



Evidence for camels (*Camelus bactrianus*) as the main intermediate host of *Echinococcus granulosus sensu lato* G6/G7 in Mongolia

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Abstract

Cystic echinococcosis (CE), the parasitic disease caused by the larval stage of *Echinococcus granulosus sensu lato* (s.l.), is a global public health problem. In Mongolia, despite wide distribution of human CE, not enough information is available on the prevalence and molecular characterization of CE in livestock and its zoonotic linkage with human cases. We investigated the distribution of human CE cases and livestock population using statistical models to get insight into the zoonotic linkage. The incidence of human CE cases increased by a factor of 1.71 for one interquartile range increment in the density of the camel population. No significant association was observed with other livestock species. The samples collected from 96 camels and 15 goats in an endemic region showed a CE prevalence of 19.7% and 6.7%, respectively. All livestock CE were *E. granulosus* s.l. G6/G7 species of the *E. granulosus* s.l. complex. The genetic diversity was investigated using the haplotype network based on full *cox1* gene analysis of the samples collected from livestock CE and nucleotide sequences previously reported from human CE and wild canids infection in Mongolia. Four haplotypes were identified within the livestock samples, two of which had not been previously reported. A common haplotype was identified among humans, camels, goats, and a wolf, all of which were within the same geographical area. A mixed infection of *E. granulosus* s.l. G6/G7 with different haplotypes in the intermediate host was identified. To the best of our knowledge, this is the most comprehensive description of the current epidemiological situation of CE in Mongolia with substantial evidence that camels might be the main intermediate host of *E. granulosus* s.l. G6/G7 in Mongolia. Moreover, our result presents the first report in the country to provide insight into the prevalence of *E. granulosus* s.l. G6/G7 in livestock.

Keywords *Echinococcus granulosus* s.l. G6/G7 · Cystic echinococcosis · Camel · Goat · Mongolia

Introduction

Cystic echinococcosis (CE) is the parasitic disease caused by the larval stage of *Echinococcus granulosus sensu lato* (s.l.).

The disease burden worldwide is estimated to be 184,000 disability-adjusted life years (DALYs), most of which are from pastoral communities due to their close contact with host animals (Budke et al. 2004; Torgerson et al. 2015). The main

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definitive host of the parasite is the dog when other canids as wolves but also in a lesser extent some felids in Africa may harbor the worms in their intestines (Romig et al. 2017). A wide range of livestock species including sheep, cattle, goats, camels, and pigs may act as intermediate hosts with the presence of hydatid cysts in the liver and/or lungs (Nakao et al. 2013a; Romig et al. 2015).

In Mongolia, nomadic pastoral farmers make up approximately one-third of the population raising more than 60 million livestock including sheep, goats, cattle, and camels, all of which are potential intermediate hosts (NSO 2017; Romig et al. 2017). Dogs are expected to be the predominant definitive host since all farmer family has more than one watch-dog (Gurbadam et al. 2010). Historically, CE prevalence was high in all parts of the country since the early twentieth century (Jezek et al. 1973; Jezek et al. 1971), but with an intensive control program supported by the former Soviet Union, the number of surgical cases decreased from 13% in 1946 to 2% in 1988 in the state hospital (Davaatseren et al. 1995; Ito and Budke 2015). The economic collapse in the 1990s followed by the privatization of veterinary sector hugely influenced the decline of control and prevention of zoonotic diseases in Mongolia (Ebright et al. 2003; Torgerson et al. 2006). In parallel, unregulated private slaughtering practices dramatically increased in rural areas of Mongolia (Gurbadam et al. 2010; McFadden et al. 2016). In slaughterhouses, infected offal is often available and consumed by free-roaming dogs, which is a major factor in perpetuating the CE transmission cycle (Craig et al. 2017). Currently, human CE cases are reported as aggregated data with other surgical diseases; therefore, official data is not available. More recent retrospective studies investigating surgical CE cases, however, reveal that almost half of all cases were children less than 15 years of age, strongly suggesting active transmission in the animal population (Bold et al. 2018).

E. granulosus s.l. is defined as a complex of five cryptic species: *E. granulosus* sensu stricto (s.s.), *Echinococcus felidis* (exclusively in Africa), *Echinococcus equinus*, *Echinococcus ortleppi*, and *Echinococcus canadensis* (Nakao et al. 2013a; Nakao et al. 2013b; Romig et al. 2017; Romig et al. 2015). The taxonomy concerning the latter species is still disputed, as it groups the previous four genotypes and strains: G6 (camel strain), G7 (pig strain), and the two cervid strains G8 and G10. Genetic analyses from mitochondrial genes and some nuclear genes have grouped genotypes G6 and G7 together in the same clade, with differentiation from the other two genotypes (Addy et al. 2017b; Yanagida et al. 2017) potentially regarding these genotypes as two different species (Laurimae et al. 2018a). Thus, the genotypes G6/G7 of *E. canadensis* will be designed here as *E. granulosus* s.l. G6/G7. Worldwide, most human cases (99.5%) are caused by *E. granulosus* s.s. and *E. granulosus* s.l. G6/G7 (Alvarez Rojas et al. 2014), while the species found in human CE cases in Mongolia are *E. granulosus* s.s. and *E. canadensis* (G6/G7 and G10) (Ito et al. 2014; Jabbar et al. 2011; Shirmen et al. 2018). One of

the few investigations on the definitive host of *Echinococcus* spp. in Mongolia found wolves infected with *E. canadensis* (G6/G7 and G10) from southern region through wild animal carcass surveys (Ito et al. 2013). There is, however, very limited information for the livestock populations due to the lack of public slaughterhouses and animal surveillance tools in the country (Chinchuluun et al. 2018; Deplazes et al. 2017). To date, there is not enough information on the molecular characterization of *Echinococcus* species causing CE in livestock (Yanagida et al. 2017). As a result, the transmission cycle of CE in Mongolia is largely unknown despite wide distribution of CE in the country. In our study, we provide the first investigation into the zoonotic linkage of CE between humans and animals in Mongolia using epidemiological and molecular approach.

Materials and methods

Data collection of human and animal population

Mongolia has 331 “soums” (smaller administrative unit of province) in 21 provinces and 1 city, Ulaanbaatar. Data on the human population was collected from the National Statistical Office (NSO) for adjustment of CE cases. We collected population data on sheep, camels, goats, and cattle as potential intermediate hosts of CE from the NSO (NSO 2017; Romig et al. 2017). Data were aggregated by livestock species by soum and year with a unit of 1000 heads. For the statistical analysis, the population density of each livestock species (expressed as number of animals per square km) was used as the exposure measure.

Data collection of human cases of CE

Data regarding patients recorded with the diagnosis of CE (ICD code 67.1–67.9) between 2006 and 2016 were extracted from the digital archive of Health Development Center of MoH. We collected information regarding age, sex, registration number, residential province, admission date, hospital name, and received treatment modalities. The analysis was restricted to surgical cases from tertiary-level hospitals, which represent approximately 98% of all surgical CE cases in Mongolia. Human cases of CE subsection were anonymized. The data was aggregated by year and province for further statistical analysis.

Data collection of molecular genetic information of human CE and definitive host

Forty-three CE cases were confirmed previously by histopathology at the National Center of Pathology in Ulaanbaatar (Ito et al. 2014). Among them, 29 cases including 17 children

were caused by *E. granulosus* s.l. G6/G7. Infections by *E. granulosus* s.l. G6/G7 were also diagnosed previously in one wolf each from both Gobi-Altai and Zavkhan provinces, which are in the southwestern part of the country (Ito et al. 2013). As the entire mitochondrial cytochrome c oxidase subunit 1 (*cox1*) was available in GenBank for these human and wolf cases, the corresponding haplotypes were included in the present study in order to obtain a more complete overview of the genetic diversity of *E. granulosus* s.l. G6/G7 in Mongolia.

Biological sample collection from intermediate host

Animal samples from camels and goats were collected from Khurmen and Khankhongor soums of Omnogobi province, which has distance of 330 km from each other. The animals were being processed as part of the normal work of the slaughterhouse. Omnogobi province is located in the Gobi Desert toward the southern part of the country, with a climate ranging from -20 to -30 °C in winter and $+30$ to $+38$ °C in summer. The province has approximately 60,000 people in an area measuring 165,000 km². The majority of this area is used for nomadic pastoral farming. The province has 2%, 6%, 0.5%, and 30% of the total sheep, goat, cattle, and camel populations in the country, respectively (Appendix 2) (NSO 2017). Omnogobi was selected for the sampling in this research because according to the previous report, it had the highest number of surgical CE cases (Bold et al. 2018).

Samples were collected from a site in Khankhongor soum and three sites in Khurmen soum in December 2016. November to December is the winter meat preparation period in Mongolia. The camels and goats included in this study were to be used as winter meat storage for the local military. A total of 96 camels and 15 goats from 9 farms were slaughtered under the supervision of a local veterinarian for the meat preparation in winter. All organs of the pleural and abdominal cavities were inspected to determine the presence of hydatid cysts. When cystic lesions were detected, the cyst contents and wall were kept in a tube containing 96% alcohol. Sampling site, animal species, and organ affected were recorded for each sample. The samples were transported to the central laboratory in a portable freezer where it was stored at -20 °C. After thawing, cyst material was incised with a scalpel. The internal layer of the cyst was applied to an FTA® card for 20 s to impregnate the paper with parasite cells (Boue et al. 2017). The FTA® cards were dried at room temperature for 24 h before shipment to the laboratory (Anses, Malzéville, France) for genetic analysis. One to four hydatid cysts per animal was sampled using the FTA card (Appendix 1).

Statistical analysis

Quantitative variables were described using mean and interquartile range (IQR) and qualitative variables using absolute

and relative frequencies. To investigate the link between human CE cases and livestock population, we developed multivariable mixed negative binomial regression models for the total number of cases and the number of cases among children under 15 years of age per province and year. As sheep, goats, cattle, and camels are all potential intermediate hosts, their population densities by province and year were included as predictor variables in the full model. The human population was used as the offset variable to adjust for differences in population size. Random intercepts at the level of provinces were also included in the models to adjust for potential geographic clustering. A potential effect of hospital accessibility on observed counts of CE cases was assessed using an indicator variable for provinces along the railroad. Significant relations were illustrated by a map showing mean surgical incidence at the province level and animal population density at the “soum” level between 2006 and 2016. All analyses and maps were done using the statistical package R v 3.4.0.

Molecular analyses and haplotype network

DNA extraction was performed as previously described using approximately 1 cm square of each impregnated FTA card (Boue et al. 2017). After lysis, the iPrep Charge Switch gDNATissue kit (Invitrogen) was used with the iPrep purification instrument to extract DNA. The entire *cox1* gene (1608 bp) was sequenced with a nested PCR using two pairs of primers to amplify the gene in one piece (Addy et al. 2017a). The nucleotide sequences were analyzed and aligned using the Vector NTI software program (Invitrogen). A network of the *cox1* haplotypes was drawn using statistical parsimony with TCS 1.2 software (Clement et al. 2000) and online tcsBU software (Murias dos Santos et al. 2016) to highlight the diversity and relationships among the different haplotypes. The *E. granulosus* s.l. G6/G7 haplotypes of the complete *cox1* gene from the humans ($n = 29$) and wolves ($n = 2$) identified in Mongolia were added to the haplotype network (Ito et al. 2013; Ito et al. 2014). In order to increase the number of sequences in the haplotype network and to place the Mongolian sequences in a broader geographical context, sequences from 94 samples from 6 intermediate host species originating from 15 different countries obtained by Laurimae et al. (2018b) were added.

Results

Statistical analysis

A total of 446 surgical cases were reported from 2006 to 2016 in Mongolia. The median age of CE cases was 23.0 years (IQR = 10.0–44.7). The percentages of males and females were 44% and 56%, respectively (p value = 0.01). The incidence of surgical cases was 1.4 per 100,000 person years. The

median densities of livestock for sheep, goats, cattle, and camels were 15.3 (IQR 9.36–24.48), 15.0 (IQR 10.76–19.92), 1.96 (IQR 1.00–3.76), and 0.10 (IQR 0.04–0.23) per 1 km², respectively. Human CE cases were significantly related to camel density in the model with all animal species (Table 1). CE incidence across all ages increased by a factor of 1.71 (1.42–2.04) for an IQR increment in camel density (i.e., 1 camel per 1 km²) and the respective increase was 2.08 (1.66–2.61), among children under 15 years of age. There were no significant associations between human CE cases and other animal species densities in the first model. In the second model with an indicator variable for provinces with railroad stations, the incidence rate ratio associated with camel density was 1.78 (1.55–2.05) and the negative association with cattle density became statistically significant. Living close to a railroad increased human CE incidence significantly by a factor of 1.6 (1.2–2.1) compared to living farther away from railroads. In provinces without railroad connections, the incidence rate ratio associated with camel density increased to 1.88 (1.61–2.20) while no significant associations were observed with other animal densities.

We also estimated and plotted marginal CE incidence rate as a function of camel population density. The positive association between camel density and human CE cases is illustrated in Fig. 1.

The highest incidences of CE were reported from southern provinces including Omnogobi, Dornogobi, Dundgobi, and Bayankhongor with 7, 3, 4, and 3 cases per 100,000 person years, respectively (Fig. 2). Northern provinces including Arkhangai, Orkhon (a former city), Dornod, and Ulaanbaatar city had the lowest case numbers per 100,000 person years. In parallel, camel population densities were also higher in southern as compared to northern provinces. The highest CE incidence and the

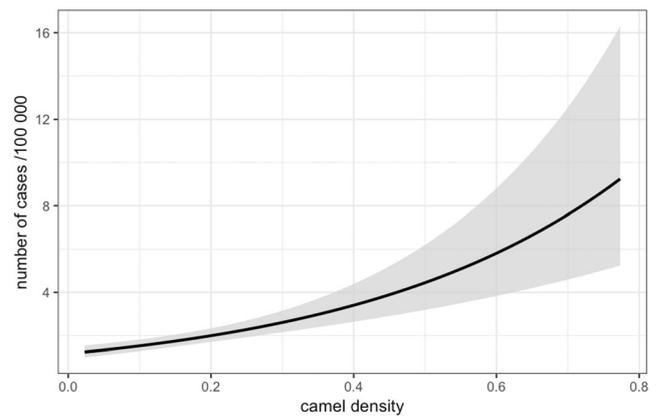


Fig. 1 The predicted incidence of surgical CE cases in humans by camel population density, 2006–2016 (number of camels per 1 km²)

highest camel population density were both observed in the province of Omnogobi.

Molecular genetic analysis

The species *E. granulosus* s.l. G6/G7 was identified in all the 33 FTA samples isolated from 19 camels and 1 goat. The CE prevalence was estimated as 19.7% (CI95% 12.4–29.2%) in camels and 6.7% (CI95% 1.7–32.0%) in goats. The nucleic sequence of the entire *cox1* gene was obtained from 32 samples as only partial results were obtained for one camel sample despite successive assays. Four haplotypes (numbered 1 to 4) were identified with one to four point-mutations (only substitutions) within each other (Appendix 1). Haplotypes 1 and 3 were reported previously in GenBank from the human samples collected in Mongolia (Ito et al. 2014). The haplotypes 2 and 4 had not been reported before. The haplotype 2 is identified as 99% (1607/1608 bp) with haplotypes EcMGL6 from Mongolia and Ec01 from African countries, while haplotype 4 is identified as 99% (1607/1608 bp) with EcMGL15 from Mongolia (Addy et al. 2017b; Ito et al. 2014). As identified in 9 of the 20 infected animals, haplotypes 1 and 2 were the most represented ones, whereas haplotypes 3 and 4 were identified in only three and one animal(s), respectively. The presence of two different haplotypes was observed in two camels with presence of haplotypes 1 and 2 in both cases. The haplotype network reveals a double star-like configuration (Fig. 3), where haplotypes designed as genotype G6 are around haplotype 1 when the haplotypes designed as G7 are hap3, hap4, haplotype 3 and haplotype 4 and those around them. Haplotype 1 identified in this study was previously described in the same species of intermediate hosts (camels, goats, human) from Iran, Argentina, Kenya, Sudan, and Mauritania but also including the haplotype Gmon from a human case of CE in Mongolia. No other correspondence with haplotypes from Laurimae et al. (2018b) was found with haplotypes 2 to 4 described in this study as well as for the haplotypes from human CE cases described by Ito et al. (2014). Concerning only Mongolia, haplotype 1 appears

Table 1 Model prediction of human CE cases and livestock density population

Covariates	Estimate ^a	95%LCL	95%UCL	P value
Camel density	1.71	1.42	2.04	<0.0 01
Sheep density	1.13	0.71	1.8	0.61
Cattle density	0.91	0.8	1.03	0.14
Goat density	0.91	0.7	1.18	0.46
Covariates	Estimate ^a	95%LCL	95%UCL	P value
Camel density	1.71	1.42	2.04	<0.0 01
Sheep density	1.13	0.71	1.8	0.61
Cattle density	0.91	0.8	1.03	0.14
Goat density	0.91	0.7	1.18	0.46

LCL lower confidence level, UCL upper confidence level

^a The estimate for the IQR increment in the density of the respective animal population

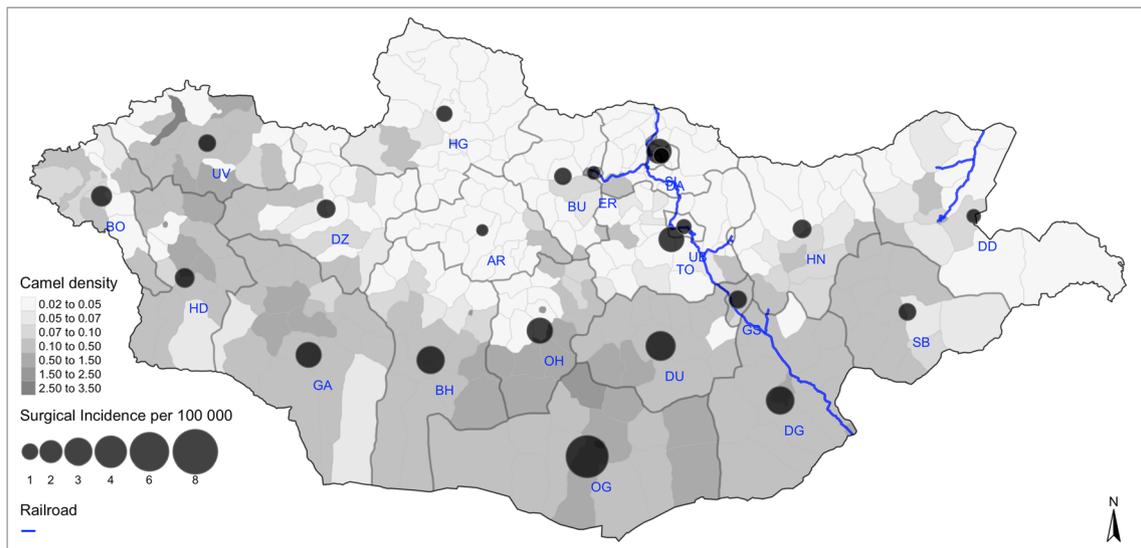


Fig. 2 Geographical distribution of camel density (in number of camels per 1 km²) and incidence (in number of cases per 100,000 person years). The average camel density between 2006 and 2016 is plotted as base-layer for each soum (gray choropleth). The mean annual incidence of surgical CE cases per 100,000 between 2006 and 2016 is plotted for each province (black circle). Abbreviations of provinces: BO Bayan-

Olgii, UV Uvs, HD Khovd, DZ Zavkhan, GA Gobi-Altai, BH Bayankhongor, OH Ovorkhangai, AR Arkhangai, HG Khubs gul, BU Bulgan, DA Darkhan, SL Selenge, ER Orkhon, UB Ulaanbaatar, DU Dundgobi, OG Omnogobi, TO Tov, DG Domogobi, HN Khentii, DD Dornod, GS Gobisumber, SB Sukhbaatar

to be predominant in Mongolia with identification in camels and humans (corresponding to haplotype EcMGL2 (Ito et al. 2014)), when one of the two wolves as definitive hosts also harbors this haplotype. There are generally few mutations between the

different haplotypes from Mongolia (with a maximum of seven), independent of the species and the origin areas of the samples. The haplotype 2 was the most prevalent in camels, along with haplotype 1 which was also found in humans. Haplotype 3 was

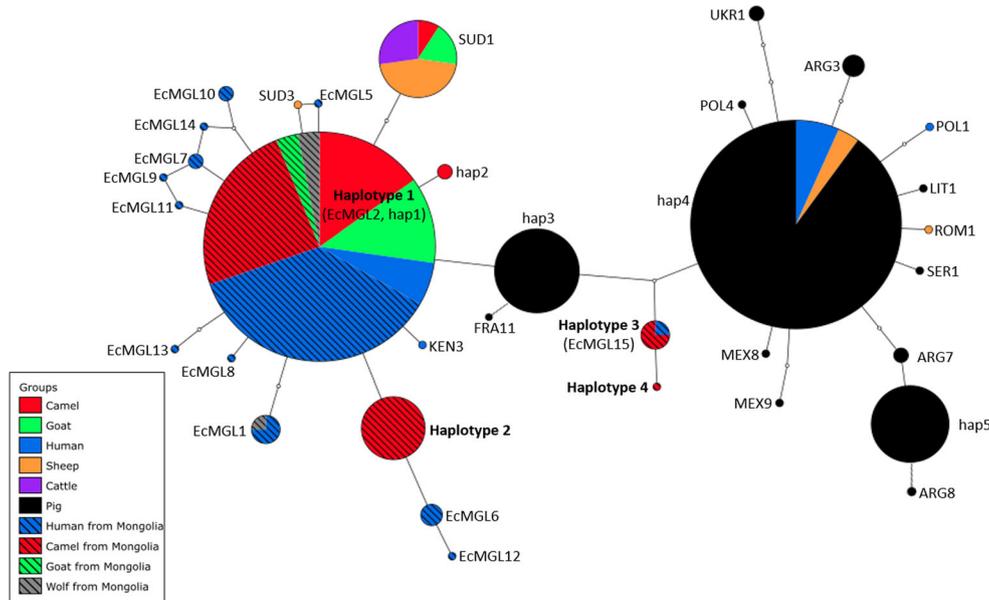


Fig. 3 Haplotype network is based on the full cox1 gene (1608 bp) using statistical parsimony of the *E. canadensis* G6/G7 samples from camels and goats (this study), humans and wolves (Ito et al. 2013; Ito et al. 2014), and 94 samples from 15 countries described by Laurimae et al. 2018b. The worldwide collection composed by G6 samples ($n = 27$) originated from Africa (Sudan, $n = 14$; Kenya, $n = 3$; Mauritania, $n = 2$), Middle East (Iran, $n = 4$), Asia (Mongolia, $n = 1$), and South America (Argentina, $n = 3$). Genotype G7 samples ($n = 67$) originated from Europe (Spain, $n = 1$;

France, $n = 27$; Italy, $n = 2$; Serbia, $n = 1$; Romania, $n = 2$; Ukraine, $n = 2$; Poland, $n = 5$; Lithuania, $n = 1$), Central America (Mexico, $n = 10$), and South America (Argentina, $n = 16$). For camels, only one sample per animal was included with the exception of two camels, which harbored two haplotypes. So, one of both haplotypes was retained. Size of the circle is proportional to the number of samples. The smaller white points represent each mutational steps/hypothetical haplotypes

found in camels and in one human case (EcMGL15). The haplotype 4 was identified only in one camel, but it differs from haplotype 3 by only one mutation. In the southern Omnogobi province, where the camels originated, haplotype 1 was identified in camels, goat, and humans (Fig. 4).

Discussion

To the best of our knowledge, the current research provides the most comprehensive description of the current epidemiological situation of both human and livestock CE in Mongolia. An important finding of our study is that we identified the common haplotype among *E. granulosus* s.l. G6/G7 in humans, camels, goats, and a wolf in the same region. This haplotype also corresponds to the main haplotype for G6 identified worldwide by Laurimae et al. (2018b). Our results provide substantial evidence indicating that camels are the main intermediate host of the *E. granulosus* s.l. G6/G7 species of the *E. granulosus* s.l. complex in Mongolia. Moreover, this is the first report from Mongolia that provides insight into the prevalence of *E. granulosus* s.l. G6/G7 in goats and camels, with confirmation of molecular species diagnostic, though the sample size and number of sampling locations needs to be increased. Interestingly, the haplotypes 3 and 4 appear to be more designed as G7 rather than G6 which was more expected. It highlights the interest to analyze additional genes or even the entire mitochondrial genomes as complete *cox1* gene has proved to potentially not correctly differentiate between G6 and G7 genotypes (Laurimae et al. 2018b). In this context,

it will be relevant to obtain more genetic information about haplotypes found in Mongolia when the only one sample from this country in the study of Laurimae et al. (2018b) resulted to be highly divergent while it correspond to the main haplotype described in this study using only *cox1*.

All cases of camel CE were caused by *E. granulosus* s.l. G6/G7. Even more importantly, a common haplotype exists between camels and humans, which is notably the main representative haplotype for both the species and is found in the same geographic area. A maximum of two mutations was identified between the haplotypes from human and animal samples. The two haplotypes M2 and M3 identified in human cases of *E. granulosus* s.l. G6/G7 using short *cox1* sequences from Jabbar et al. (2011) correspond to haplotypes 1 and 2 and haplotypes 3 and 4, respectively, as both pairs are identical for the 363 bp concerned. A higher genetic diversity may be expected in this regard if camels comprised a larger sample, including additional areas, possibly leading to the identification of other shared haplotypes from previously identified human cases, since camels appear to be the main intermediate hosts for *E. granulosus* s.l. G6/G7 in Mongolia. The molecular investigation conducted on human CE cases in Mongolia found that most children cases were caused by the *E. granulosus* s.l. G6/G7 species, while those of adults were caused by both *E. granulosus* s.l. G6/G7 and *E. granulosus* s.s. (Ito et al. 2014; Shirmen et al. 2018). Therefore, our results indicate the importance of camel role in current burden of echinococcosis in Mongolia. However, further research is required to assess the prevalence of CE in sheep and goats, in order to confirm this observation.

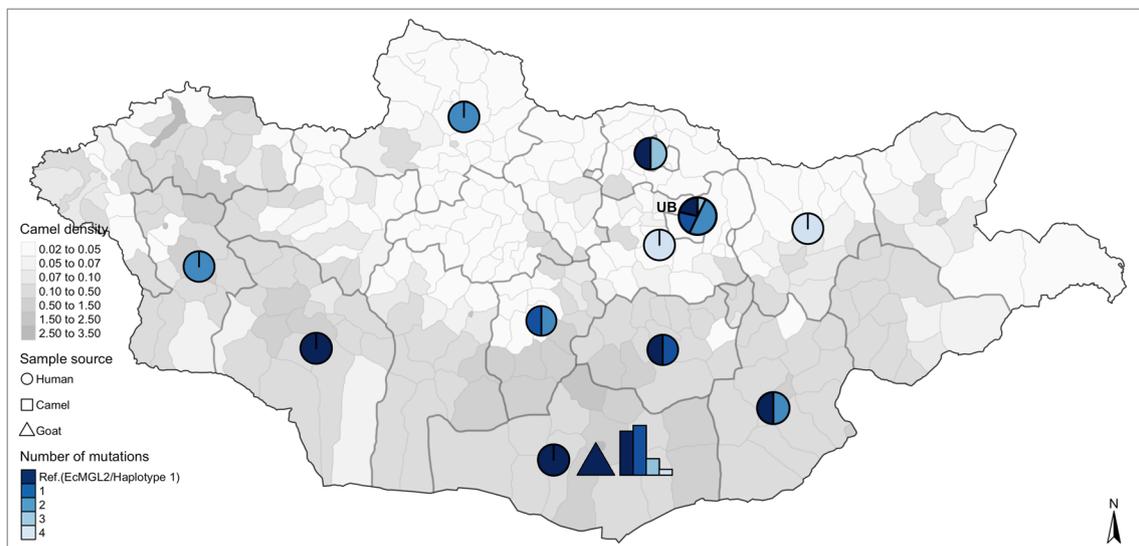


Fig. 4 Geographical distribution of haplotypes of *E. granulosus* s.l. G6/G7 in Mongolia. The average camel density between 2006 and 2016 is plotted as a base-layer for each “soum” (gray choropleth). Distributions of *E. canadensis* G6/G7 from human, camel, and goat cases are presented by pie chart, bar graph (square), and triangle point, respectively. The genetic

differences in both human and animal samples are presented by number of mutations (blue color scale) compared to haplotype EcMGL2 (Ito et al. 2014) (haplotype 1 in current study) as a reference. UB Ulaanbaatar, the capital city of Mongolia

This study provides the first report on *E. granulosus s.l.* G6/G7 infection of goats in Mongolia (Deplazes et al. 2017). Goats are the main animals that share pasture with camels in Mongolia (Appendix 2). Our statistical analysis, however, did not find an association between human CE cases and goat population density. Although our results might be suggesting that goats are more of a spillover host for *E. granulosus s.l.* G6/G7, we cannot draw firm conclusions without additional parasite data of CE-infected goats from other southern provinces with a high prevalence of human CE. Further investigation is required to clarify the role of goats in the transmission of CE and *E. granulosus s.l.* G6/G7, including comparisons of the grazing and/or drinking behavior of and ecology among these livestock (Lawson and Gemmell 1983).

Interestingly, in the liver cysts of two camels included in the sample, two different *cox1* haplotypes (1 and 2) were identified, indicating mixed infections. This could be explained by successive infections, simultaneous infection with eggs from a definitive host harboring worms with both haplotypes, or possibly even contamination of the same area by two definitive hosts harboring worms with different haplotypes. Mixed infection was previously reported for *E. granulosus s.s.* in studies that tested multiple cysts from the same intermediate host, but this occurs infrequently (Boufana et al. 2014; Casulli et al. 2012; Umhang et al. 2014; Umhang et al. 2013). To the best of our knowledge, this is the first time that the presence of mixed infections of *E. granulosus s.l.* G6/G7 with different haplotypes has been confirmed in the intermediate host. These mixed infections involve the two most frequent haplotypes identified in the camels described here (Appendix 1).

We identified the common haplotype in one of the two wolves which were previously described (Ito et al. 2013). There might be marked differences concerning the accessibility of intermediate hosts' offal, for definitive hosts to be infected. Camel offal is more likely to be left in the field during the slaughtering process owing to its large volume and can become accessible to wild canids and stray dogs, whereas offal from small ruminants would be collected by the herders to feed their own dogs. Another consideration is that the previously reported human CE samples were collected in 2009, while our field survey was conducted in 2016, which might also highlight consistency regarding the source of infection in the same geographic region.

We were unable to consider infections in dogs due to the absence of existing data. Every rural family has at least one to two watch-dogs. In particular, the children in these rural areas are highly exposed to dogs, as discussed in the national stakeholder meetings (Gurbadam et al. 2010). A nationwide surveying of dogs and livestock remains an urgent task to gain an overview of the current CE endemicity in Mongolia. While *E. granulosus s.s.* is still an important agent in Mongolia, in areas with high camel density, the presence of *E. granulosus s.l.* G6/G7 needs to be considered as an equally significant threat to public health, especially for children. The insights provided by this paper

suggest that priority interventions should aim at affecting behavioral change by improving hand hygiene, proper disposal of offal at home, private slaughtering, and deworming of dogs.

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Compliance with ethical standards

Ethics statement This work presented here was approved by the Medical Ethics committee of Mongolia (July 2014) and WHO ERC (27 Nov 2015).

Ethical approval The animals were being processed as part of the normal work of the abattoir. The routine investigation of local veterinary office on animal carcass do not require ethical approval in Mongolia.

Conflict of interest The authors declare that there is no conflict of interest.

Research data Statistical data of hospital discharge is available upon request from the National Center for Zoonotic Disease (NCZD). The director of NCZD, Dr. Tsogbadrakh Nyamdorj, is the point of contact. Email address is: tsogbadrakh@nczd.gov.mn.

All relevant data regarding animal sample is within the manuscript and its Supporting Information files.

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