



Surveillance of *Echinococcus* isolates from Qinghai, China



Junying Ma^{a,1}, Hu Wang^{a,1}, Gonghua Lin^{b,*}, Fang Zhao^b, Chao Li^a,
Tongzuo Zhang^b, Xiao Ma^a, Yongguo Zhang^c, Zhibin Hou^d, Huixia Cai^a,
Peiyun Liu^a, Yongshun Wang^a

^a Qinghai Institute for Endemic Disease Prevention and Control, Xining 811602, China

^b Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China

^c Welfare Charity Hospital of Qinghai Province, Xining 810007, China

^d Xining No. 2 People's Hospital of Qinghai Province, Xining 810003, China

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ABSTRACT

Echinococcosis is highly endemic over large parts of the Qinghai–Tibet Plateau (QTP), China. Based on a large number of samples, we present data on the current presence, host distribution, and genetic diversity of *Echinococcus* in the Qinghai Province, located in the northeastern corner of the QTP and constituting >25% of the area of the plateau. We used 521 samples (including 451 newly collected samples and 70 samples from our previous study) from humans, yaks, sheep, goats, dogs, fox, plateau pikas, and voles in 36 counties, and genotyped them using the mitochondrial DNA marker cytochrome oxidase subunit I (cox1) gene and the maximum parsimony and Bayesian reconstruction methods. Based on the 792 bp sequence matrix, we recorded 177 variable sites; 157 were parsimony-informative. A total of 105 haplotypes (H1–H105) were detected, of which H1–H15 and H90–H104, H16–H17, H18–H89, and H105 belonged to *Echinococcus shiquicus*, *Echinococcus multilocularis*, *Echinococcus granulosus*, and *Echinococcus canadensis*, respectively. Our results showed that, (i) the Qinghai Province was under a high burden of *Echinococcus* epidemiology; (ii) *E. granulosus* was the main echinococcosis threat to the local people, and the followed is *E. multilocularis*; (iii) there are a considerable number of haplotypes shared by domestic animals (sheep, yaks, and dogs) and humans, demonstrating the close relationship between human and domestic animals epidemiology; (iv) the threat of *E. shiquicus* on humans and livestock can be mostly ignored, while the infection risk of *E. canadensis* echinococcosis should not be neglected.

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1. Introduction

Echinococcosis, which is caused by members of the genus *Echinococcus* (Cestoda: Taeniidae), is a serious helminthic zoonosis in humans, livestock, and wildlife. To

date there are nine valid *Echinococcus* species: *E. granulosus*, *E. equinus*, *E. canadensis*, *E. felidis*, *E. ortleppi*, *E. multilocularis*, *E. oligarthrus*, *E. vogeli*, and *E. shiquicus* (Nakao et al., 2013). The life cycle of each species involves two mammals, including an herbivorous or omnivorous intermediate host (rodents, ungulates, humans, etc.) and a carnivorous (canids and felids) host (Eckert et al., 2001). In animals and humans, infection with the intermediate cystic stage of all species of *Echinococcus* causes severe disease symptoms, incapacity, and even death (Jenkins et al., 2005).

* Corresponding author. Tel.: +86 15909717366; fax: +86 971 6143282.

E-mail address: lingonghua@gmail.com (G. Lin).

¹ These authors contributed equally to this article.

Table 1The host and geographical origin of 521 *Echinococcus* samples used in this study.

ID	County	Human	Yak	Sheep	Goat	Dog	Fox	Pika	Vole	Sum
1	Banma	2					1	12		15
2	Chengduo	19				5		13	1	38
3	Dari	10	5			10		18	1	44
4	Datong	1								1
5	Gande	5	20					1		26
6	Gangcha	11		1						12
7	Geermu			1				3		4
8	Gonghe	2	8	14				7		31
9	Guide	1								1
10	Guinan							6		6
11	Haiyan	4	1	22				1		28
12	Henan	3	12	16						31
13	Hualong	2								2
14	Huangyuan	1								1
15	Huangzhong	4								4
16	Jianzha	2								2
17	Jiuzhi	5	15			3		24	3	50
18	Ledu	2								2
19	Maduo	7								7
20	Maqin	12	13	13		3		8	1	50
21	Menyuan	1	1					2		4
22	Nangqian	4								4
23	Qilian	5		1				1		7
24	Qumalai	1	2							3
25	Tanggula						1			1
26	Tianjun	2	1	5						8
27	Tongde	3	20	1				2		26
28	Tongren	4	1					9		14
29	Wulan	6	6	5						17
30	Xinghai	2	11	9				6		28
31	Xining	9		3						12
32	Xunhua	3								3
33	Yushu	11		3	2					16
34	Zaduo	9								9
35	Zeku	7								7
36	Zhiduo	3	2	1				1		7
	Sum	163	118	95	2	21	2	114	6	521

A few *Echinococcus* species (especially *E. granulosus* and *E. multilocularis*) are associated with a considerable global health burden in humans and a substantial financial burden on livestock industries (Torgerson and Macpherson, 2011).

Echinococcosis is highly endemic over large parts of the Qinghai–Tibet Plateau (QTP), China (Craig et al., 2008). Qinghai Province is located in the northeastern corner of the QTP, constituting >25% of the area of the plateau, and has been repeatedly demonstrated to have a considerable burden of echinococcosis (Ma et al., 2008; Wang et al., 2014). Species of *Echinococcus* prevailing in the province have been clarified by molecular taxonomic studies using mtDNA markers (e.g. Yang et al., 2005; Xiao et al., 2005; Ma et al., 2008; Li et al., 2008; Nakao et al., 2010; Zhong et al., 2014). However, sampling in most of these studies has been either geographically restricted or had a limited sample size, both largely restricting our knowledge of the prevailing *Echinococcus* in this region. A previous study from our lab collected and genotyped 70 samples from the Qinghai Province (Ma et al., 2012). Since then, we have strived to collect every accessible sample from all regions of the province, and have finally gathered several hundred more samples. Based on this large number of samples, we present data on the current presence, host distribution, and genetic diversity of *Echinococcus* in Qinghai Province.

2. Materials and methods

2.1. Parasite samples

As mentioned in our previous study (Ma et al., 2012), larval and adult specimens of *Echinococcus* spp were collected from sheep (*Ovis aries*), yaks (*Bos grunniens*), goats (*Capra aegagrus hircus*), pikas (*Ochotona curzoniae*), voles (*Microtus fuscus*), unclaimed dogs (*Canis lupus familiaris*, at necropsy), Tibetan foxes (*Vulpes ferrilata*, at necropsy), and humans (from surgical cases at local hospitals) in Qinghai, China. *Echinococcus* tapeworms collected at necropsy from canine intestines were unwound in tap water (to wash away the dirt) and then fixed in 95% ethanol. Hydatid cyst tissues from intermediate hosts were collected at livestock slaughter or from trapped small mammals and fixed in 95% ethanol.

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the larval and adult samples using a spin column kit (DNeasy tissue kit; Qiagen, Germany). The partial mitochondrial *cox1* sequences were amplified in a 50 µL reaction volume and the PCR products

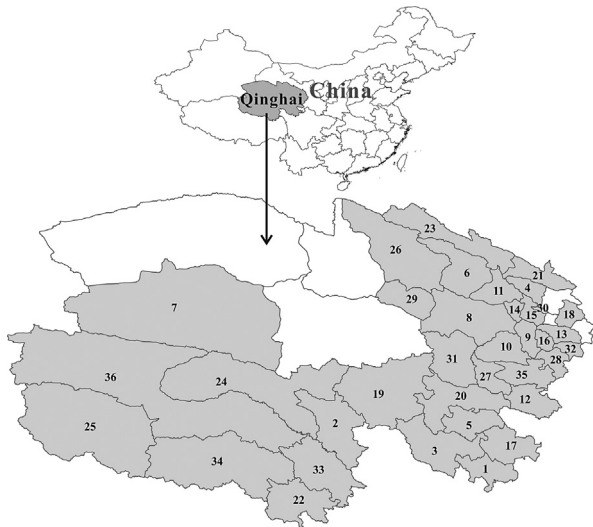


Fig. 1. Sampling sites (gray counties) of *Echinococcus* in the Qinghai Province (the numbers 1–36 correspond the county IDs in Table 1).

were purified and sequenced using the same primers used for amplification (see Ma et al., 2012 for details).

2.3. Data processing

Sequences were aligned using CLUSTAL X (Thompson et al., 1997) with the default settings and refined manually. These new sequences and our formerly published sequences were combined for data analysis. The number of variable sites and haplotype distributions were determined using DnaSP v5 (Librado and Rozas, 2009). We also calculated the haplotype share indices between each host: (i) The percentage of shared haplotypes (PH) = $N_S / (N_1 + N_2 - N_S) \times 100\%$; where: N_1 , number of haplotypes from host 1; N_2 , number of haplotypes from host 2; and N_S , number of shared haplotypes between host 1 and 2. (ii) The percentage of individuals involved in shared haplotypes (PI) = $I_S / (I_1 + I_2) \times 100\%$; where: I_1 , number of individuals from host 1; I_2 , number of individuals from host 2; and I_S , number of individuals from either host 1 or host 2 which were involved in shared haplotypes.

Both maximum likelihood (ML) and Bayesian Inference (BI) methods were applied for tree reconstruction. The PhyML version 3.1 (Guindon et al., 2010) was used to reconstruct ML trees with bootstrap replicates of 1000, which is generally considered as a reasonable number of replicates. MrBayes version 3.2.2 (Ronquist et al., 2012) was used to reconstruct trees with 10,000,000 generations, sufficient to meet the 0.01 criteria of standard deviation of split frequencies. The best-fit nucleotide substitution models (TPM1uf+I+G for ML reconstruction and HKY+I+G for BI reconstruction) were determined by AIC implemented in jModelTest 2 (Darriba et al., 2012). Some other cox1 sequences were downloaded from GenBank for phylogenetic analyses; nine *Echinococcus* species were studied: *E. granulosus* (NC_008075), *E. multilocularis* (NC_000928), *E. shiquicus* (NC_009460), *E. canadensis* (NC_011121), *E. equinus* (NC_020374), *E. ortleppi* (NC_011122),

E. oligarthrus (NC_009461), *E. felidis* (NC_021144) and *E. vogeli* (NC_009462). *Taenia solium* (NC_004022) was used as outgroup.

3. Results

A total of 521 samples (including 451 newly collected samples and 70 samples collected in our previous study (Ma et al., 2012)) from 36 different counties were used in this study. The geographical distribution of *Echinococcus* is shown in Table 1 and Fig. 1. The aligned 792 bp sequence matrix of the 521 partial cox1 genes contained 177 variable sites, of which, 157 were parsimony-informative. A total of 105 unique haplotypes (H1–H105) of *Echinococcus* species were identified (deposited in GenBank under accession no. JF906137–JF906170 and KM576144–KM576214). The relationships between the 105 haplotypes and the samples are shown in the supplementary Table S1. The 521 cox1 sequences and 105 haplotypes are shown in the supplementary files Sequence521.fas and H105+others.fas, respectively.

The ML and BI reconstruction methods showed a similar topology (Fig. 2, and Supplementary files, ML.tre and BI.tre). The major nodes involving the 105 haplotypes in both trees exhibited high bootstrap values of either 99% or 100%. The haplotypes were separated into four groups: (1) *E. shiquicus*, H1–H15 and H90–H104, 116 samples; (2) *E. multilocularis*, H16–H17, 39 samples; (3) *E. granulosus*, H18–H89, 360 samples; and (4) *E. canadensis*, H105, 6 samples (Fig. 2). The distributions of the four species in their hosts are listed in Table 2.

The N_S , I_S , PH, and PI values are shown in Table 3. Humans shared haplotypes with yaks, sheep, goat, and dogs. Based on the PH and PI values, humans had the closest relationship with sheep, followed by yaks and dogs. Three haplotypes were found to be shared by humans, herbivores, and carnivores: (1) H16 (*E. multilocularis*) was shared by 17 humans, 16 dogs, and 3 voles; (2) H18 (*E. granulosus*) was shared by 53 humans, 53 yaks, 47 sheep, and 1 dog; and (3) H23 (*E. granulosus*) was shared by 16 humans, 7 yaks, 8 sheep, and 1 dog.

4. Discussion

In this study we performed a molecular surveillance of a large number of *Echinococcus* isolates from the Qinghai Province, China. The collection of the parasite involved 36 counties, covering 86% of the 42 counties in the province, indicating the high burden of *Echinococcus* epidemiology throughout the region. A total of 360 samples were identified as *E. granulosus*, totaling 69% of the 521 samples. In fact, of the 163 human samples, 145 (89%) were *E. granulosus*, indicating this species as the main echinococcosis threat to the local people.

Tibetans live as seminomadic pastoralists with sheep, yaks, and dogs as their main domestic animals. Animal infection rates of *E. granulosus* in yaks and in sheep were extremely high throughout the whole QTP regions (Heath et al., 2005). Moreover, local Tibetan pastoralists usually feed their dogs with fresh internal organs (offal) of yaks and sheep, which represent the main transmission route

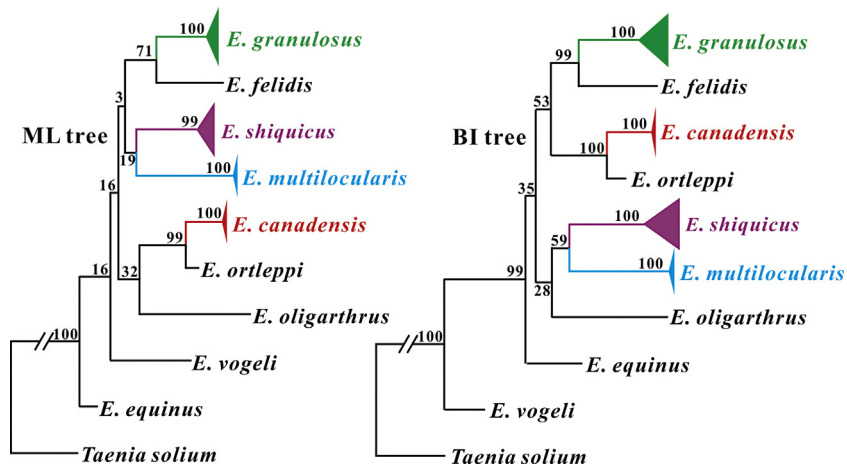


Fig. 2. Phylogenetic trees based on the maximum likelihood (ML) and Bayesian Inference (BI) methods (the numbers show bootstrap percentage values in the major nodes).

for *E. granulosus*. Our study shows a large number of haplotypes shared by humans with yaks or sheep (Table 3). Moreover, there were two haplotypes shared by humans, yaks, sheep, and dogs (H18 shared by 53 humans, 53 yaks, 47 sheep, and 1 dog; H23 shared by 16 humans, 7 yak, 8 sheep, and 1 dog), further showing the close relationships between human and domestic animals.

A total of 39 samples were determined as *E. multilocularis*. Interestingly, only humans, dogs, and voles were found to carry this particular species, indicating that the vole-dog recycling system, but not the ungulate-dog system, was the main threat of *E. multilocularis* infection in humans. Moreover, haplotype H16 was shared by 17 humans, 16 dogs, and 3 voles, further supporting the importance of voles in human-associated echinococcosis. In the QTP, besides

house dogs, a large number of stray dogs are also tolerated by pastoralists and Buddhist monks (Craig et al., 2008). These dogs likely ingest voles and contribute to the transmission of *E. multilocularis* to humans. In addition, since there was a large number of haplotypes shared by dogs and other animals (especially voles) (Table 3), better control of both house dogs and stray dogs is necessary for preventing the transmission of echinococcosis in this region.

E. shiquicus produces cysts similar to cystic echinococcosis or polycystic echinococcosis, but of unknown zoonotic status (Xiao et al., 2005). Foxes and pikas are natural terminal and intermediate hosts, respectively, of *E. shiquicus*. Although a previous study showed that *E. shiquicus* could also infect dogs (Boufana et al., 2013), in the current study, we did not find any other hosts other than foxes and pikas

Table 2
Distribution (species and hosts) of the 521 *Echinococcus* specimen.

	<i>E. granulosus</i>	<i>E. multilocularis</i>	<i>E. shiquicus</i>	<i>E. canadensis</i>	Sum
Human	145	17	0	1	163
Yak	118	0	0	0	118
Sheep	92	0	0	3	95
Goat	0	0	0	2	2
Dog	5	16	0	0	21
Fox	0	0	2	0	2
Pika	0	0	114	0	114
Vole	0	6	0	0	6
Sum	360	39	116	6	521

Table 3
Number (N_s) and percentage (PH) of shared haplotypes between each two hosts (lower-left triangle) and number (I_s) and percentage (PI) of individuals involved in shared haplotypes between each two hosts (upper-right triangle).

	Human	Yak	Sheep	Goat	Dog	Fox	Pika	Vole
Human	–	188; 66.29%	186; 72.09%	3; 1.82%	104; 56.52%	0; 0%	0; 0%	20; 11.83%
Yak	12; 17.65%	–	162; 76.06%	0; 0%	64; 46.04%	0; 0%	0; 0%	0; 0%
Sheep	13; 25.49%	12; 25.00%	–	5; 5.15%	57; 49.14%	0; 0%	0; 0%	0; 0%
Goat	1; 2.38%	0; 0%	1; 4.55%	–	0; 0%	0; 0%	0; 0%	0; 0%
Dog	3; 6.82%	3; 7.50%	2; 8.00%	0; 0%	–	0; 0%	0; 0%	19; 70.37%
Fox	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	–	2; 6.25%	0; 0%
Pika	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	29; 33.33%	–	0; 0%
Vole	1; 2.33%	0; 0%	0; 0%	0; 0%	1; 16.67%	0; 0%	0; 0%	–

that were infected by this parasite. Our results suggest that, as expected, the threat of *E. shiquicus* on humans and livestock can be mostly ignored.

E. canadensis is sporadically distributed worldwide and is an underestimated cause of human echinococcosis (Nakao et al., 2013). To our knowledge, no case of this species has been reported in the QTP regions. Unexpectedly, however, we detected six cases (1 human, 3 sheep, and 2 goats) that shared the same haplotype (H105), indicating that the echinococcosis situation in this region is much more complex than expected. Moreover, the six samples we collected were from five different counties, suggesting this species is widely distributed and the infection risks of *E. canadensis* should not be neglected.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetpar.2014.11.012>.

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