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Surveillance of *Echinococcus* isolates from Qinghai, 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.

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).

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Table 1
The 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