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Research paper

Detection of *Giardia duodenalis* assemblage E infections at the Tibetan Plateau Area: Yaks are suitable hosts

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ABSTRACT

The prevalence of *Giardia duodenalis* (*G. duodenalis*) assemblages in yaks is poorly known. The present study examined 297 fecal samples from weaned yak, 4–7 months of age, from 3 different farms, in Tibetan Plateau Area (TPA) of the Qinghai Province in Western China. The prevalence of infection was determined by light and immunofluorescence microscopy, and nested-PCR. PCR was performed for the small subunit ribosomal RNA (SSU) amplified 16 positive for *G. duodenalis* products. The prevalence of *Giardia* species was 5.0% (15/297) on light microscopic analysis, 6.1% (18/297) on immunofluorescence test (IFT) and 5.4% (16/297) on nested-PCR. The overall prevalence with the three methods was 5.5%. Ten of the 16 PCR products have been successfully sequenced. Sequence results and phylogenetic analysis of the 18S rRNA sequence data using MEGA5.0 and DNAstar7.0 identified all samples of interest as *G. duodenalis* assemblage E. This study revealed for the first time the presence of *G. duodenalis* in yaks from the Qinghai province in China and confirmed that yak is a suitable host for *Giardia* parasites. The results provide useful information about *G. duodenalis* prevalence and the epidemiological significance of yak a suitable host to harbor *Giardia* infections.

1. Introduction

Giardia duodenalis (*G. duodenalis*) is common and widespread intestinal protozoan parasite of both humans and animals (Karanis and Ey, 1998; Plutzer et al., 2010; Feng and Xiao, 2011). Giardiasis infections result from fecal-oral transmission of *Giardia* cysts, usually via water, food, or direct contact (Karanis et al., 2007; Baldursson and Karanis, 2011; Plutzer et al., 2010; Xiao and Fayer, 2008). Therefore, the role of livestock as reservoirs of *G. duodenalis* and its potential threat to public health are of increasing concern (Thompson, 2004). To date, *G. duodenalis* is considered a multi-species complex, comprising at least eight distinct genotypes or assemblages (A-H) based on molecular genetic analyses at limited and highly conserved loci (Ryan and Caccio, 2013). Assemblages A and B are zoonotic, whereas C, D, E, F, G, and H are defined as host restricted (Li et al., 2016; Thompson, 2000). In China, the molecular studies of *Giardia* have been reported in dairy cattle, sheep and goats, dogs, rabbits, monkeys, wild animals (Tibet antelope, Pika and Zokor Sokhor) and human (Feng and Xiao, 2011; Li et al., 2013; Ma et al., 2014; Ye et al., 2012; Zhang et al., 2012). However, there have been only two studies of *G. duodenalis* infection in yaks (*Bos grunniens*) in China (Qi et al., 2015; Song et al., 2016) and less is known about the prevalence and molecular characteristics of this pathogen in yaks. Yaks are members of bovid family, but they reside at a higher altitude than any other member of the family (2500 to 6000 m). They live throughout the Himalaya region of southern central Asia, the Qinghai-Tibetan plateau and as far north as Mongolia and Russia, where the air pressure is high and the temperature and oxygen content are low. Most of them are domesticated *B. grunniens* but there is also a small population of wild yaks *B. mutus*. On the Qinghai Province, the yak population, about 5 million, is the highest in the word (Sun et al., 2011). Milk, meat, bones, wool and leather from yak sought-after products for many local people. Therefore, research into the parasites that infect yaks is important for the habitants of these regions. The need to ensure the maintenance of domestic animal diversity, therefore, cannot be overstated (Wiener et al., 2003). Previous studies have indicated that *Giardia* species could affect yaks (Qi et al., 2015; Song et al., 2016). However, to our knowledge only two studies for *Giardia* infections in yaks (Ma et al., 2014; Qi et al., 2015) from Qinghai province of China with limited information on species and subspecies have been published before. The aim of the present study was to determine the preva-

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lence of *G. duodenalis* in yaks in Qinghai province, in Tibetan Plateau Area (TPA) of China, and characterize it at the molecular level, using genetic relationships of *G. duodenalis* inferred from partial SSU RNA sequence data.

2. Materials and methods

2.1. Ethics statement

This study was performed strictly according to the Chinese Laboratory Animal Administration Act 1988. Our protocol was reviewed and approved by the Research Ethics Committee of Qinghai University, Xining PR China. All fecal specimens were collected from yaks with the permission of owners.

2.2. Sample collection

A total of 297 fresh fecal samples were collected between September 2015 and November 2015 from yaks aged 4–7 months on seven farms in 3 areas (Qilian, Haiyan, Gangcha counties) in Qinghai Province of TPA, northwestern China (Fig. 1). Yaks in these areas were kept outdoors and shared pastures with sheep, goats and wild animals. Fecal samples were collected with sterile gloves, placed into a disposable plastic tub, with the age of each animal being recorded and transported to the laboratory in the Qinghai Academy for Animal Sciences and Veterinary Medicine, Qinghai University, Xining, China. The samples were stored at −20 °C until use.

2.3. Giardia *cysts concentration and microscopic examination*

Microscopic examinations using bright light or immunofluorescence were performed on all samples after discontinuous sucrose gradient flotation technique as described previously (Gomez-Couso et al., 2006). *Giardia* cysts concentrated with the later method were detected with Lugol's iodine staining technique and microphotographs were acquired under x400 magnification, using an Olympus CHA Bright-field microscope equipped with a LY-WN-HP-CCD digital camera.

2.4. Immunofluorescence test (IFT)

The concentrated pellet was mixed and 25 μl of each concentrated sample was transferred to a microscope slide, fixed with methanol and stained with iso-thiocyanate-conjugated anti-*Giardia* spp. monoclonal-labeled antibody (Cellabs Biotechnology, Australia). The slide was covered with a coverslip, and the entire coverslip area was examined by fluorescence microscopy under x400 magnification, using Nikon Eclipse Ni fluorescence microscope equipped with a Nikon DS-Ri2 camera. Samples, that fulfill the criteria for *Giardia* cysts based on size, shape and fluorescence where identified as *Giardia* species cysts based on the manufacture guidelines and Karanis et al. (1996).

2.5. DNA extraction

Fecal specimens were washed three times in distilled water with centrifugation at 3000xg. Genomic DNA was extracted from pellet using a QIAamp Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer-recommended protocol, with the addition of eight freeze-thaw cycles (after the ASL lysis buffer step, samples were freezed at liquid nitrogen and then thawed at 90 °C for eight times, to brake the cyst wall of *Giardia*). The extracted DNA was stored at −20 °C.

2.6. PCR assays

Shortly after DNA extraction, *Giardia* spp PCR was performed using a two-step nested-PCR amplification of the SSU rRNA gene using primers Gia2029 (5′-AAGTGTGGTGCAGACGGACTC-3′) and Gia2150c (5′-CTGCTGCCGTCCTTGGATGT-3′) for the primary PCR, and RH11 (5′-CATCCGGTCGATCCTGCC-3′) and RH4

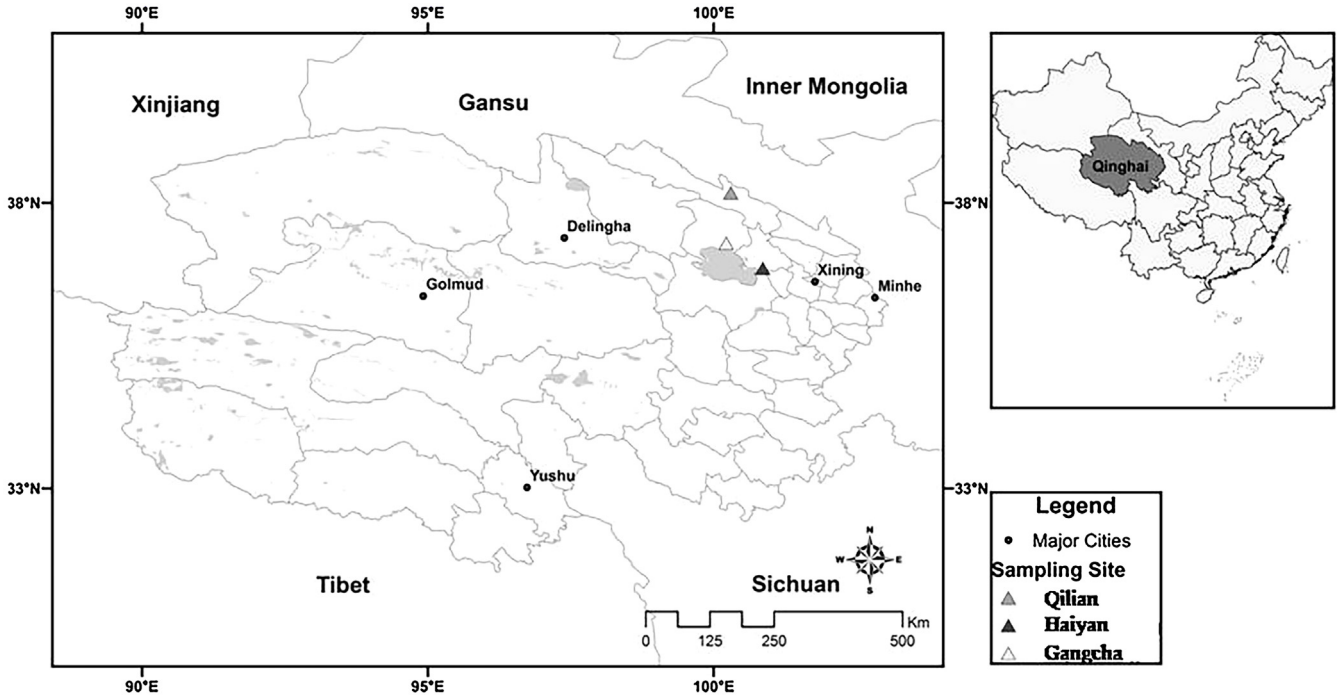

Fig. 1. Counties and the specific locations at the Qinghai TPA, in which specimens were collected during this study. The specific sampling locations are three villages in the counties of interest and they are noted in triangle. The major cities of Qinghai province are also noted in the map. (Map was kindly drafted by Environ. Eng. Ioannis Kontogeorgos).