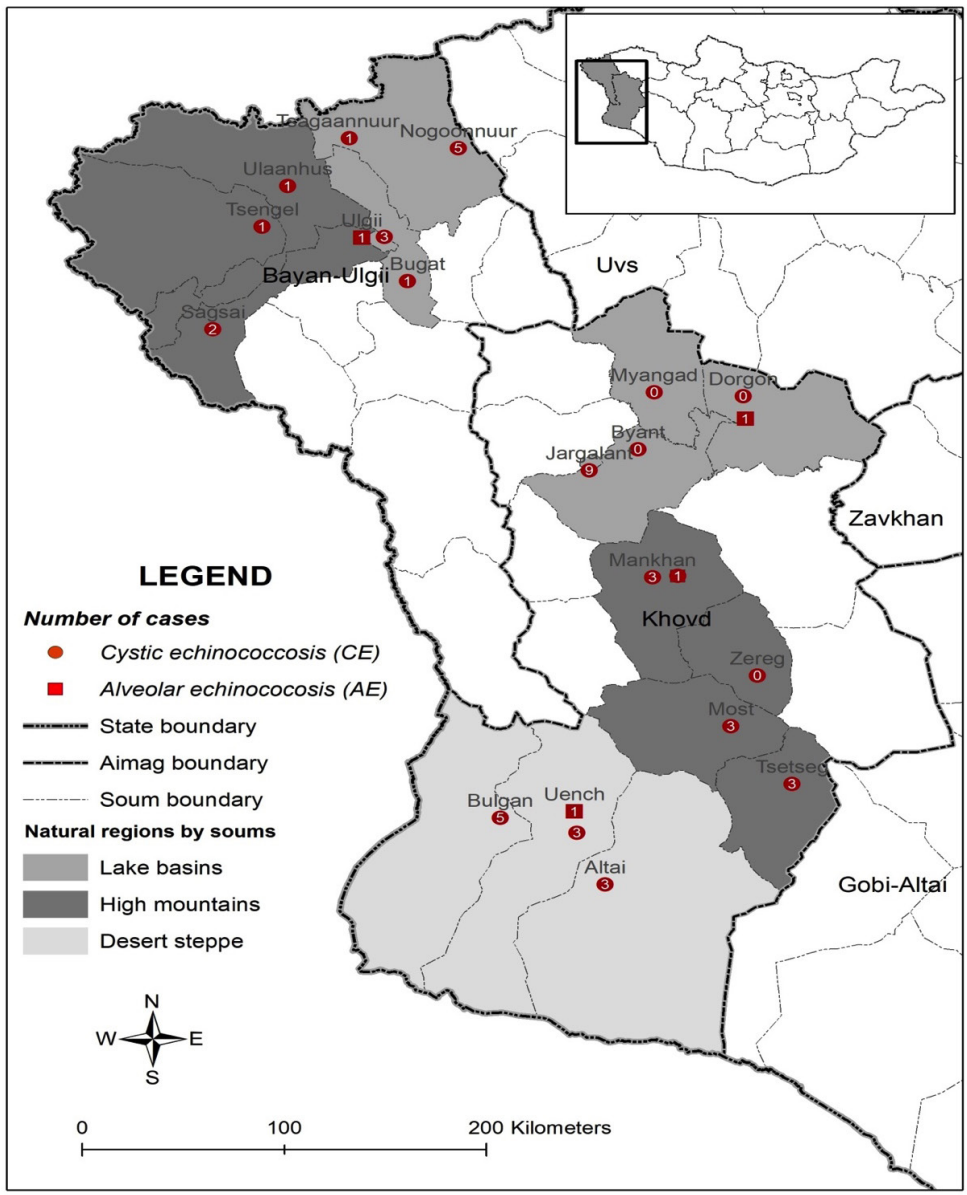


Figure 1. Map showing areas surveyed in Bayan-Ulgii and Khovd provinces and the number of cases of human echinococcosis identified.

First Central Hospital and National Center for Maternal and Child Health.

Serology test

Patients who had CE by ultrasound were recommended to have a serology test, as advised by the WHO[13]. Of the patients who had CE by ultrasound, 97.8% (n=88) had a serology test. Participants gave 2 ml venous blood sample for detection of antibodies against *E. granulossus*. We used a commercial Echinococcus IgG-ELISA (Nova Lisa TM) kit according to the manufacturer's instructions. Result were measured in Nova Tec Units (NTU) with a Cut-off: 10 NTU; Grey zone: 9-11 NTU; Negative: less than 9 NTU; Positive: greater than 11 NTU. When a grey zone result was encountered, the test was repeated 2-4 weeks later with a fresh serum sample.

DNA analysis

Genomic DNA was extracted from two samples of ethanol-fixed cysts from hepatic CE patients using a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 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[14,15]. 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). The sequences obtained were edited using Geneious Pro version 7.0.4 (created by Biomatters at http://www.geneious.com), and multiple alignments of each *cox* 1 haplotype were made by the program MAFFT with the homologous sequences of other Echinococcus species available in the GenBank database[14,16].

Statistical analysis

Quantitative variables were assessed to check the normality by Shapiro-Wilk test. For continuous variables, a mean and standard deviation were reported when the data was normally distributed, while median and range were reported when the distribution was not normal. Categorical variables were compared, using the Chi-square test when appropriate. A two-tailed p<.05 was considered statistically significant. All statistical analysis were performed using SPSS (SPSS Inc., IBM, Ch) and Microsoft Excel.

Ethical statements

Ethical approvals were obtained from the Medical Ethical Review Committee at the Ministry of Health, Mongolia. Informed consent (for adults) and informed assent (for children under 18 years old) forms were developed in local language. After providing an oral explanation of our study and answering the potential participant's questions, the written informed consent to participate in this research was obtained.

Results

Cases of echinococcosis in Bayan-Ulgii, Khovd provinces

The study involved 1063 people living in Bayan-Ulgii and Khovd provinces with the age of 5-96, the mean age of 45.5±14 years old. A total of 45 echinococcosis cases, Bayan-Ulgii n=15 (5.0%) and Khovd n=30 (3.9%) were identified during our screening. Of the 12 newly diagnosed cases of CE, 33.3% were in Bayan-Ulgii, 66.7% were in Khovd. Of 29 cases with previously diagnosed CE, 35.5% were in Bayan-Ulgii, 65.5% were in Khovd. Of 4 AE cases, 25% were in Bayan-Ulgii and 75% in Khovd. A summary of the cases and their demographic characteristics, including both new and previously diagnosed cases are shown in Table 1.

Table 1. Demographic characteristics of the study population among Echinococcosis and Healthy Groups (N=1063)

	Total (N)	CE N (%)	Healthy N (%)	p-value
Provinces				
Bayan-Ulgii	300	15 (33.3)	285 (28.0)	.685
Khowd	763	30 (66.7)	733 (72.0)	
Age				
<18	27	7 (15.6)	20 (2.0)	.0001
>=18	1036	38 (84.4)	998 (98.0)	
Sex				
Male	331	17 (37.8)	314 (30.8)	.529
Female	732	28 (62.2)	704 (69.2)	
Education				
Primary/Tertiary	143	6 (13.3)	141 (13.9)	.356
Secondary	799	39 (86.7)	760 (74.7)	
Occupation				
Herders and non-professional	629	24 (53.3)	605 (59.4)	.711
Others	434	21 (46.6)	413 (40.6)	
Total	1063	45 (4.2)	1018 (95.8)	

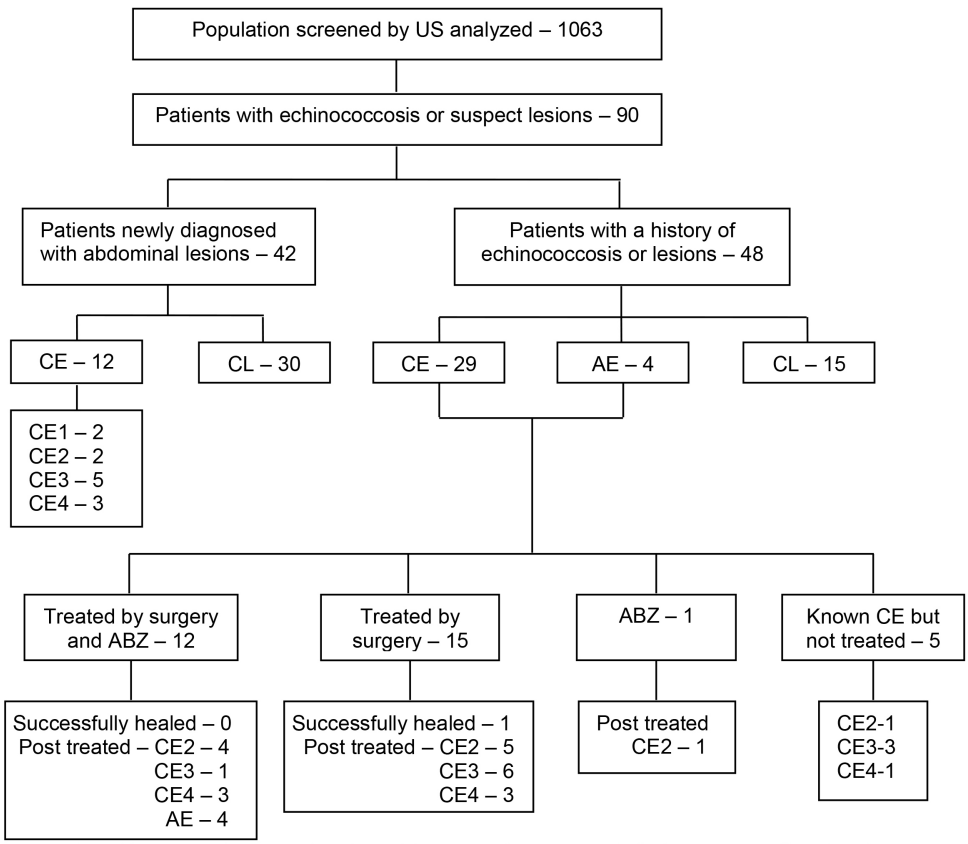
CE: Cystic Echinococcosis; *p-value calculated with Chi-Square Test

CE and AE were more common in people over 18 years of age (p<.0001). There was no statistically significant difference in the occurrence of CE between the populations of Bayan-Ulgii and Khovd (p<.685), between genders (p<.529), between different education levels (p<.356), and between the groups of people with different occupations (p<.711).

The 18 soums in Bayan-Ulgii and Khovd providences are located in different geoclimatic 3 zones defined as the desert steppe zone, high mountainous zone and lake basin zone. To study the effect of these zones on EC and AE occurrence, the cases were stratified based on zone. In desert steppe zone there were 12 cases (Bulgan soum 5, Altai 3, and Uench 4) representing a total 26.7% of all cases. In the high mountainous zone there were also 12 cases (Mankhan soum 4, Must 1, Tsetseg 3, Sagsai 2, Ulaankhus 1, Tsengel 1, and Zereg 0) forming a total 26.7% cases. In lake basin zone there were 21 cases (Nogoon nuur soum 5, Tsagaan nuur 1, Ulgii 4, Bugat 1, Jargalant 9, Durgun 1, Buyant 0, and Myangad 0), representing a total of 46.6% cases. The three geoclimatic zones all had patients with the AE. This showed that AE is prevalent in all of the geoclimatic zones of western Mongolia p<.05 (Figure 1).

WHO classification by ultrasound screening with serology test

We demonstrated by ultrasound that 90 of 1063 subjects (8.46%) had abdominal cysts, of which 46.6% (42 of 90) were newly diagnosed cases. Of these 42 patients, 12 (28.6%) had CE and 30 (71.4%) had a cystic lesion (CL), which is a non parasitic cyst as defined in the WHO classification. Out of 12 CE cases, four (33.3%) were in the CE1-2 (active stages of parasitization), five (41.7%) were in the CE3 (transitional stage), while three (25%) were CE4 (inactive stage) and none were CE5 (dead cyst stage). Out of 90 patients with abdominal cysts, 53.3% (48/90) had been diagnosed before and were being monitored by the SHCs. Of them (n=48), 29 (60.4%) were CE, four (8.3%) were AE, and 15 (31.3%) were with CL. Twenty-four of the 29 had been treated. As a result of the treatment, only 1 (4.2%) fully was cured, 10 (41.6%) were still in the active stage CE2, 6



CE: Cystic echinococcosis, AE: Alveolar echinococcosis, CL: Cystic lesion, ABZ: Albendazole

Figure 2. CE cases findings by ultrasound results and among CE treated and untreated patients

Table 2. WHO classification of ultrasound with serology test

Ultrasound results			ELISA results, by number		
Classifications		N=90	Positive	Negative	Total
Liver	CE1	2	1	1	2
	CE2	13	12	1	13
	CE3	15	13	2	15
	CE4	10	7	3	10
Treated CE		1	1	1	1
AE		4	1	1	2
Echinococcosis		45	34	9	43
CL		45	9	36	45
Total		90	43	45	88

CE: Cystic echinococcosis, AE: Alveolar echinococcosis, CL: Cystic lesion

Table 3. Sero-positive rate in ultrasound results of patients with previous treatments

Ultrasound results			ELISA results, by number (%)		
Classifications		N=28	Positive	Negative	Total
Liver	CE2	10	9 (90)	1 (10)	10 (100)
	CE3	7	6 (85.7)	1 (14.3)	7 (100)
	CE4	6	4 (66.7)	2 (33.3)	6 (100)
Treated CE		1		1 (100)	1 (100)
AE		4	1 (50.0)	1 (50.0)	2 (100)
Total		28	20 (76.9)	6 (23.1)	26 (100)

CE: Cystic echinococcosis, AE: Alveolar echinococcosis

(25%) were at the inactive stage CE4, and 7 (29.2%) were the cases that were transitioning from active into inactive stage CE3. Among those who had not been treated, 20% (1/5) were at the stage of CE2, 60% (3/5) were CE3, 20% (1/5) CE4 (Figure 2). All four patients who had treatment for AE were not cured. A total of 55 cysts were identified, mostly located in liver (92.7%), mainly in the right hepatic lobe (70.6%). Only one subject had cysts in both liver and kidney while another single subject had cysts in a lung. The median number of CE cysts per subject was 1 (IQR = 1–2, min 1, max 3). The median diameter of CE cysts was 3.6 cm (IQR = 2.7–4.7 cm, min 1.7cm, max 7.9 cm).

Eighty-eight CE and CL positive patients by ultrasound underwent serologic testing except. This did not include 2 patients with AE. Out of 43 echinococcosis cases, 34 were positive, 9 were negative but of 45 cystic lesion (CL) cases, 9 were positive, 36 were negative by serology test respectively (Table 2).

Indirect ELISA of patient serum was done to identify Echinococcus IgG among those patients who had been treated (Table 3). Out of those patients who had CE and AE treatment (n=28), 26 had ELISA. IgG was positive in 76.9% of these patients, which is higher than our expectation. In terms of CE classification, there was a higher seropositivity rate among patients with CE2 (90%) and CE3 (85.7%), followed by CE4 (66.7%). Echinococcus IgG among those who had treated was 14.48-67.0 NTU in CE2, 34.5-57.9 NTU in CE3, and was 12.6-44.4 NTU in CE4.

Molecular identification of Echinococcus species causing CE

Human CE species in the Bayan-Ulgii were identified by extracting genomic DNA for sequencing from two post-surgical specimens from hepatic CE patients. Nucleotide sequences of the *cox* 1 gene (1608-1609 bp) were determined from the two

specimens, and consequently two haplotypes were obtained. Phylogenetic analysis showed that 2 specimens (2 haplotypes) were *E. granulossus* s.s. (G1) and *E.canadensis* (G6/7). BLAST search revealed that the sequences were 100% identical to the *cox* 1 gene sequence of *E. granulossus* s.s. (G1) and *E. canadensis* (G6/7), (Accession Number KY766890, AB893246 and LC184603, AB893259 respectively).

Discussion

In this study, first we determined cases of human echinococcosis in Bayan-Ulgii and Khovd provinces. These are the most remote provinces of the Mongolia on the far western side of the country adjacent to the Altai mountains to the west and are sandwiched between China to the south and Russia to the north.

In these provinces, 45/1063 (4.23%) of people were infected with echinococcosis. Little published information seems to exist on the human echinococcosis epidemiological situation in Mongolia. Galbardrakh analyzed 846 CE cases from 21 hospitals in Mongolia from 1964 to 1970 and estimated that the prevalence rates of CE were 1.3/10000 in western provinces, 0.7-1.2/10000 in central provinces and 0.1-0.6/10000 in southern provinces[17].

Wang Y et al. compared the prevalence human echinococcosis of Hobusesar of Xinjang (China) bordering Bulgan soum of Khovd province (Mongolia)[6]. In Bulgan soum in the ultrasound prevalence of confirmed CE was relatively low, at 0.2% (4/1609). By contrast, the ultrasound prevalence of confirmed human CE in the community at Hobukesar was 2.7% (49/1844). The data from the mass screening of these communities in western Mongolia and northwest China confirmed that human CE was endemic and a potential public health problem[6]. Our study found the higher number of CE cases in comparison with previous study. However, our seroprevalance is slightly lower than the seroprevalence in Khovd and Bayan Ulgii, reported in a cross-sectional study of 334 semi-nomadic pastoralists[7]. They identified a 5.2% seroprevalence using ELISA for antibodies to the highly specific Echinococcus granulosus antigen B. These researchers found that only 10% of the adult subjects (aged ≥15 years) had heard of the disease and only 5% recognized hydatid cysts in their livestock[7]. These differences can be explained by the methods used for the investigations.

Our results for CE infections in Bulgan soum of Khovd and Hobukesar are similar to those in China, and with the 4 new CE cases in Bulgan soum of Khovd we concluded that prevalence of CE is 0.2%[6]. We identified 4 patients with CE in Bulgan soum of Khovd with CE. Out of them 1 had surgery, 2 had CE2, and 2 had CE3. One patient with CE3 had negative serology test result, the remaining 3 patients were positive. Of the total study population, patients with current echinococcosis in the liver was 3.9% in Khovd, 5.0% in Bayan-Ulgii. The CE incidence rate is 1.04% and 1.33% in the Khovd and Bayan-Ulgii study population, respectively. These provinces are quite similar in terms of the geographical location and socio-economic status, consequently the incidence is also similar. Our and other's results suggest that the CE incidence is similar among closer locations with similar socio-economic status[18].

Next, we found 12 (26.7%) of cases in desert steppe zone, 12 (26.7%) in the high mountainous zone, and 21 (46.6%) in lake basins, respectively. Another study also found AE in Microtus limnophilus in the area around Khar Us Lake in Khovd[8]. The most recent study we could identify found a high prevalence of alveolar echinococcosis in China[5]. These results suggest that AE exists in the intermediate host. Further studies need to determine the prevalence of echinococcosis in both definitive and intermediate hosts in western Mongolia.

To our knowledge, this is the first research in these two provinces of Mongolia identifying and classifying CE by ultrasound screening in line with WHO classifications, identifying Echinococcus IgG using ELISA testing to confirm ultrasound results, and assessing CE treatment outcomes. We performed a retrospective review of patients' medical records who had surgery for CE in three tertiary level hospitals in Ulaanbaatar city and then recommended to introduce and implement the WHO-IWGE expert consensus recommendations at all levels of the health care system to triage patients into the four WHO recommended treatment modalities. Implementation of these recommendations at the primary and secondary health care levels saves transport costs, travel time for patients and allows a substantial proportion of patients to stay near or in their homes during treatment[10].

In this study, we performed ELISA serologic testing on CE patients identified by ultrasound. Out of 43 echinococcosis cases, 34 (79%) were seropositive, 9 (20.9%) were seronegative but out of 45 cystic lesion (CL) cases, only 9 (20%) were seropositive, and 36 (80%) were seronegative. The high frequency of positive

IgG test results in patients with CE was higher than we expected and when compared to the low frequency of positive test results in patients with CL suggests that IgG testing may be useful in monitoring the transformation from CE to CL and vis versa. They also suggest that seropositive CL patients undergo US and repeat serologic testing a few months later to check for progression of their disease.

The comparison of ultrasound and serology test results shows that the seropositive rate was higher among CE2-3 patients (67.0 NTU), while it is decreasing in CE4 patients (3.6 NTU). Serology can also be used for complementary diagnosis both clinically and in community surveys, and it is important to correlate ultrasound and serological prevalence for both human AE and CE. A standardized clinical classification has been created for CE by the WHO Informal Working Group on Echinococcosis[13]. These classification systems are important to provide some indication of developmental or regressive changes in the lesion either naturally or after therapy. Such studies can provide useful insights on the immunological events that are associated with different clinical outcomes of the disease. For CE, seropositive rates are increased in the active (early) stage to the transitional (middle) stage and then decreased in the inactive (later) stages. It is possible that these morphological types, which represent a progression in the development of cysts from a highly viable unilocular cyst (CE1 and CE2) to a degenerating primary cyst (CE3) and finally to an inactive (CE4) or dead (CE5) cyst, may reflect the differing profiles of antigens produced and released to the host immune system by different developing stages of human CE[19,20]. Thus, it is important to improve the accurate diagnosis rate before operation by the combined use of different methods in the diagnosis of human echinococcosis[21].

We confirmed *E. canadensis* G6/7 and *E. granulossus* s.s. G1 by evaluating the *cytochrome c oxidase subunit* I gene of mitochondrial DNA 2 patients in Bayan-Ulgii showing that these exist in western Mongolia, which is consistent with findings by previous studies[22,23]. These species were also found in the Xinjiang Uygur Autonomous Region of China, 45 having *E. granulossus* G1, 2 having *E. canadensis* G6 genotype[24]. This finding indicates that these species are common both in Mongolia and neighboring China. A similar result has been reported in 4 patients who had histopathologically confirmed alveolar echinococcosis. The diagnosis in these four cases were confirmed by the molecular identification (evaluating the *cytochrome c oxidase subunit* I gene of mitochondrial DNA). Interestingly, three of these four cases were from western Mongolia, in particular from Bayan-Ulgii, Khovd, Uvs[9].

Our study has some limitations. The 1063 people involved in this study could not all have serology testing due to resource constrains. Only 88 patients had serology test to confirm the positive ultrasound results. Moreover, the regional Diagnostic and Treatment Centre in Khovd had some of the CE operations done at local hospitals, and we could not perform molecular identification of these cysts. Another limitation is the imbalance of human population from two provinces in the study. However, the sample size was statistically conclusive.

Future studies are needed to identify cases of human echinococcosis in other socioeconomic regions and asymptomatic cases in Mongolia. It is necessary to define risk factors, the definitive and intermediate hosts, to treat dogs with antihelminthics drugs regularly to control the spread of echinococcosis and to implement management and prevention plans to reduce its occurrence.

In conclusion, to our knowledge this is the first study reporting a high incidence of echinococcosis is recorded in western Mongolia. To our knowledge, it also the first time the WHO classification of CE patients in western Mongolia. The haplotypes identified in the Bayan-Ulgii population were *E. canadensis* and *E. granulossus* s.s.

Conflict of Interest

The authors state no conflict of interest.

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References

1. McManus D, Zhang W, li J, Barley PB. Echinococcosis. Lancet 2003; 362: 1295-304. doi:10.1016/S0140-

6736(03)14573-4.
2. Vuitton D, Zhou H, Bresson-Hadni S, Wang Q, Piarroux M, Raoul F, et al. Epidemiology of alveolar echinococcosis with particular reference to China and Europe. Parasitol 2003; 127: S87-107.
3. Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 2010; 114: 1-16. doi: 10.1016/j.actatropica.2009.11.001.
4. World Health Organization. The control of neglected zoonotic diseases: community based interventions for NZDs prevention and control: report of the third conference organized with ICONZ, DFID-RiU, SOS, EU, TDR and FAO with the participation of ILRI and OIE: 23-24 November 2010, WHO Heaquarters, Geneva, Switzerland 2011 [accessed on 15 September 2018]. Available at: https://apps.who.int/iris/handle/10665/44746.
5. Zhang W, Zhang Z, Wu W, Shi B, Li J, Zhou X, et al. Epidemiology and control of echinococcosis in central Asia, with particular reference to the People's Republic of China. Acta Tropica 2015; 141: 235-43. doi: 10.1016/j.actatropica.2014.03.014.
6. Wang Y, He T, Wen X, Li T, Waili TT, Zhang W, et al. Human cystic echinococcosis in two Mongolian communities in Hobukesar (China) and Bulgan (Mongolia). Trans R Soc Trop Med Hyg 2005; 99: 692-8.
7. Watson-Jones D, Craig P, Badamochir D, Rogan M, Wen H, Hind B. A pilot, serological survey for cystic echinococcosis in north-western Mongolia. Ann Trop Med Parasitol 1997; 91: 173-7.
8. Gardner SL, Dursahinhan AT, Rácz GR, Batsaikhan N, Ganzorig S, Damdinbazar D, et al. Sylvatic species of Echinococcus from rodent intermediate hosts in Asia and South America. Occasional Papers, Museum of Texas Tech University 2013. Available at:http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1769&context=parasitologyfacpubs.
9. Ito A, Agvaandaram G, Bat-Ochir OE, Chuluunbaatar B, Gonchigsenghe N, Yanagida T, et al. Histopathological, serological, and molecular confirmation of indigenous alveolar echinococcosis cases in Mongolia. Am J Trop Med Hyg 2010; 82: 266-9.
10. Bold B, Hattendorf J, Shagj A, Tserendovdon B, Ayushkhuu Ts, Luvsandorj A, et al. Patients with cystic echinococcosis in the three national referral centers of Mongolia: A model for CE management assessment. PLoS negl trop dis 2018; 12: e0006686. doi: 10.1371/journal.pntd.0006686.
11. EpiInfo StatCalc program Sample size. U.S. Department of Health & Human Services [accessed on 05 March 2016]. Available at: www.cdc.gov/epiinfo/.
12. Mongolian Statistical Information Service. Enumberation of human population 2016 [accessed on 01 March 2016]. Available at: www.1212.mn.
13. Group WHOIW. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. Acta Trop 2003; 85: 253-61.
14. Nakao M, Sako Y, Yokoyama N, Fukunaga M, Ito A. Mitochondrial genetic code in cestodes. Mol Biochem Parasitol 2000; 111: 415-24.
15. Konyaev SV, Yanagida T, Nakao M, Ingovatova GM, Shoykhet YN, Bondarev AY, et al. Genetic diversity of Echinococcus spp. in Russia. Parasitol 2013; 140: 1637-47. doi: 10.1017/S0031182013001340.
16. Transform biological data into knowledge and actionable insights. North America. Biomatters [accessed 02 May 2017]. Available at: http://www.geneious.com.
17. Galbadrakh D. Epidemiology and Prevention of Cystic Echinococcosis in the Republic of Mongolia [dissertation]. Ulaanbaatar, Mongolia: Mongolian National University of Medical Sciences; 1972.
18. Tamarozzi F, Hou A, Morales ML, Giordani MT, Vilca F, Mozo K, et al. Prevalence and risk factors for human cystic echinococcosis in the Cusco region of the Peruvian highlands diagnosed using focused abdominal ultrasound. Am J Trop Med Hyg 2017; 96: 1472-7. doi:10.4269/ajtmh.16-0882.
19. Yang Y, Craig P, Ito A, Vuitton DA, Giraudoux P, Sun T, et al. A correlative study of ultrasound with serology in an area in China co-endemic for human alveolar and cystic echinococcosis. Trop Med Int Health 2007; 12: 637-46.
20. Lissandrin R, Tamarozzi F, Piccoli L, Tinelli C, Silvestri AD, Mariconti M, et al. Factors influencing the serological response in hepatic Echinococcus granulosus infection. Am J Trop Med Hyg 2016; 94: 166-71. doi: 10.4269/ajtmh.15-0219.

21. Piccoli L, Tamarozzi F, Cattaneo F, Mariconti M, Filice C, et al. Long-term sonographic and serological follow-up of inactive echinococcal cysts of the liver: hints for a "watch-and-wait" approach. PLoS negl trop dis 2014; 8: e3057. doi: 10.1371/journal.pntd.0003057.
22. Jabbar A, Narankhajid M, Nolan MJ, Jex AR, Campbell BE, Gasser RB. A first insight into the genotypes of Echinococcus granulosus from humans in Mongolia. Mol Cell Probes 2011; 25: 49-54.
23. Ito A, Dorjsuren T, Davaasuren A, Yanagida T, Sako Y, Nakaya K, et al. Cystic echinococcoses in Mongolia: molecular identification, serology and risk factors. PLoS Negl Trop Dis 2014; 8: e2937. doi: 10.1371/journal.pntd.0002937.
24. Bart J, Abdukader M, Zhang YL, Lin RY, Wang YH, Nakao M, et al. Genotyping of human cystic echinococcosis in Xinjiang, PR China. Parasitology 2006; 133: 571-9.