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Short Communication

A Pilot Study on Deworming Wild Foxes for *Echinococcus* spp. in Qinghai, China

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Abstract

Background: Wild foxes play an important role in echinococcosis epidemics. There have been a few studies investigating *Echinococcus* spp. in the Qinghai-Tibet Plateau area, China, but none on the prevention and control of *Echinococcus* spp. in wild foxes.

Methods: From 2016 March through December 2019, two wild fox dens were selected as two test sites based on prior long-term camera screening and observation. Anthelmintic praziquantel tablets were placed near the two dens of wild foxes, and the wild foxes freely consumed the anthelmintic drugs. Morphological methods were used to detect initially the parasite species, and PCR molecular methods were used to identify accurately parasite and host species.

Results: Parasite eggs of *E. multilocularis* (2/11, 18.2%) were found in 11 fecal samples. Importantly, the eggs of *E. multilocularis* (1/21, 4.8%) were found again in the feces of the foxes one year later; moreover, the eggs of *E. multilocularis* (2/19, 10.5%) still existed in the feces of the foxes two years later.

Conclusion: Wild foxes were repeatedly infected with *E. multilocularis* and that deworming for prevention and control is required at least twice per year. Prevention and control methods for echinococcosis in wild foxes were explored, providing a scientific basis for the prevention and control of echinococcosis in wild animals.

Introduction

Hydatid disease is a zoonotic parasitic disease caused by the larva of *Echinococcus* spp.. *Echinococcus* spp. display a

fixed life cycle between definitive hosts (carnivores, such as dogs, foxes and wolves) and intermediate hosts (such as humans, livestock



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and some wild animals) (1). The most important and widespread species of the genus *Echinococcus* in the Qinghai-Tibet Plateau area in western China are *E. granulosus* sensu stricto and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE) respectively (2), representing public health threats. The Qinghai-Tibet Plateau area has the highest prevalence of echinococcosis in the world and is located in the northern part of China (3). Dogs are the most important definitive hosts and sources of infection (4), as the infection rate of *E. granulosus* sensu stricto in dogs is as high as 64.56% (5). The *Echinococcus* spp. infection rate in Qinghai Province was confirmed as the highest by antigen testing of dog feces in 2012-2016 (6).

Recently, measures such as monthly drug delivery and the deworming of all dogs were implemented by the Qinghai Provincial Government, resulting in the effective control of *Echinococcus* spp. infections in domesticated dogs, humans and livestock; the hydatid infection rate was greatly reduced (4). However, wild canines, especially wild foxes (*Vulpes vulpes*, *V. corsac* and *V. ferrilata*), are also important definitive hosts and contribute to the spread of echinococcosis, especially *E. multilocularis* and human AE (7, 8). Increases in the numbers of red foxes (*V. vulpes*) were associated with increases in the number of new cases of AE in human populations in Switzerland and Poland (9, 10), suggesting that the transmission of AE in human populations is related to the number of wild foxes and their activities. In Japan, a deworming experiment for wild foxes in urban landscapes that involved baiting with anthelmintic praziquantel, was established and implemented (11).

There have been a few reports investigating *Echinococcus* spp. in the Qinghai-Tibet Plateau area, but no studies on the prevention and control of *Echinococcus* spp. in wild foxes have been reported. Recently, with the ecological environment improving and fox population increasing, the habitat of the wild foxes and

territory used by herdsmen have rapidly started to overlap, and given the consequent increase in close contact between foxes and humans, there is an urgent need to establish effective echinococcosis control strategies, as performed in Japan (12), to minimize the risk of infection in humans and livestock. In addition, at least 16 papers have focused on the control of *E. multilocularis* by baiting foxes in highly endemic areas of Europe or Japan (13), but no studies have been carried out in Qinghai-Tibet Plateau endemic areas of China.

In this study, the investigation of wild fox feces near caves and the deworming of wild foxes were carried out over three years. Two wild fox dens were selected as two test sites based on prior long-term camera screening and observation.

Materials and Methods

Ethics statement

This study was performed in accordance with the Law of the People's Republic of China on Wildlife Protection. Before the initiation of the experiments, the protocol of the current study was reviewed and approved by the Institutional Animal Care and Use Committee of the Qinghai Academy of Animal Sciences and Veterinary Medicine and the Forestry and Grassland Bureau of Qinghai Province and was subsequently conducted under permit. No animals were harmed during the experimental process.

The site

The study area was located at an altitude of approximately 3,000 meter in Haiyan County of Haibei Prefecture, Qinghai Province, in the eastern Qinghai-Tibet Plateau area. Before this experiment was carried out, a previous study showed that approximately 50% of fox faeces collected in this area contained *E. multilocularis* eggs. The dominant landscape of the study area was pasture, with yaks, Tibetan sheep, herdsmen, pikas and voles living in the same

environment. The 50 km² study area was the same before and after deworming in the experimental baited section.

The deworming

From 2016 March through December 2019, the two dens were confirmed to belong to wild foxes (*V. ferrilata*) by PCR of the host feces in the study area. The anthelmintic bait consisted of praziquantel (50 mg), meat and gelatine. Small holes 15-20 cm in length, 10 cm in width and 6-9 cm in depth were dug 5 to 6 m away from both sides of the fox dens. The praziquantel was combined with a small amount of lamb meat, and then placed directly into the hole. Infrared cameras were set up at both fox dens and were used for the observation of wild fox behaviours and the monitoring of their intake of praziquantel. The fox faeces surrounding the fox dens were collected for analysis (before deworming). Given the large area, scattered wildlife and effective deworming effect, the dosing procedure was performed again three days later to ensure as much as possible that these groups of wild foxes consumed praziquantel. Ten days after the second administration, the faeces surrounding the fox dens were collected for the detection of *Echinococcus* spp. eggs. Feces were collected for egg detection at the time of administration and 6 months, 1 year, and 2 years after the administration of praziquantel.

Preparation and analysis of samples

During the study, fecal samples suspected of belonging to the wild foxes were collected regularly, and the collected feces were kept in a -80 °C freezer for more than 2 weeks. Then, the fecal samples were processed by the sucrose flotation and precipitation method (7).

Molecular experiments were used to monitor the prevalence of taeniid parasites (especially *E. multilocularis*) in the foxes. First, the host species in each experiment was determined, and then the parasites were identified in the host feces. The effect of deworming was evaluated by comparing the experimental

data at different time points. The feces suspected of belonging to the wild foxes were rinsed with ASL buffer (QIAamp DNA Stool Mini Kit, Qiagen, Germany) according to the literature (14), and host genomic DNA on the surface of the feces was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Approximately 1 g of wild fox feces was used for parasite egg examination; tapeworm and nematode eggs were detected by the sucrose flotation method (1.27 g/cm³ (1.27 g/ml)), and the sediments were tested for trematode eggs. Eggs were classified and identified by egg morphology. Twenty to forty collected eggs were used for genomic DNA extraction with a QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan). PCR targeting the D-loop domain was performed with the universal primers prL: 5'-CACCATTAGCACCCAAAGCT-3' and prH: 5'-CCTGAAGTAGGAACCAGATG-3' (product=540 bp) to identify the host species (14). PCR was performed to amplify the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene and the ITS2 gene with the following tapeworm universal primers: *cox1*-2575: 5'-TTT*TTTGGGCATCCTGAGGTTTAT-3' and *cox1*-3021: 5'-TAAAGAAAGAACATAATGAAAATG-3' (product=444 bp) (15) and ITS2-LC1: 5'-CGAGTATCGATGAAGAACGCAGC-3' and ITS2-HC2: 5'-ATATGCTTAAGTTCAGCGGG-3' (product=450~550 bp, according to the species) for the identification of the parasite species (16). The amplification products (10 µl) were analysed by 1.0% agarose gel electrophoresis in 1× tris-acetate-EDTA (TAE) buffer (pH 8.3) containing 10 µl of Gelstain per 100 ml, and the positive PCR products were sequenced by Shanghai Biotech Bioengineering Technology Co., Ltd..

Results

Before baiting with praziquantel was performed, 37 faecal samples suspected of belonging to the foxes were collected from around the two dens, and 11 samples were identified as having come from Tibetan foxes (*V. ferrilata*) according to PCR molecular analyses. Eggs of *E. multilocularis* (2/11, 18.2%) and *Taenia crassiceps* (1/11, 9.1%) were found in 11 feces

samples (Table 1). *Alaria alata* (1/17, 5.9%) was discovered in a faecal sample 6 months after the second drug administration. Importantly, eggs of *E. multilocularis* (1/21, 4.8%) were found again in the feces of the foxes one year later; moreover, eggs of *E. multilocularis* (2/19, 10.5%) still existed in the feces of the foxes two years later. Additional details are shown in Table 1.

Table 1: Animal species were identified by DNA in the faeces and the examination of eggs in the feces

Time	Number of faecal samples examined	Number of animals identified	Found parasites		
			<i>E. multilocularis</i>	<i>T. crassiceps</i>	<i>A. alata</i>
Before deworming	37	11 (<i>V. ferrilata</i>)	2	1	0
10 days after the second deworming	19	11 (<i>V. ferrilata</i>)	0	0	0
6 months after the second deworming	27	17 (<i>V. ferrilata</i>)	0	0	1
1 year after the second deworming	32	21 (<i>V. ferrilata</i>)	1	0	1
2 years after deworming	29	19 (<i>V. ferrilata</i>)	2	0	2

Two Tibetan fox families were captured on camera by two sets of infrared cameras; 2 adult foxes and 4 juvenile foxes were photographed at the first experimental site, and sometimes a Pallas's cat (*Otocolobus manul*) also appeared. Two adult foxes and sometimes 1-4 badgers (*Meles meles*) were photographed at the second experimental site. Other mammals, including humans, yaks and sheep, were also photographed; these animals may potentially maintain echinococcosis epidemics as intermediate or definitive hosts.

The wild foxes freely consumed the bait with praziquantel, which was confirmed with on-site infrared cameras. No *Echinococcus* spp. eggs (0/17) were found within 6 months after deworming, suggesting that deworming was effective, but the eggs of *E. multilocularis* were detected in the feces of the foxes one and two years later.

Discussion

Both wild foxes and wolves can be infected with *E. multilocularis* (7). In addition, tapeworm eggs are excreted in gravid segments, which is a limitation of the egg test method. Experiments have shown that dosing with praziquantel for deworming can greatly improve the positive detection rate of tapeworm eggs (17). In this study, *E. multilocularis* eggs (2/11) were detected in fox feces before deworming, indicating that the wild foxes in the area were infected with *E. multilocularis*. One year after deworming, *E. multilocularis* eggs were again detected in the fox feces, suggesting that the wild foxes had been reinfected and that deworming should be performed regularly. Moreover, baits impregnated with praziquantel have been used for *E. multilocularis* (18, 19), with the intent of interrupting the parasite's transmission cycle in wildlife (20). This study confirmed

that wild foxes consume praziquantel embedded in attractants based on infrared camera images and on-site confirmation. *E. multilocularis* eggs were not detected in the faeces of the wild foxes for one year after deworming, indicating that praziquantel has a good deworming effect and can be used to control echinococcosis in wild foxes. However, the eggs of *E. multilocularis* were detected in the feces of the foxes one and two years later. This result suggested that wild foxes were repeatedly infected with *E. multilocularis*, which is fundamentally the same result as in previous studies performed in Europe; the foxes were not fully dewormed, and *E. multilocularis* rapidly recovered its parasitic capacity (13). Therefore, deworming for prevention and control is required at least twice per year. In future experiments, the experimental area should be increased, and the numbers of fox dens should also be increased; if possible, the foxes should be tagged with GPS trackers.

With the gradual improvement in the ecological environment of the Qinghai-Tibet Plateau and the increased awareness regarding animal protection, the numbers of animals have been constantly increasing. The ranges of wild foxes and wolves are encroaching on the living environments of people. Therefore, the risk of echinococcosis transmission by wild canines is increasing, and additional attention should be given now and in the future.

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Conflict of Interest

The authors declare no conflicts of interest.

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