



# Brief report prevalence of *Echinococcus* species in wild foxes in parts of Qinghai Province, China

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## Abstract

Echinococcosis, caused by tapeworms of the genus *Echinococcus*, is a zoonotic parasitic disease. Various *Echinococcus* spp. are endemic and distributed in the Qinghai Province of China. Currently, few studies on the prevalence of *Echinococcus* spp. in the wild foxes are available. Hence, the aim of the study was to evaluate the prevalence of *Echinococcus* spp. in wild foxes in highly endemic areas of Qinghai Province, China. A total of 600 wild canid fecal samples were collected from Yushu, Qilian and Guinan in the study region, and 521 samples were successfully molecularly identified as wild foxes (Tibetan fox: 448, red foxes: 70, corsac fox: 3). Among the wild foxes, 5.57% (29/521) tested positive for *Echinococcus* spp. The prevalence rates of *Echinococcus* spp. in wild foxes in the Yushu, Qilian and Guinan areas were 2.51%, 15.22% and 0.96%, respectively. Furthermore, sequencing analysis indicated that *E. multilocularis* was the most prevalent species, occurring in 4.03% (21/521) of the wild foxes. Compared to *E. granulosus* occurring in 0.58% (3/521) of the foxes, *E. shiquicus* occurred in 1.54% (8/521), and *E. shiquicus* was first reported with 2.17% (3/138) prevalence in the Qilian area, indicating its transmission range is expanding. The current results provide useful epidemiological data for understanding and monitoring the dissemination of *Echinococcus* spp. by wild foxes in Qinghai Province, China.

**Keywords** *Echinococcus* spp. · Zoonosis · Epidemiology · Wild fox · PCR

## Introduction

Echinococcosis is caused by the larval stages of the genus *Echinococcus*, which may cause severe illness and even life-threatening conditions in intermediate hosts (Moro and Schantz 2009; Bhutani and Kajal 2018). In echinococcosis

cases, *E. granulosus* and *E. multilocularis* are the most common and dangerous pathogens to humans, causing cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively (Moro and Schantz 2009; Craig et al. 2019; Fu et al. 2021). CE and AE are the most common echinococcoses worldwide and pose significant threats to public health and safety in the pastoral areas of Qinghai Province (Craig et al. 2019; Wang et al. 2020).

Adult *Echinococcus* tapeworms are parasitic in the small intestines of domestic and wild definitive hosts (canids-dogs and foxes), and their pathogenic eggs are excreted with the feces of the definitive hosts, with the subsequent contamination of water, vegetables, or other foods and the environment (Wang et al. 2008; Craig et al. 2019). Metacestodes are larval stages of *Echinococcus* cestodes, which develop in the internal organs (livers and lungs) of intermediate hosts (such as rodents and aberrant humans) when they ingest contaminated drinking water and foods (Moro and Schantz 2009; Craig et al. 2019; Lass et al. 2020; Gao et al. 2022). From the perspective of the threats of *Echinococcus* eggs

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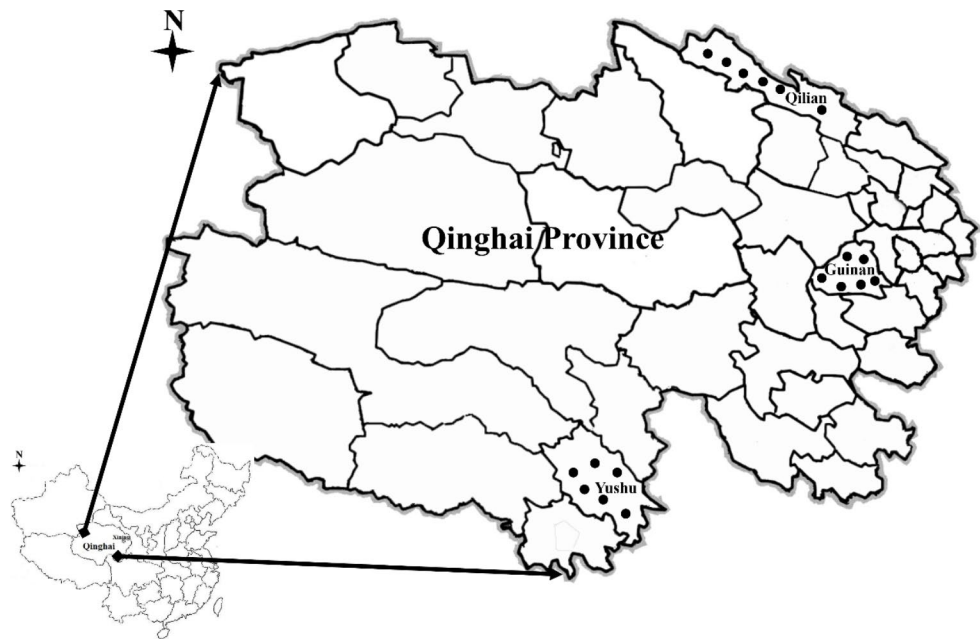
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**Fig. 1** Distribution of the locations (●) of samples collection in this study. The Qinghai Province located on the Qinghai Tibetan Plateau of China



to humans, the contamination of eggs is the most important part of parasitic life.

The incidence of echinococcosis (CE and AE) is highly endemic in the Qinghai-Tibet Plateau (Craig et al. 2019; Cai et al. 2021). *Echinococcus* spp. was positive in wild foxes at 5.5% based on the copro-DNA PCR method in Qinghai Province in 2014~2015 (Cai et al. 2021). Additionally, five of 36 (13.9%) wild foxes were infected with *Echinococcus* based on the rapid diagnostic strip during the period 2007–2011 (Dang et al. 2017). Foxes play a role as the main definitive host of *E. multilocularis*, and the migration of foxes influences its spread. The survey resulted in a prevalence rate of mature *E. multilocularis* parasites at 42% out of 50 fox intestines, and the prevalence of fecal contamination with *E. multilocularis* was 10.5% (63/600) in the county of Erzurum in Turkey (Avcioglu et al. 2021). In the protected area of the Tatra National Park in southern Poland, the prevalence of *E. multilocularis* was 4.4% in 91 screened red fox feces by nested PCR (Gawor et al. 2021). Recently, *E. multilocularis* (maximum prevalence  $\pm$  95% CI: 33.3%  $\pm$  15.4%) was detected in 36 Tibetan fox feces collected in Shiqu County (Sichuan Province, China), eastern Tibetan Plateau (Zuo et al. 2020). In China, after a large deworming and control effort in dogs, the prevalence of *E. multilocularis* decreased. However, there has been limited research on non-domesticated carnivores such as foxes, which may pose a potential threat to humans. So, the epidemiological investigation of *E. multilocularis* in foxes was imminent. In this study, the prevalence of *Echinococcus* spp. in wild foxes was investigated in the Yushu, Hainan and Haibei prefectures of Qinghai Province using the copro-PCR method.

## Materials and methods

### Study area

In the present study, fecal samples were collected from wild canids in parts of Qinghai Province on the Qinghai-Tibet Plateau of China (Fig. 1). Yushu County is located in the eastern part of the Qinghai-Tibet Plateau and borders to the Tibet Autonomous Region in the east and southeast. The average altitude is 4493.4 m, with only cold and warm seasons. Guinan County is located in the Hainan Tibetan Autonomous Prefecture of the northeastern part of Qinghai Province, between the West Tilt Mountains and the Yellow River, adjacent to the Huangnan Tibetan Autonomous Prefecture in the east. Qilian County is located in Haibei Tibetan Autonomous Prefecture of Qinghai Province and located in the hinterland of the middle section of the Qilian Mountains and adjacent to the ancient Silk Road.

### Specimen collection

From January 2018 to December 2020, a total of 600 wild fresh canid fecal samples were collected next to fox dens on the wild grassy hills and grasslands, where yaks and sheep grazed, and local people may also occasionally arrive. With great help from local government veterinarians and local herdsman, who knew location and scope of activity of foxes and wolves and had extensive experiences in finding and identifying the feces of the above wild animals. Each sample was individually collected and stored in labeled plastic zip lock bags at -80 °C for more than 1 week to inactivate *Echinococcus* oncospheres reducing the potential risk of

**Table 1** The fox species and numbers in the Qinghai Tibetan Plateau Area

	Red fox ( <i>Vulpes vulpes</i> )	Tibetan fox ( <i>Vulpes ferrilata</i> )	Corsac fox ( <i>Vulpes corsac</i> )	Total
Yushu area	279	0	0	279
Qilian area	43	92	3	138
Guinan area	27	77	0	104
Total	349	169	3	521

laboratory infection, and then, they were stored at -20 °C before subsequent extraction and examination.

## Molecular analyses

First, each fecal sample was eluted with lysis solution, especially the membranes on of feces, and then, the eluent and precipitate were extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Genomic DNA was used to identify the origin of carnivorous species by molecular identification with the primers prLand prH described by Nonaka et al. (2009). Then the positive samples were used for further research, and the copro-DNA sample was extracted from 200 ~ 300 mg of each fecal sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. All copro-DNA samples were amplified using the universal primers 2575 and 3021 for tapeworm identification again (Bowles et al. 1992). At the same time, all the copro-DNA samples were submitted to *Echinococcus* spp. identification by a multiplex PCR assay with species-specific primers described by Liu et al. (2015). Then, all the positive PCR products were sequenced by Shanghai Biotechnology Co. (Shanghai, China) and confirmed by comparison against reference sequences in the GenBank database to determine the *Echinococcus* spp. Statistical analysis was carried out using Open Source Epidemiologic Statistics Software (<http://www.openepi.com/Proportion/Proportion.htm>).

**Table 3** Prevalence and species results for *Echinococcus* spp. in foxes in Qinghai Province

	Samples	<i>E. multilocularis</i> (%; 95% CI)	<i>E. granulosus</i> (%; 95% CI)	<i>E. shiquicus</i> (%; 95% CI)	Total (%; 95% CI)
Yushu	279	3 (1.08%; 0.37–3.11)	2 (0.72%; 0.20–2.58)	5 (1.79%; 0.77–4.13)	7 (2.51%; 1.2–5.09) (3 co-infection)
Qilian	138	17 (12.32%; 7.84–18.84)	1 (0.72%; 0.13–3.99)	3 (2.17%; 0.74–6.20)	21 (15.22%; 10.17–22.14) (no co-infection)
Guinan	104	1 (0.96%; 0.17–5.25)	0 (NA)	0 (NA)	1 (0.96%; 0.17–5.25) (no co-infection)
Total	521	21 (4.03%; 2.65–6.08)	3 (0.58%; 0.20–1.68)	8 (1.54%; 0.78–3.00)	29 (5.57%; 3.90–7.88) (3 co-infection)

**Table 2** The numbers of positive sample detected using the 2575 and 3021 primers in the fox fecal samples

	Foxe fecal samples	Positive sample	Positive rate % (95% CI)
Yushu area	279	22	7.89% (5.27–11.65)
Qilian area	138	57	41.30% (33.43–49.65)
Guinan area	104	31	29.81% (21.86–39.19)
Total	521	110	21.11% (17.83–24.82)

## Results and discussion

Of the 600 collected fecal samples, 521 were molecularly identified as positive samples. The geographic region of the wild fox samples was as follows: 279 Tibetan fox samples were collected in Yushu; 92 Tibetan fox, 43 red fox and 3 corsac fox samples were collected in Qilian; and 77 Tibetan fox and 27 red fox samples were collected in Guinan (Table 1).

For the prevalence of parasites in wild canids detected by the 2575 and 3021 primers, 110 samples (110/521, 21.11%) from wild foxes were positive. More specially, 22 (22/279, 7.89%), 57 (57/138, 41.30%) and 31 (31/104, 29.81%) fox fecal samples from Yushu, Qilian and Guinan were positive (Table 2). All the samples were identified again according to the multiplex PCR assay, and special attention was given to those positive samples. In terms of the prevalence of *Echinococcus* spp. in the wild foxes in the geographic region, seven (7/279, 3.58%) *Echinococcus* spp.-positive samples contained 3 (3/279, 1.08%) *E. multilocularis*, 2 (2/279, 0.72%) *E. granulosus* and 5 (5/279, 1.79%) *E. shiquicus* in Yushu; two fecal samples were identified to be infected with *E. multilocularis* and *E. granulosus*, and one was coinfection with *E. multilocularis* and *E. shiquicus*. Twenty-one (21/138, 15.22%) *Echinococcus* spp.-positive samples contained 17 (17/138, 12.32%) *E. multilocularis*, 1 (1/138, 0.72%) *E. granulosus* and 3 (3/138, 2.17%) *E. shiquicus* in Qilian. Only one (1/104, 0.96%) *Echinococcus* spp.-positive sample and one (1/104, 0.96%) *E. multilocularis* sample were found in Guinan (Table 3). Among these, 21, 3 and 8 were positive for *E. multilocularis*, *E. granulosus* and *E. shiquicus* in the three areas, respectively.

The results showed that Yushu is a highly endemic area of *Echinococcus* spp., and there have been a few reports of the

prevalence of *Echinococcus* spp. in wild foxes and domestic and stray dogs (Boufana et al. 2013; Cheng et al. 2016; Cai et al. 2021). However, there have been few reports of *Echinococcus* spp. in Guinan and Qilain. Therefore, the present study was used to monitor the dynamic prevalence of *Echinococcus* spp. and to evaluate prevalence and emergence in new areas.

The Chinese government has implemented the national echinococcosis control program for domestic dog deworming and sheep vaccinating (Xiao 2021). It was very obvious that the prevalence rate of *Echinococcus* spp. in domestic dogs significantly decreased from 40% (even high up to 70% in Yushu and Golog) to 10.7%–13.0% in Qinghai (He 2000). Previous reports showed that the prevalence rates of *Echinococcus* spp. in dogs were 12.5% (Guo, 2014) and 44.19% (Dang, 2017) in Chengdu area; the prevalence rates of *Echinococcus* spp. in dogs in Qilian area was 47.6% (Zhang, 2007); and in Guinan area, *E. granulosus* has not been detected since 2012, except that *E. granulosus* was detected in feces with 28.33% prevalence (Danzheng 2014). However, research on the prevalence of *Echinococcus* spp. in wild canids is still relatively limited, and there is currently speculation that the transmission of *Echinococcus* spp. in wild animal enclosures was entering livestock enclosures. There have been some reports on the prevalence of *Echinococcus* spp. in wild canids, with 5.5% prevalence in wild foxes detected by Cai et al. (2021), 3.7% in wild foxes detected by Wei et al. (2020), and *E. shiquicus* (8%) and *E. multilocularis* (3%) in wild foxes necropsied and identified by Li et al. (2013).

Although the prevalence of infection in wild foxes was low, the large population of wild foxes and other canids (wolves) in the pastoral areas of Qinghai Province that were in close contact with herdsman and livestock must be considered new potential sources and risk factors for the transmission of *Echinococcus* spp. to humans (Gawor et al. 2021; Khan et al. 2021). The results of the multiplex PCR in the current study showed that the Qilian area had a high prevalence (21/138, 15.22%) of *Echinococcus* spp. in wild foxes in Qinghai Province. Another notable point was that *E. shiquicus* was first reported in the Qilian area, which was the only discovered species reported surrounding the Qinghai and Sichuan areas (Zhu et al. 2020), so the endemic area of *E. shiquicus* is expanding.

The prevalence of *Echinococcus* spp. is affected by detection methods, such as DNA extraction, egg production and shedding (Maksimov et al. 2017; Skrzypek et al. 2020). To ensure accurate detection, genomic DNA was extracted from wild fox fecal samples using a commercial kit with inhibitEX tablets, and two detection methods were performed for identification with wide coverage, strong specificity and multiple infection tests. Definitive hosts are involved in egg dispersion mechanism, with the maturation and residence time of adults occurring inside wild foxes and egg production and shedding occurring in the feces of adult worms and hosts (Kapel et al.

2006; Al-Sabi et al. 2008). The occurrence of *Echinococcus* spp. in foxes was reported in this study as quite low, and all the above reasons may make it more difficult to detect *Echinococcus* spp. in the wild fox fecal samples. Namely, the potential occurrence of *Echinococcus* spp. infections in foxes may have been underestimated.

It is important to note that the negative results presented in this study, such as negative results in Guinan, did not preclude the risk of infections for wild canids in some places and some definitive hosts in Qinghai Province. Preventive measures and a high degree of attention have been paid to the prevalence of *Echinococcus* spp. in domestic dogs across Qinghai Province (Liu et al. 2017). Future work aiming to characterize the prevalence among such wild foxes and wolves would provide valuable information on the potential and increased exposure risk for livestock and even humans.

In conclusion, the wild foxes are an important part of wild reservoirs of *Echinococcus* spp. in Qinghai, confirming that the wild cycle of *Echinococcus* spp. is of equal importance as the transmission of the domestic cycle. Therefore, we suggest that further epidemiological surveys should target *Echinococcus* spp. isolates from voles, pikas and human intermediate hosts and from wild definitive hosts in the same ecological environment in Qinghai. At the same time, our results also provide crucial information for the control and prevention of wild type echinococcosis in Qinghai Province, China.

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**Author contribution** XYZ, YF, and HD conceived the study and wrote the manuscript. XYZ, YJM, ZHG, XYS and ZL carried out the experiments and analyzed the data. GQJN, XHW and WMJ contributed to the collection of the fecal samples. XYZ, YF, ZL, ZHG and HD contributed material, discussions of the results and to the final version of the manuscript. All authors provided critical feedback.

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**Data availability** The datasets supporting the conclusions of this article are included within the article.

## Declarations

**Declarations** The fecal samples of wild animals were collected strictly, according to the legal requirement guide for the Care and Use of Laboratory Animals of the Ministry of Health, China and the Law of the People's Republic of China on Wildlife Protection. No animals were harmed during the experimental process

**Conflict of interest** The authors declare no competing interests.

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