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Research paper

# Multilocus genotyping of *Giardia duodenalis* in Tibetan sheep and yaks in Qinghai, China

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#### ABSTRACT

Giardia duodenalis is a common gastrointestinal protozoon in mammals. Although many studies have been reported on the distribution of G. duodenalis genotypes in sheep and cattle raised under intensive farming, few studies are available on the distribution of G. duodenalis in Tibetan sheep and yaks, which are raised free ranging in a continental plateau climate. In this study, 495 fecal specimens from Tibetan sheep and 605 from yaks were collected from eight counties in Qinghai, China and analyzed for G. duodenalis by PCR targeting the  $\beta$ -giardin (bg), glutamate dehydrogenase (gdh), and triosephosphate isomerase (tpi) genes. Based on PCR positivity at the bg locus, G. duodenalis occurrence rates were 13.1% (65/495) in Tibetan sheep and 10.4% (63/605) in yaks. DNA sequence analysis identified the presence of G. duodenalis Assemblages A (in 10 Tibetan sheep and 2 yaks) and E (in 51 Tibetan sheep and 60 yaks). In addition, mixed infections of the two were identified in four Tibetan sheep and one yak. Among the sequences obtained in this study, 1, 10, and 2 new subtypes of Assemblage E were detected at the bg, gdh and tpi loci, respectively. Based on sequences from the three loci, 28 multilocus genotypes (MLGs) were obtained, including 27 MLGs in Assemblage E and one MLG in Assemblage A. Each MLG was found in no more than seven animals, with most MLGs forming host-specific clusters in phylogenetic analysis except for one cluster including MLGs from both Tibetan sheep and yaks. Only two MLGs were found in both sheep and yaks. The above results demonstrate a high subtype diversity of G. duodenalis Assemblage E in Tibetan sheep and yaks raised in a traditional animal husbandry system and suggest that only limited cross-species transmission of G. duodenalis occurs between yaks and sheep sharing pastures.

#### 1. Introduction

Giardiasis, caused by the infection of *Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*), is a common enteric disease of various mammals (Feng and Xiao, 2011; Karanis and Ey, 1998; Plutzer et al., 2010). It causes diarrhea, abdominal pain, bloating, and weight loss in humans and farm animals (Einarsson et al., 2016). In humans, the parasite is transmitted mainly via ingestion of contaminated water and food or direct contact with infected animals (Baldursson and Karanis, 2011; Efstratiou et al., 2017; Feng and Xiao, 2011; Karanis et al., 2007; Plutzer et al., 2010). Cloven-hoofed animals (cattle, sheep, pigs, etc.) are believed by some to be reservoirs for *G. duodenalis* infection in humans. Thus, giardiasis is considered a zoonotic disease and

*G. duodenalis* infection in farm animals has some public health potential.

Thus far, eight assemblages (A–H) of *G. duodenalis* have been identified, with two of them (A and B) having a broad host range and the remaining (C–H) mostly in specific groups of animals (Feng and Xiao, 2011; Ryan and Caccio, 2013). In hoofed livestock, especially sheep and cattle, Assemblage E is the dominant genotype (Gillhuber et al., 2013; Gomez-Munoz et al., 2012; Liu et al., 2015; Wang et al., 2017; Wang et al., 2016b; Zhang et al., 2012), although infections with Assemblages A and B have also been detected. A few studies have shown a common occurrence of Assemblage A or B in sheep and cattle (Lalle et al., 2005; Wang et al., 2014; Ye et al., 2015).

Recently, multilocus genotyping (MLG) at the  $\beta$ -giardin (bg),

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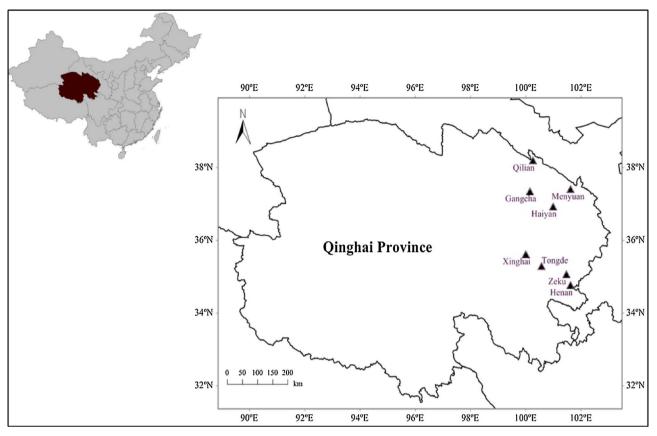


Fig 1. Counties (A) in Qinghai Province, northwestern China, examined in this study for Giardia duodenalis infection in Tibetan sheep and yaks.

#### Table 1

Distribution of Giardia duodenalis genoty	pes and subtypes in Tibetan sh	neep and yaks in Qinghai Province	e, China by counties.

Location	No. positive/No. examined (%)		Genotype or subtype						
	sheep	yak	sheep			yak			
			bg	gdh	tpi	bg	gdh	tpi	
Tongde	14/86 (16.3)	6/102 (5.9)	E (12), A1 (1)	E (8), A1 (1)	E (8), A1 (2)	E (5)	E (5)	E (2)	
Zeku	4/45 (8.9)	16/67 (23.9)	E (3)	E (4)	E (2)	E (16)	E (13)	E (9)	
Menyuan	5/58 (8.6)	14/136 (10.3)	E (3), A1 (2)	E (2), A1 (1)	E (1), A1 (1)	E (10), A2 (1)	E (10)	E (7)	
Xinghai	21/120 (17.5)	10/125 (8.0)	E (14), A1 (6)	E (6), A1 (4)	E (3), A1 (7)	E (10)	E (5)	E (5), A1 (1)	
Qilian	9/66 (13.6)	16/143 (11.2)	E (7), A1 (2)	E (5), A1 (1)	E (5), A1 (1)	E (16)	E (9)	E (11)	
Henan	2/31 (6.5)	1.0/32 (3.0)	E (2)	E (1)	E (1)	A2 (1)	0	0	
Haiyan	8/51 (15.7)	0	E (8)	E (6)	E (6)	0	0	0	
Gangcha	2/38 (5.3)	0	E (2)	0	0	0	0	0	
Total	13.5 (55/406)	10.4 (63/605)	E (51), A1 (11)	E (32), A1 (7)	E (26), A1 (11)	E (57), A2 (2)	E (42)	E (34), A1 (1)	

glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) loci has been used in some studies on the distribution of *G. duodenalis* genotypes and subtypes (Caccio et al., 2008; Gomez-Munoz et al., 2012; Qi et al., 2015; Wang et al., 2016b). Because PCR assays targeting these loci have been shown to have different sensitivities and occasionally genotyping results (Fava et al., 2013; Geurden et al., 2008; Liu et al., 2012; Nolan et al., 2010; Wang et al., 2014), MLG is often used in recent molecular epidemiologic studies of *G. duodenalis* in humans and animals.

Many studies have reported the distribution of *G. duodenalis* genotypes in sheep and cattle raised under intensive farming environment (Abeywardena et al., 2014; Jafari et al., 2014; Li et al., 2016; Liu et al., 2015; Peng et al., 2016; Wang et al., 2016a; Wang et al., 2014; Zhang et al., 2012). However, there are no studies on *G. duodenalis* in Tibetan sheep, and only a few on *G. duodenalis* in yaks (Qi et al., 2015; Song et al., 2016; Wang et al., 2017). Both Tibetan sheep and yaks are raised free range under a continental plateau climate (i. e., high altitude, cold climate and low rainfall), sharing pastures with each other and wildlife.

The main objectives of this study were 1) to identify the occurrence and distribution of *G. duodenalis* genotypes in Tibetan sheep and yaks; 2) to assess the public health potential of *G. duodenalis* in these animals; and 3) to assess the extent of cross-species transmission of infections between Tibetan sheep and yaks using subtyping based on sequence analysis of three genetic loci (*bg*, *gdh* and *tpi*).

#### 2. Materials and methods

#### 2.1. Ethics statement

Permission was obtained from the owners of yaks and Tibetan sheep before the collection of fecal specimens. The field study was approved by the Ethic Committee of the Qinghai Academy of Veterinary Medicine Download English Version:

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