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Short Communication

An epidemiological survey of echinococcosis in intermediate and definitive hosts in Qinghai Province, China

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Abstract. In order to understand the epidemiological status of alveolar and cystic echinococcosis in intermediate and definitive hosts in Qinghai Province, China, during the period 2007–2011, we investigated the infection in humans and animals, including yaks, Tibetan sheep, Tibetan dogs, and wild foxes distributed in different counties around the province. Sera from local residents were examined using a rapid serodiagnostic kit to detect specific antibodies against *Echinococcus*. Seropositive samples were confirmed with B-scan ultrasonography and X-ray examinations. Yaks and Tibetan sheep were checked at slaughterhouses, and cysts and suspicious lesions were collected for analysis. A rapid diagnostic strip was used to detect *Echinococcus* adults in Tibetan dogs. Positive dogs were dewormed and the parasites collected. Wild foxes were trapped and necropsies performed with particular attention to the intestine. Forty-eight of 735 (6.4%) humans tested were positive and 475 of 854 (55.6%) Tibetan sheep and 85 of 352 (24.15%) yaks were infected with *Echinococcus*. Across different counties, 214 of 948 (22.57%) Tibetan dogs were positive, and five of 36 (13.9%) wild foxes were infected with *Echinococcus*. Molecular studies showed that all the infections detected in humans, domestic yaks, and Tibetan sheep were the G1 genotype (*E. granulosus*), whereas the parasites from Tibetan foxes and Tibetan dogs were *E. shiquicus* and *E. multilocularis*, respectively. In conclusion, *Echinococcosis* is hyperendemic in Qinghai Province in both its intermediate and definitive hosts and the G1 genotype of cystic *Echinococcus* is the dominant strain.

Echinococcosis (hydatid disease) is a zoonotic disease caused by the larval stage of cestodes of the genus *Echinococcus* (Taeniidae). To date, the genus *Echinococcus* consists of nine valid species (Nakao *et al.*, 2013). Among these species, *E. granulosus* and *E. multilocularis* cause serious life-threatening diseases, cystic echinococcosis (CE) and alveolar echinococcosis (AE) worldwide. China is one of the most hyperendemic countries for echinococcosis throughout the world (Wang *et al.*, 2014). Recent studies have shown that the infection rates for both CE and AE are high in many parts of northwestern China, especially in areas where the main agricultural activity is animal husbandry (McManus *et al.*, 2003; Yang *et al.*, 2005; Craig *et al.*, 2006).

Qinghai Province, in northwestern China, is situated in the Eurasia Hinterland, at an altitude of over 3000 meters above sea level. The Qinghai–Tibet plateau, with its special geography and economy, provides suitable conditions for the biological hosts and transmission of *Echinococcus*, leading to very high prevalences of both CE and AE in humans, livestock, and wild animals (Wang *et al.*, 2002). Recent reports have indicated that in Qinghai Province, *Echinococcus* infection has become more complex because mixed infections with a new species, *E. shiquicus* have been detected (Xiao *et al.*, 2005).

Against this background, the status of *Echinococcus* infections in its intermediate and definitive hosts was investigated during the period 2007–2011 in different counties across Qinghai Province to better understand its epidemiological status.

The study was performed in eastern Qinghai pasturing area (Haiyan, Guinan, Gangcha, Xinghai, Gonghe, and Huzhu counties), southwestern Qinghai pasturing area (Chengduo, and Yushu counties) and eastern Qinghai agricultural area (Huzhu County). A rapid serodiagnostic kit (batch number: 070531; Beisiming Co Ltd Xingjiang) was used to examine serum samples from 735 people in Haiyan County. This is a 3 min rapid dot immunogold filtration assay used for the serodiagnosis of human CE and AE. It is based on four native antigen preparations: crude and partially purified hydatid cyst fluid extracts from *E. granulosus* (EgCF and AgB), *E. granulosus* protoscolex extract (EgP), and *E. multilocularis* metacestode antigen (Em2) (Feng *et al.*, 2010). B-scan ultrasonography (3.5 MHz) (Mindray BN73AA3856, Shenzhen, China) and X-ray examinations (50 mA) were performed to confirm the 48 seropositive individuals (27 males and 21 females, 15–80 years old). For detecting the infectious ration of livestock, six private slaughterhouses in Haiyan, Gangcha, Guinan, and Gonghe counties (Table 1), visual inspection and palpation were used to check for cysts in the livers and lungs of Tibetan sheep and yaks over 1 year old. In detail, the palpated Tibetan sheep were 1–6 years old, predominantly 1.5–3 years old; and the yaks were 1–7 years old, predominantly 4–6 years old. The cysts and suspected cysts were stored in 75% ethanol for genotyping. For detecting the coproantigen secreted by adult *Echinococcus* worms was used to investigate *Echinococcus* infections in Tibetan dogs in Yushu, Chengduo, Gonghe, Xinghai, and Huzhu counties. Fecal samples were analyzed according to the manufacturer's instructions. Fecal samples were collected from dogs within 24 h and ground. An arecoline purge with 1% arecoline hydrobromide was administered orally to positive dogs with a drencher, at the recommended dose of 1.5 mL/10 kg. Fecal samples were collected 2 h later and incubated in an oven (70ºC, 24 h) to inactivate the oncospheres. The heat-treated fecal samples were then washed several times with physiological salt solution. The precipitated proglottids were collected and stored in 75% ethanol for genotyping.

With the permission of the Ministry of Forestry, China, wild foxes were hunted by local hunters in Haiyan and Chengduo counties. The foxes were stored at –80°C for at least 10 days to inactivate the *Echinococcus* eggs, and the intestine was checked at necropsy in the laboratory. The intestine was washed several times with physiological solution containing 5% formalin. The precipitated proglottids were collected and stored in 75% ethanol for genotyping. To identify the fox species, about

25 mg of fox muscle or liver tissue was taken from each fox carcass. DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Japan). D-loop primers prL (5'-CACCATTA GCACC-3') and prH (5'-CCTGAAGTAGG AACCA-3') were used for PCR amplification, with an initial denaturation step at 94°C for 15 min, followed by 35 cycles (94°C for 1 min, 56°C for 1 min, and 72°C for 1 min), and an extension step for 5 min at 72°C. The PCR products (20 µl) were separated by electrophoresis in a 2% agarose gel and visualized with ethidium bromide staining and subsequent UV excitation. The PCR products were purified for sequencing with a PCR Purification Kit (Qiagen).

The samples used for *Echinococcus* genotyping are from Human, yak and Tibetan sheep (Table 4). Total genomic DNA was extracted from the cysts and adult worms with a QIAamp DNA Mini Kit (Qiagen). PCR amplification of the DNA samples was performed immediately. The mitochondrial gene *cox1* (443 bp) was amplified using primers *cox*1-F (5'-TTTTTTGGGCATCC TGAGGTTTAT-3') and *cox*1-R (5'-TAAA GAAAGAACATAATGAAAATG-3'), as previously described (Bowles *et al.*, 1995). PCR was performed with the HotStarTaq Master Mix Kit (Qiagen), with an initial denaturation step at 95°C for 15 min, 35 cycles (94°C for 1 min, 48°C for 1 min, and 72°C for 1 min), and an extension step for 10 min at 72°C. The PCR products were separated by electrophoresis in a 2% agarose gel and visualized with ethidium bromide staining and subsequent UV excitation. The PCR products were purified with a PCR Purification Kit (Qiagen) and sequenced. The samples were genotyped by comparison with reference sequences (accession numbers: G1, AB491440; G2, M84662; G3, M84663; G4, AF346403; G5, AB235846; G6, AB208063; G7, AB235847; G8, AB235848; G10, AF525457). The nucleotide sequences of the PCR-amplified DNAs were determined with a 3130 Genetic Analyzer (Applied Biosystems) using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. The primers used in each PCR were used as the cycle sequencing primers. The sequences obtained were subjected to a BLAST sequence similarity search (National Center for Biotechnology Information) to identify the genotypes of *Echinococcus*. The sequences and those registered in GenBank were aligned with the CLC Sequence Viewer 6.1 (CLC bio Japan).

In total, 735 people, 845 Tibetan sheep, 352 yaks, 948 Tibetan dogs, and 15 wild foxes were examined during this epidemiological investigation conducted in six counties of Qinghai Province, China. Serological tests showed that 48 of the 735 (6.53%) humans were positive for *Echinococcus* antibodies, some of whom had both CE and AE, with the cystic form (+++) being stronger than the alveolar form (+). Four of the 735 samples were judged to be suspicious (very weak color reaction in the test spot for the AE Em2 antigen). B-scan ultrasonography and X-ray examinations confirmed the presence of cystic lesions in the livers or lungs of the seropositive individuals. However, there were no lesions in the four suspected cases.

The 854 Tibetan sheep and 352 yaks (all > 1-year-old) were examined in Haiyan, Gangcha, and Gonghe counties (Table 1). The prevalence of *Echinococcus* in the Tibetan sheep and yaks was 55.62% (475/854) and 24.15% (85/352), respectively. Among the 475 seropositive Tibetan sheep, 196 (46.45%) had liver-type echinococcosis and 226 (53.55%) had lung-type echinococcosis. Among the 352 seropositive yaks, 24 (28.24%) had liver-type echinococcosis and 61 (77.76%) had lung-type echinococcosis (Table 1). The results for the Tibetan dogs examined in Gangcha, Haiyan, Gonghe, Chengduo, Yushu, and Huzhu counties reveal a prevalence of 22.57% (214/948). (Table 2)

Five of the 36 (13.9%) wild foxes examined in Guinan, Gangcha, Haiyan, and Chengduo counties were infected with *Echinococcus* (Table 2): one of 27 (3.7%) red foxes was infected with *E. multilocularis* and three of nine (33.3%) Tibetan foxes were infected with *E. granulosus* (Table 3). However, the prevalence of *Echinococcus* among wild foxes varied across counties. Those from Chengduo County had the highest infection rate (66.7%), followed by those from Haiyan County (4.7%), whereas those from

Table 1. The prevalence (%) of *Echinococcus* in sheep and yak from four counties in the Qinghai Province

County	% in Sheep				% in Yak			
	N	Over all	Liver	Lung	N	Over all	Liver	Lung
Haiyan	324	74.69	65.70	34.30	175	26.86	27.66	72.34
Gangcha	50	76.00	10.53	89. 47	85	21.18	33.33	66.67
Gonghe	380	37. 37	20.70	76. 76	46	23.91	27.28	72.72
Guina	100	53.00	60.38	39.62	46	19.56	22.22	77.78

N: number of hosts examined.

Table 2. The prevalence (%) of *Echinococcus* in the Tibetan dog (using coproantigens) and adult worms in the red fox from seven counties in the Qinghai Province

County	Tibetan dog		Wild fox		
	N	%	N	%	Rang in worm nuber
Gangcha	282	10.64	5	0	0
Haiyan	172	16.28	21	4.7	116
Chenduo	86	44.19	6	66.7	1–1670
Gonghe	288	31.94	–	–	–
Yushu	94	27.66	–	–	–
Huzhu	26	0.00	–	–	–
Guinan	–	–	4	0	0

Note: "–" represents "not examined".
N: number of hosts examined.

Table 3. Fox species and species of infected *Echinococcus* in investigated counties

County	Fox species	Number of necropsied fox	Number of fox infected with *E. multilocularis* (burden)	Number of fox infected with *E. granulosus* (burden)
Gangcha	Red fox	4	0	0
	Tibetan fox	1	0	0
Haiyan	Red fox	19	1 (116)	0
	Tibetan fox	2	0	0
Chengduo	Red fox	–	–	–
	Tibetan fox	6	0	3 (833-1,640)
Guinan	Red fox	4	0	0
	Tibetan fox	–	–	–

Note: "–" represents "not examined".

Table 4. Genotyping of analyzed DNA samples from human and livestock hosts for *Echinococcus granulosus*, *Taenia hydatigena* abd *Fasciola hepatica*

Host	Sample (cyst)	Positive/ examined	DNA sequence (bp)	Blast results	Genotype
Human	liver	15/15	332–377	*E. granulosus*	G1
	lung	4/4	330–385	*E. granulosus*	G1
	abdomen	3/3	350–361	*E. granulosus*	G1
Yak	liver	9/27	219–401	*E. granulosus*	G1
	liver	1/1	270	*F. hepatica*	
	liver	16/19	344–399	*E. granulosus*	G1
Tibetan sheep	liver	4/14	353–370	*E. granulosus*	G1
	liver	1/1	361	*T. hydatigena*	
	lung	4/10	244–356	*E. granulosus*	G1
	lung	1/1	304	*T. hydatigena*	

Guinan and Gangcha counties were free of *Echinococcus* infection (Table 2). The mean intensity of infection of *Echinococcus* among wild foxes from different counties ranged between 1 and 1670, with those from Haiyan county displaying the lowest mean intensity of infection (116), whereas those from Chengduo county had the highest mean intensity of infection (1–1670) (Table 2). One of 19 red foxes from Haiyan was infected with 116 adult *E. multilocularis* compared with 3 of 6 Tibetan foxes from Chenduo harboring a range of 833 - 1640 adults (Table 3).

The genotypes of the cysts in the intermediate hosts are determined by PCR. When the *cox1* gene was amplified, the cysts infecting 22 patients (15 patients with liver-type, four patients with lung-type, and three patients with abdomen-type infection) were shown to be *E. granulosus* (G1 type) (Table 4).

Of the 28 yaks with liver-type echinococcosis, nine were infected with *E. granulosus* (G1 type) and one with *Fasciola hepatica*. Of the 19 yaks with lung-type echinococcosis, 16 were infected with *E. granulosus* (G1 type).

Of the 26 infected Tibetan sheep, four of the 15 sheep with liver-type echinococcosis were infected with *E. granulosus* (G1 genotype) and one with *Taenia hydatigena*. Similarly, four of the 11 sheep with lung-type echinococcosis were infected with *E. granulosus* and one with *Taenia hydatigena*.

When the 36 wild foxes (definitive hosts) were genotyped, 27 were red foxes and nine were Tibetan foxes. Red foxes were only trapped in Haiyan County and Tibetan foxes were only trapped in Chengduo County. A gene comparison revealed that the red foxes were infected with *E. multilocularis* and four of the six Tibetan foxes were infected with *E. shiquicus*. About 10,195 *Echinococcus* adults were collected from one stray dog and DNA (*cox1*) amplification revealed them to be *E. multilocularis*.

Qinghai Province is located on the central Qinghai–Tibetan Plateau, where the geographic conditions provide a unique ecosystem for the survival of various creatures living together, including yaks, dogs, wild foxes, and sheep. In this environment, the livelihoods of local residents depend largely on livestock and dogs. These interactions between man, livestock (sheep and yaks), dogs, and wild foxes provide an environment conducive to the transmission of *Echinococcus* (Craig *et al.*, 2003). A lack of knowledge about the prevention of echinococcosis increases its prevalence and severity in the Qinghai–Tibet region (Yu *et al.*, 2008).

Our survey in the Qinghai Lake region revealed a prevalence of *Echinococcus* in humans of about 6.53%. This is lower than but similar to the 13.7% reported in people in the southern part of Qinghai Province (Han *et al.*, 2009). The prevalence of *Echinococcus*

infection in the Qinghai–Tibet region suggests that the environment is highly contaminated with *Echinococcus* eggs, thus threatening the lives and health of the inhabitants of the region.

The infection rate of *E. granulosus* was higher in Tibetan sheep (55.0%) than in yaks (24.15%). Of the 203 hydatid cysts collected from Tibetan sheep, few (41/203) were found in the liver, whereas a substantial proportion were found in the lungs (162/203). This variation in cyst formation between Tibetan sheep and yaks and the cyst distributions in the sheep organs (liver versus lungs) warrants further study to clarify the reasons for these differences.

An examination of fecal samples from 948 Tibetan dogs across six counties revealed a mean infection rate of 22.57%. However, the infection rate was 44.19% in Chengduo County (in the southern part of Qinghai). A similar high infection rate was reported previously in the southwest of Qinghai Province by Zhang *et al.* (2007), implying that effective prevention and control measures for *Echinococcus* infection in this region are essential.

No dogs from agricultural regions were infected with the *Echinococcus* tapeworm, whereas in the pastoral areas, the infection rate ranged from 10.64% to 44.19%. This is probably attributable to the differences in the dietary habits of the residents of each farming area. In agricultural regions, livestock are captive, with little chance of contacting dogs, and the dogs are fed cooked wheaten food. In contrast, the Tibetan people in pastoral areas eat beef (from yaks) and mutton (from sheep) and feed their dogs on visceral matter, thus increasing the chance of infection in dogs, with cysts conferring either liver-type or lung-type echinococcosis.

Genotyping (based on the *cox1* gene) the DNA from cysts revealed that all the humans, yaks, and Tibetan sheep tested were infected with *E. granulosus* (G1 genotype), suggesting that only one genotype of *E. granulosus* is prevalent in Qinghai Province. This warrants further investigation of a larger sample from a broader region to establish whether other genotypes are present in the area.

Among the hydatid cysts collected from yaks and Tibetan sheep, 1/3 of them had white cavities. A molecular analysis revealed that all of them were hydatid cysts. Therefore, during necropsy and meat inspection, great attention must be paid to distinguishing these cysts from calcification and nodules, because *F. hepatica* and *Cysticercus tenuicollis* also form cysts or nodules in the liver and lung.

Echinococcus multilocularis was detected in foxes and stray dogs in Haiyan, Heka, and Chengduo counties. This can be explained by the fact that the two species of predators feed on pikas and other rodents. This food chain completes the developmental cycle of *Echinococcus*, presenting a challenge for the prevention and control of echinococcosis. A study by Vaniscott *et al.* (2011) demonstrated that the risk of *Echinococcus* infection is much higher in dogs than in foxes on the Qinghai–Tibetan Plateau.

In this study, 10,195 tapeworms were collected from a stray dog trapped in Chengduo County, where a new species of tapeworm, *E. shiquicus*, was first reported by Xiao *et al.* (2005). The same tapeworm was found in the lungs of pika (*Ochotona curzonia*) (Xiao *et al.*, 2006). It is likely that mixed infections of *E. shiquicus* and other species complicate the diagnosis and control of echinococcosis.

In conclusion, this study extends our understanding of the epidemiology of echinococcosis in its intermediate and definitive hosts in Qinghai Province. It is now very clear that echinococcosis is hyperendemic on the Qinghai–Tibetan Plateau, especially in southern Qinghai, and that the predominant genotype is G1, *E. granulosus*. To effectively control echinococcosis, attempts must be made to create an awareness of it among local residents, enabling them to recognize the importance of its prevention, especially by eliminating the infection through the regular deworming of dogs. It has previously been recommended that local health and veterinary departments should encourage local residents to develop hygienic habits (Xiao *et al.*, 2013).

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Conflict of interest
None.

Ethical standards
This project was approved by the Qinghai University Ethics Committee. All protocols used for specimen collection were consistent with the Rules for Animal Care of the Academy of Animal and Veterinary Medicine, Qinghai University of Animal and Veterinary Sciences.

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