**Original Article**

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

Jun-Ke Song 1,2, Dan Wang 1, Mei Ren 1, Fan Yang 1, Pin-Xue Wang 1, Min Zou 1, Guang-Hui Zhao 1, *Qing Lin 1,2*

1. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi Province 712100, China
2. State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, Qinghai Province 810016, China

| Received  | 11 Jun 2020 |
| Accepted  | 12 Aug 2020 |

**Abstract**

**Background:** *Giardia duodenalis* is an important opportunistic zoonotic intestinal protozoan, 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 isomerase (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*.

**Keywords:** *Giardia duodenalis*; Multilocus sequence typing (MLST); Yaks; Prevalence

*Correspondence Email:* yllinqing@126.com

Available at: [http://ijpa.tums.ac.ir](http://ijpa.tums.ac.ir)
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, electron-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 isomerase (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 °C.
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 °C.

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)            |
|          | Xining     | 192           | 0.93% (4)        | A (3) E (1)      |
|          | Datong     | 190           | 2.11% (4)        | E (4)            |
|          | Haibei     | 182           | 0.62% (1)        | E (1)            |
|          | Haiyan     | 162           | 2.13% (1)        | E (1)            |
|          | Hainan     | 162           | 2.13% (1)        | E (1)            |
|          | Tienjin    | 47            | 2.13% (1)        | E (1)            |
|          | Huangnan   | 218           | 2.75% (6)        | E (6)            |
|          | Zeku       | 52            | 3.85% (2)        | E (2)            |
|          | Henan      | 270           | 2.96% (8)        | E (8)            |
|          | Subtotal   | 270           | 2.96% (8)        | E (8)            |
|          | Yushu      | 50            | 2.00% (1)        | E (1)            |
|          | Yushu      | 50            | 2.00% (1)        | E (1)            |
|          | Chengduo   | 40            | 5.00% (2)        | E (2)            |
|          | Subtotal   | 90            | 3.33% (3)        | E (3)            |
|          | Maqin      | 69            | 0% (0)           | A (3) E (18)     |
|          | Dari       | 7             | 0% (0)           | A (3) E (18)     |
| 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 Tianshu 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.
Acknowledgements

This study was supported by the Project of Qinghai Science & Technology Department (2016-ZJ-754 and 2016-ZJ-Y01) and the Open Project of State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University (2017-ZZ-08). We thank the owners of the yaks for their collaboration.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Lee MF, Cadogan P, Eytle S, et al. Molecular epidemiology and multilocus sequence analysis of potentially zoonotic Giardia spp. from humans and dogs in Jamaica. Parasitol Res. 2017; 116(1): 409-14.
2. Di Cristanziano V, Santoro M, Parisi F, et al. Genetic characterization of Giardia duodenalis by sequence analysis in humans and animals in Pemba Island, Tanzania. Parasitol Int. 2014; 63(2): 438-41.
3. Squire SA, Yang R, Robertson I et al. Molecular characterization of Cryptosporidium and Giardia in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana. Infect Genet Evol. 2017; 55: 236-43.
4. Zheng G, Alsarakihi M, Liu Y, et al. Genotyping of Giardia duodenalis isolates from dogs in Guangdong, China based on multi-locus sequence. Korean J Parasitol. 2014; 52(3): 299-304.
5. Faubert G. Immune response to Giardia duodenalis. Clin Microbiol Rev. 2000; 13(1): 35-54.
6. Xiao S, Li G, Wang B, et al. Molecular identification of the first isolate of calf-derived Giardia from mainland of China. Chinese Journal of Zoonoses. 2006; 22(9): 861-3.
7. Plutzer J, Ongerth J, Karanis P. Giardia taxonomy, phylogeny and epidemiology: Facts and open questions. Int J Hyg Environ Health. 2010; 213(5): 321-33.
8. Heyworth MF. Giardia duodenalis genetic assemblages and hosts. Parasite. 2016; 23: 13.
9. Ryan U, Cacciò SM. Zoonotic potential of Giardia. Int J Parasitol. 2013; 43(12-13): 943-56.
10. Aloisio F, Filippini G, Antenucci P, et al. Severe weight loss in lambs infected with Giardia duodenalis assemblage B. Vet Parasitol. 2006; 142(1-2):154-8.
11. Qi M, Cai J, Wang R, et al. Molecular characterization of Cryptosporidium spp. and Giardia duodenalis from yaks in the central western region of China. BMC Microbiol. 2015; 15: 108.
12. Jin Y, Fei J, Cai J, et al. Multilocus genotyping of Giardia duodenalis in Tibetan sheep and yaks in Qinghai, China. Vet Parasitol. 2017; 247: 70-6.
13. Thompson RC. The zoonotic significance and molecular epidemiology of Giardia and giardiasis. Vet Parasitol. 2004; 126(1-2):15-35.
14. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev. 2011; 24(1): 110-40.
15. Asher AJ, Hose G, Power ML. Giardiasis in NSW: Identification of Giardia duodenalis assemblages contributing to human and cattle cases, and an epidemiological assessment of sporadic human giardiasis. Infect Genet Evol. 2016; 44: 157-61.
16. Song G, Qin S, Zhao G et al. Molecular characterization of Giardia duodenalis from white yaks in China. Acta Parasitol. 2016, 61(2): 397-400.
17. Wang G, Wang G, Li X et al. Detection of Giardia duodenalis assemblage E infections at the Tibetan Plateau Area: Yaks are suitable hosts. Acta Trop. 2017; 169: 157-62.
18. Feng Y, Gong X, Zhu K, et al. Prevalence and genotypic identification of Cryptosporidium spp., Giardia duodenalis and Enterocytozoon bieneusi in pre-weaned dairy calves in Guangdong, China. Parasites & Vectors. 2019; 12(1): 41.
19. Jin Y, Zhang X, Li X, et al. Prevalence and molecular characterization of Giardia duodenalis in cattle and sheep from the Qinghai-Tibetan Plateau Area (QTPA), northwestern China. Vet Parasit. 2018; 250: 40-4.
20. Wang G, Wang G, Li X, et al. Prevalence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in 1-2-month-old
highland yaks in Qinghai Province, China. Parasitol Res. 2018; 117(6): 1793-800.

21. Li K, Shahzad M, Zhang H, et al. Socio-economic burden of parasitic infections in yaks from 1984 to 2017 on Qinghai Tibetan Plateau of China-A review. Acta Trop. 2018; 183: 103-9.

22. Wang H, Zhao G, Chen G, et al. Multilocus genotyping of Giardia duodenalis in dairy cattle in Henan, China. PLoS One. 2014; 9(6): e100453.

23. Minetti C, Taueenan W, Hogg R et al. Occurrence and diversity of Giardia duodenalis assemblages in livestock in the UK. Transbound Emerg Dis. 2014; 61(6): e60-7.

24. Hu S, Liu Z, Yan F, et al. Zoonotic and host-adapted genotypes of Cryptosporidium spp., Giardia duodenalis and Enterocytozoon bieneusi in dairy cattle in Hebei and Tianjin, China. Vet Parasitol. 2017; 248: 68-73.

25. Zhang X, Tan Q, Zhao G, et al. Prevalence, Risk Factors and Multilocus Genotyping of Giardia intestinalis in Dairy Cattle, Northwest China. J Eukaryot Microbiol. 2016; 63(4): 498-504.

26. Wang X, Wang R, Ren G et al. Multilocus genotyping of Giardia duodenalis and Enterocytozoon bieneusi in dairy and native beef (Qinchuan) calves in Shaanxi province, northwestern China. Parasitol Res. 2016; 115(3): 1355-61.

27. Fan Y, Wang T, Koehler AV, et al. Molecular investigation of Cryptosporidium and Giardia in pre- and post-weaned calves in Hubei Province, China. Parasit Vectors. 2017; 10(1): 519.

28. Li J, Wang H, Wang R, et al. Giardia duodenalis Infections in Humans and Other Animals in China. Front Microbiol. 2017; 8: 2004.

29. Cacciò SM, Ryan, U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. 2008; 160(2): 75-80.

30. Sulaiman IM, Fayer R, Bern C, et al. Triosephosphate isomerase gene characterization and potential zoonotic transmission of Giardia duodenalis. Emerg Infect Dis. 2003; 9(11): 1444-52.

31. Gillhuber J, Pallant I, Ash A, et al. Molecular identification of zoonotic and livestock-specific Giardia-species in faecal samples of calves in Southern Germany. Parasit Vectors. 2013; 6(1): 346.

32. Lalle M, Jimenez-Cardosa E, Cacciò S M, et al. Genotyping of Giardia duodenalis from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. J Parasitol. 2005; 91(1): 203-5.

33. Cacciò S M, Beck R, Lalle M, et al. Multilocus genotyping of Giardia duodenalis reveals striking differences between assemblages A and B. Int J Parasitol. 2008; 38(13): 1523-31.

34. Wang X, Cai M, Jiang W, et al. High genetic diversity of Giardia duodenalis assemblage E in pre-weaned dairy calves in Shanghai, China, revealed by multilocus genotyping. Parasitol Res. 2017; 116(8): 2101-10.

35. Uehlinger FD, Greenwood SJ, O’Handley R, et al. Prevalence and genotypes of Giardia duodenalis in dairy and beef cattle in farms around Charlottetown, Prince Edward Island, Canada. Can Vet J. 2011; 52(9): 967-72.

36. Kakandewa C, Siwila J, Nalubamba KS, et al. Prevalence of Giardia in dairy cattle in Lusaka and Chilanga districts, Zambia. Vet Parasitol. 2016; 215: 114-6.

37. Huang J, Yue D, Qi M, et al. Prevalence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in dairy cattle in Ningxia, northwestern China. BMC Vet Res. 2014; 10: 292.

38. Liu G, Su Y, Zhou M, et al. Prevalence and molecular characterization of Giardia duodenalis isolates from dairy cattle in northeast China. Exp Parasitol. 2015; 154: 20-4.

39. Xiao L, Fayer R. Molecular characterization of species and genotypes of Cryptosporidium and Giardia and assessment of zoonotic transmission. Int J Parasitol. 2008; 38(11): 1239-55.

40. Volotão AC, Costa-Macedo LM, Haklad FS, et al. Genotyping of Giardia duodenalis from human and animal samples from Brazil using beta-giardin gene: a phylogenetic analysis. Acta Trop. 2007; 102(1): 10-9.

41. Traversa D, Orranto D, Milillo P, et al. Giardia duodenalis sub-Assemblage of animal and human origin in horses. Infect Genet Evol. 2012; 12(8): 1642-6.

42. Gomez-Puerta LA, Lopez-Urbina MT, Alarcon V, et al. Occurrence of Giardia duodenalis assemblages in alpacas in the Andean region. Parasitol Int. 2014; 63(1): 31-4.