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Original Article

Seasonal Prevalence and Novel Multilocus Genotypes of *Giardia duodenalis* in Yaks (*Bos grunniens*) in Qinghai Province, Western China

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Abstract

Background: *Giardia duodenalis* is an important opportunistic zoonotic intestinal protozoon, which could parasitize yaks. However, a few studies have been conducted on the seasonal infection of *G. duodenalis* in yaks in China.

Methods: Overall, 1,027 fecal samples were collected from yaks of two age groups in seven cities of Qinghai Province, China at four seasons between May 2016 and Sep 2017. The prevalence and assemblages were analyzed by nested PCR and multilocus sequence typing (MLST).

Results: The overall prevalence of *G. duodenalis* was 2.04% (21/1027) based on triose phosphate isomease (tpi) locus. No significant differences in prevalence of the organism in yaks were found among different sampling areas. Additionally, same result was also presented in different seasons. However, there was statistically significant difference between young yaks within 6 months (8.33%, 4/48) and adult yaks over 6 months (1.73%, 17/979). The assemblage A recognized as a zoonotic assemblage (n=3) was found in yaks (>6 months) from Xining, while assemblage E (n=18) was detected from yaks in six cities. There were 5, 2 and 3 *G. duodenalis* subtypes detected positive at the tpi, the β-giardin (bg), and the glutamate dehydrogenase (gdh) loci, with 2, 2 and 3 novel subtypes, respectively. Three samples were successfully sequenced at all three loci, forming 1 assemblages A multilocus genotype (MLG) and 2 assemblages E MLGs, not reported.

Conclusion: This study indicated a zoonotic potential of *G. duodenalis* in yaks from Qinghai Province and provides basic information about the epidemiology of *G. duodenalis*.

Introduction

Giardia is a very common enteric parasite of humans and many animals (1-4). This protist, firstly described by Leeuwenhoek in 1681 (5), and was identified by Xiao et al in 2006 in China for the first time (6). Although six species of *Giardia* were distinguished based on light microscopic, electro-microscopic characteristics and molecular biology (7), *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*) is the only *Giardia* species that could cause human infection (8), which transmits through the fecal-oral route, via direct or indirect contact with feces (9).

With infection by *G. duodenalis*, the most common clinical syndromes are known as diarrhea, weight loss, and impairment to the feed efficiency (7, 10). So far, *G. duodenalis* is regarded as a multispecies complex encompassing 8 genetic assemblages (A-H) which differ in host distribution (11, 12). Among them, assemblages A, B and E have been found in yaks from Qinghai Tibetan plateau before (11, 12), with assemblage E seen as the genotype responsible for the most *G. duodenalis* infection in bovid family (11, 13-20). It is considered as one of the main causes of diarrhea in cattle infected with not only the host-specific assemblage E but also the zoonotic assemblages A and B (8-10), causing a great loss to the breeding industry.

Yaks are the natural hosts of *G. duodenalis* (11, 16-17). Yaks as "boat of the plateau" are living in the highest altitude of the world and are raised free range under the plateau climate with high pressure and low temperature (21). The largest population of yaks in the world, approximately 5 million, are in Qinghai Tibetan plateau, not only serving as a tool of transportation, but also supplying milk, wool, and fur for the local people (20, 21). Although they are a small proportion of the world's cattle industry, but they are the major economic animals in the Tibetan plateau and important for the residents. *G. duodenalis* infections on yaks would cause severe diarrhea, serious growth retardation and even death.

In recent years, limited and highly conserved loci have been used to detect the prevalence and genotypic distribution of *G. duodenalis* by PCR amplifications, like small subunit ribosomal RNA (SSU rRNA), triose phosphate isomease (tpi), the β-giardin (bg), and the glutamate dehydrogenase (gdh) gene loci (22-25), and the result varied according to the diverse region, season, host age and species in cattle infection (26-28). Since PCR assays of different gene loci have different sensitivities and a single gene amplification may not supply enough information to define the genotype of *G. duodenalis*, multilocus sequence typing (MLST) has been developed to confirm the genetic differences among assemblages of *G. duodenalis* (29).

There are many studies about the prevalence and genotype of *G. duodenalis* in yaks (11, 12, 16), but few research was done on the seasonal prevalence and multilocus genotyping. The purpose of the present study was to investigate the prevalence and assemblages of *G. duodenalis* in yaks from different areas, seasons and host ages, and to evaluate the potential threat of this pathogen to the local public health.

Materials and Methods

Sampling

Overall, 1,027 fresh fecal samples were collected from yaks between May 2016 and Sep 2017, in 7 areas of Qinghai Province, western China (Fig. 1). These yaks are raised free-range in Tibetan Plateau. The fresh fecal samples of yaks were collected into individual plastic bags, labeled with the location, date, breed and number, then transported to laboratory and stored in 2.5% potassium dichromate at 4 ℃.

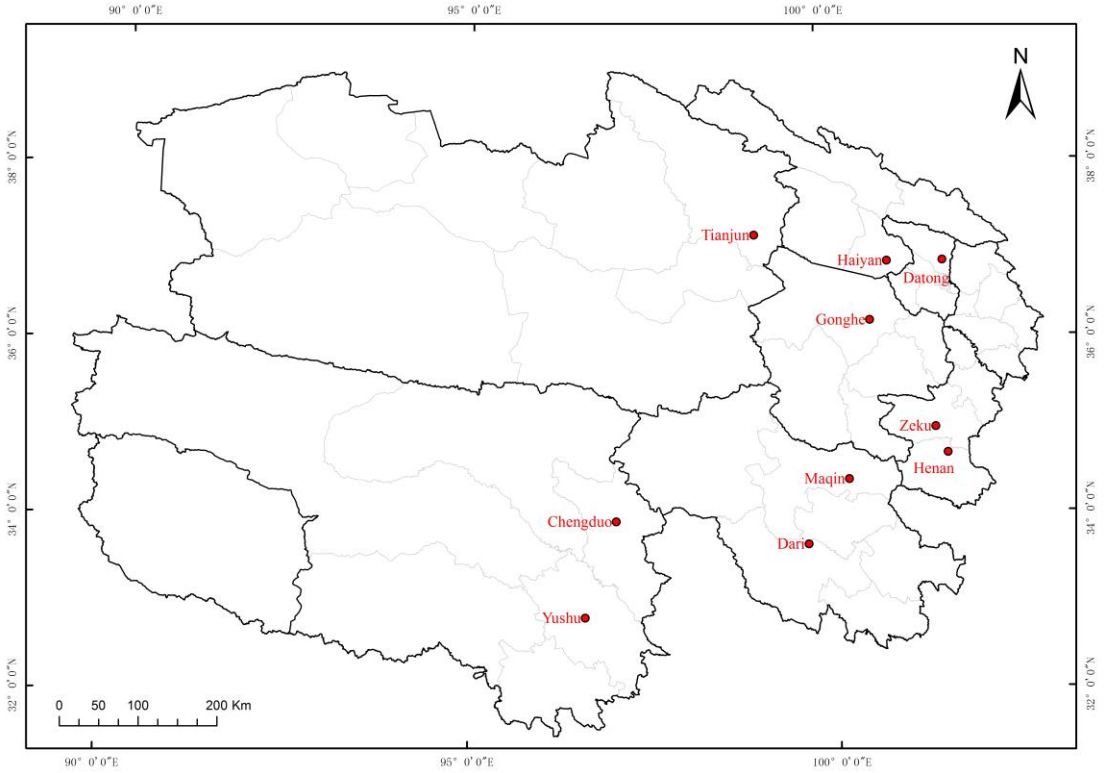

Fig. 1: Counties in Qinghai Province, western China, where samples were collected in this study

DNA Extraction and Amplification

For DNA extraction, each sample was washed several times with distilled water by centrifugation at 3000g to wash out the potassium dichromate. Genome DNA was extracted with an E.Z.N.A.® Stool DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer-recommended protocol and stored at -20 ℃.

To determine the species and genotype of *G. duodenalis*, all the extracted DNA was amplified by nested PCR at tpi gene under the protocol and primers referenced from Sulaiman with the amplified fragment about 530 bp (30).

MLST and Sequencing

The positive samples were amplified at bg gene and gdh gene to identify subtypes at each gene locus (31-33). The positive nested PCR productions were sent to Shanghai Sangon Biotechnology Company for sequencing on an ABI PRISM 3730 XL DNA Analyzer (Applied Biosystems, USA). The sequences were compared with reference sequences in the GenBank database using Basic Local Alignment Search Tool (BLAST).

Statistical analysis

The differences between prevalence of the regions, seasons and ages were analyzed by the method of chi-square test with SPSS Statistics ver.21.0 (IBM Corp. New York, NY). The difference was considered statistically significant when $P<0.05$.

Results

PCR amplification at tpi gene showed the overall prevalence of *G. duodenalis* in yaks was 2.04%, ranging from 0% to 3.33% in 7 areas (Table 1). Although the differences in prevalence of the seven sampling areas and four

different seasons were not significant, there was statistically significant difference between young yaks within 6 months (8.33%, 4/48) and adult yaks over 6 months (1.73%, 17/979).

Table 1: Occurrence of *Giardia duodenalis* according to the animal age, season and area from yaks in Qinghai Province, China

Variable	*Categories*		*No. of sample*	*Prevalence (No.)*	*Assemblages (No.)*	
Age	< 6 months		48	8.33% (4)		E (4)
	> 6 months		979	1.73% (17)	A (3)	E(14)
Season	Spring		215	0.93% (2)		E (2)
	Summer		355	2.25% (9)	A (3)	E (6)
	Autumn		254	2.36% (6)		E (6)
	Winter		203	1.97% (4)		E (4)
State	Xining	Datong	192	2.08% (4)	A (3)	E (1)
/County	Haibei	Haiyan	190	2.11% (4)		E (4)
	Hainan	Gonghe	162	0.62% (1)		E (1)
	Haixi	Tianjun	47	2.13% (1)		E (1)
	Huangnan	Zeku	218	2.75% (6)		E (6)
		Henan	52	3.85% (2)		E (2)
		Subtotal	270	2.96% (8)		E (8)
	Yushu	Yushu	50	2.00% (1)		E (1)
		Chengduo	40	5.00% (2)		E (2)
		Subtotal	90	3.33% (3)		E (3)
	Guoluo	Maqin	69			
		Dari	7			
		Subtotal	76	0% (0)		
Total			1027	2.04% (21)	A (3)	E (18)

Of the 21 *G. duodenalis* positive samples at tpi locus, 2 genotypes were identified, including assemblages A (14.29%, 3/21) and assemblages E (84.71%, 18/21). Only 1 subtype (accession no. MH230890) of assemblages A was identified as identical to the reference sequence JQ688289 (subtype A1). Four subtypes of assemblages E were identified (accession no. MH230886-MH230889), while MH230886 and MH230887 showed 100% similarity to the sequences referenced from GenBank with accession numbers of KY769100 and KY633482, respectively. The other two sequences not reported before were similar to the referenced sequence KY769100 with substitutions at positions 148 and 494, respectively.

Among the 21 *G. duodenalis* positive samples at tpi locus, 4 samples were positive at bg locus, and 5 samples were positive at gdh locus. Two subtypes of assemblage A were found at the bg (n=1) and gdh (n=1) loci, respectively. Of which 1 subtype sequence at the bg locus (accession no. MH230882) showed 99% similarity to the reference sequence of subtype A1 (EU726988) and the other sequence of the gdh locus (accession no. MH230885) showed 99% similarity to the referenced sequence subtype A1 (KF843930). And one novel subtype of assemblages E, named as E1 in present study, was identified at bg locus (accession no. MH230881), which was 99% similar to the reference sequence (KT922249). Two novel subtypes of assemblage E identified at gdh locus (accession no. MH230883- MH230884), named as E1 and E2, were 99% similar to the reference sequences AB692776 and AB692774.

Only 3 samples were successfully subtyped at all three gene loci altogether, forming 1 assemblage A MLG and 2 assemblages E MLGs. The A MLG was A1A1A1 (at the tpi, bg, gdh,

respectively). Besides, the E MLGs were E1E1E1 and E1E1E2, not reported before.

Discussion

As the most important economic animals in plateau area, yaks have been reported as suitable hosts for many kinds of parasites. For bovid family, the infection rates of *G. duodenalis* can be up to 60.1% in dairy cattle from Shanghai (11, 34). In present study, the infection rate of *G. duodenalis* in yaks from seven places of Qinghai Province was 2.04%, which was similar to the prevalence of yaks from central western region of China (2.9%) and white yaks from Tianzhu county of Gansu Province (1.92%) (11, 17). However, it is lower than the prevalence in previous studies, which were 5.4% and 10.4% on *G. duodenalis* in yaks in Qinghai Province (12, 17). Moreover, the result we investigated was lower than other studies about *G. duodenalis* infection in cattle from other areas in China and other counties, such as 4.58% in dairy cattle from Ningxia Province, 40.19% in cattle from Canada and 34.5% in cattle from Zambia (25, 35, 36). The reason may be that most samples we collected were from adult yaks, while according to reports, the infection rate of calves is much higher than the average infection rate of cattle (37, 38). In addition, low parasite load might prevent the amplification of some loci analyzed.

The significant difference between the infections of two age groups of yaks indicated that the infection of *G. duodenalis* is more common in the young yaks. The results were coordinated with what reported previously in the central-western region of China (11), which shows that the prevalence of *G. duodenalis* is related to age. However, the sample size of young yaks in this study is relatively small, and this conclusion needs further investigation. Judging from the infection rate of *G. duodenalis* in different seasons, the infection of *G. duodenalis* existed all year round, particularly during summer and autumn, which was similar to the description by Xiao et al. (39). However, the difference in prevalence of *G. duodenalis* in different seasons is not significant.

Additionally, in this study, 76 fecal samples were collected from Guoluo area, where the detection of *Giardia* has never been conducted so far. No *G. duodenalis* infection was found in Guoluo area. The data contributed to understanding the prevalence of *G. duodenalis* in yaks.

Sequence analysis confirmed that assemblage A and assemblage E of *G. duodenalis* both exist in yaks in Qinghai Province. Assemblage E is the dominant *G. duodenalis* genotype as previously reported (11, 16-17). Assemblage A, the major zoonotic genotype, was found in three samples in this study. Meanwhile, all sequences of assemblage A from the three loci were subtype A1 found in human (40), dairy cattle (26), sheep (12), horses (41), alpacas (42). Even though it is more common in animals than humans are, there is still potential for yaks transmitting *G. duodenalis* to humans.

Two, two and three novel subtypes were found at tpi, bg and gdh loci in this study, respectively, which showed the polymorphism of nucleotides in *G. duodenalis*. However, only 3 samples were successfully sequenced at all loci. The present study formed 1 assemblage A MLG and 2 assemblage E MLGs which were not identical to the results in previous study in Qinghai Province (35).

Conclusion

Both zoonotic assemblage A and non-zoonotic assemblage E of *G. duodenalis* were identified in yaks in Qinghai Province, northwestern China. This study could contribute to a better understanding of the epidemiology of *G. duodenalis* in yaks in Qinghai Province and provide basic information for the prevention and treatment of giardiasis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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