Molecular detection and identification of *Giardia duodenalis* in cattle of Urmia, northwest of Iran

Farnaz Malekifard*, Minoo Ahmadpour

Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

**Article Info**

**Abstract**

*Giardia duodenalis* is one of the most prevalent intestinal protozoa infecting humans and domestic animals. The aim of this study was to identify subspecies of *G. duodenalis* by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method from fecal samples of naturally infected cattle in the Urmia, West Azerbaijan province, Iran. Overall, 246 fecal specimens were collected from the cattle (diarrheic and healthy) and microscopically examined for *G. duodenalis*. The PCR-RFLP analysis of glutamate dehydrogenase (*gdh*) locus was used to identify the genotypes found in cattle. In this method, 432 bp expected size was amplified and then specific restriction *Nla* IV enzyme was used for subspecies detection. Totally, 23 (9.34%) specimens were microscopically positive for giardia cyst out of 246 examined samples. The PCR-RFLP analysis revealed that 19 samples (82.60%) have the genotype E and 4 samples (17.39%) belong to the subgroup AI. Our findings indicated that *G. duodenalis* infection is prevalent in cattle of Urmia and the non-zoonotic genotype E predominates in cattle in this region.

**Key words:** Cattle *Giardia duodenalis* Glutamate dehydrogenase Iran PCR-RFLP

© 2018 Urmia University. All rights reserved.

*Correspondence:*

Farnaz Malekifard, DVM, PhD
Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
E-mail: fmalekifard@urmia.ac.ir

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.
Introduction

*Giardia duodenalis* (syn *Giardia intestinalis*) is an important protozoan parasite infecting a wide range of vertebrates including humans and domestic animals. It has been found in the feces of calves, beefs and dairy cattle worldwide. Giardiasis clinical manifestations in cattle are relatively variable, ranging from the absence of symptoms to persistent diarrhea, mucoid and fatty stool, weight loss and growth rate reduction. Cattle have been considered as potential sources of giardiasis in humans through direct contact and/or surface water supplies contamination.

*G. duodenalis* is now considered as a species complex comprising at least seven major genotypes (A–G). The molecular analysis of cattle isolates from different geographical locations has demonstrated that only *G. duodenalis* genotype E and the zoonotic genotypes (A and B) are associated with cattle infections. Recently, molecular techniques have been applied for *G. duodenalis* detection and genotyping in animals and humans. The use of molecular diagnostic techniques in the genotypic specifying of *G. duodenalis* has led to increased recognition of the diversity of parasites infecting humans and animals and role of animals in the transmission of human giardiasis.

Specifying of *G. duodenalis* genotypes has performed based on the characterization of the small subunit ribosomal RNA (SSU-rRNA), β-giardin (*bg*), glutamate dehydrogenase (*gdh*) and triose phosphate isomerase (*tpi*) genes. The *gdh* gene is useful for genotypic analysis of *G. duodenalis* parasites from mammals.

In Iran, although giardiasis has been reported in cattle in some regions, but there is not any data about *G. duodenalis* genotypes in cattle. Therefore, the main objective of the current study was to determine the genotypes of *G. duodenalis* isolates from cattle in Urmia, northwest of Iran using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) assay on the organism’s glutamate dehydrogenase (*gdh*) gene.

**Materials and Methods**

**Study area and sample collection.** This study was performed from February to September 2015 in Urmia, in northwest of Iran. Cattle fecal samples were collected from the rectum of each animal using an individual disposable latex glove. Each sample was placed in a plastic specimen cup with a screw-on lid, labeled and transported to the to the Laboratory of Parasitology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran within 2 hr after collection. The age, sex and consistency of the fecal samples were recorded for each animal. The samples were stored at 4 °C and processed within 24 hr.

**Light microscopy examination.** Giardia cysts were identified microscopically in fecal smears and partially concentrated by sucrose flotation. The concentrated cysts were stored in sterile distilled water without adding any preservatives, up to two weeks at –20 °C.

**DNA extraction.** DNA was extracted by phenol-chloroform-isooamyl alcohol (PCI) on concentrated fecal by sucrose gradient samples according to Rayani *et al.* with some modifications. Also, freeze-thaw was used for cyst wall disruption in some samples. Briefly, 200 μL of sediment concentrated cysts sample and 200 μL 3.00% Triton X100 were mixed and incubated in a water bath at 75 °C for 1 hr. Then, 200 μL of lysis buffer and 10 μL of proteinase K were added to 200 μL of homogenate and incubated at 37 °C overnight. The parasite DNA was extracted with PCI and precipitated with ethanol. The purified DNA pellets were dissolved in 100 μL of double-distilled water and stored at –20 °C for subsequent PCR reactions.

**PCR amplification.** In the PCR reaction, the 432 bp fragment of the *gdh* genes was amplified using the forward primer (GDHiF) 5’-CAG TAC AAC TCT GCT CTC GG-3’ and the reverse primer (GDHiR), 5’-GTT GTC CCT GCA CAT CTC C-3’. The PCR amplification was done using a programmable thermal cycler (Eppendorf, Hamburg, Germany). Amplification reaction was modified as follows, the PCR mix consisted of 1X buffer containing 1.50 mM MgCl₂ (Cinaclon, Tehran, Iran), each deoxynucleotide triphosphate at the concentration of 100 μM, 10 ng of DNA and 2.50 U of Taq DNA polymerase (Cinaclon, Tehran, Iran). Cycling parameters were 10 min at 94 °C (initial heat activation step), followed by 50 cycles of 35 sec at 94 °C, 35 sec at 61 °C and 50 sec at 72 °C, with a final extension of 7 min at 72 °C. Positive and negative controls were included in each PCR to validate results. Cysts were utilized as the templates for the positive controls and distilled water was utilized as the template for negative controls.

**The RFLP of the gdh gene.** All PCR positive specimens were subjected to RFLP analysis. *G. duodenalis* genotypes were determined by RFLP analysis as previously described. The RFLP analysis was carried out by digesting 8 μL of PCR products with 1.50 U of NlaIV enzyme (Vivantis, Vilnius, Lithuania) in 2 μL of 10X enzyme buffer in a final volume of 20 μL for 3 hr at 37 °C.

The PCR products and restriction fragments were separated respectively by horizontal electrophoresis in 1.50 and 2% agarose gels with ethidium bromide staining. A 100-bp DNA ladder (Fermentas, Darmstadt, Germany) was used as a size marker.

**Statistical analysis.** The prevalence of *G. duodenalis* infection in cattle was compared based on the different age groups, sex and diarrheic or none-diarrheic groups using the chi-square test. Data were analyzed using SPSS (version 17; SPSS Inc., Chicago, USA). A value of *p* < 0.05 was considered as statistically significant.
Results

Giardia duodenalis cysts were detected microscopically in 9.34% (23/246) of fecal samples (Fig. 1). The number of infected cattle based on age, sex and consistency of the fecal samples is summarized in Table 1. Statistically, there was no significant correlation between infection rate and sex factor ($p > 0.05$). The results indicated that infection with *G. duodenalis* is more prevalent in younger animals than older ones ($p < 0.05$), (Table 1). Fecal samples were classified according to the consistency as diarrheic (17/246) and non-diarrheic (229/246). *G. duodenalis* was detected in 52.94% (9/17) of diarrheic cattle and 6.11% (14/229) of non-diarrheic cattle. Thus, the prevalence of *G. duodenalis* in diarrheic cattle was significantly higher than non-diarrheic cattle ($p < 0.05$).

The PCR amplification. The gdh gene was successfully amplified from 23 (9.34%) samples. A 432 bp fragment of gdh gene was amplified in the PCR using GDHf and GDHRr primers (Fig. 2A).

The RFLP method. The PCR-RFLP analysis in all *G. duodenalis* positive samples using NlaIV enzyme revealed that 19 samples (82.60%) have the genotype E and 4 samples (17.39%) belong to the subgroup Al (Fig. 2B). Genotype B was not detected in this study.

Discussion

Giardiasis as a zoonotic infection can transmit to humans from infected cattle, sheep, cats and dogs. Zoonotic pathogenic species of giardia found in cattle can infect humans through contact with cattle and their feces and via drinking water contaminated with cattle faeces. There are several reports on the occurrence of *G. duodenalis* infections in cattle in different geographic regions, but little is known about *G. duodenalis* infection rates and genotypes in cattle in Iran.

![Fig. 1. Giardia spp. cysts in the purified fecal samples (Black arrows, 100×).](image)

![Fig. 2. A) Electrophoretic separation of PCR product from DNA amplified at the gdh locus of *G. duodenalis* on an ethidium bromide stained 1.50% agarose gel. Lane M: 100 bp gene ruler (Fermentas); Lane 1: Positive control; Lane 2: Negative control; Lanes 3-5: The PCR products from examined samples (432 bp fragment); B) The NlaIV digestion of PCR products on an ethidium bromide stained 2% high resolution agarose gel. Lane M: 100 bp gene ruler (Fermentas); Lane1: *G. duodenalis* genotype A1; Lanes 2-4: *G. duodenalis* genotype E.](image)

The present study was performed to detect the *G. duodenalis* from fecal samples of naturally infected cattle in the Urmia, northwest of Iran and determine the genetic characterization of these isolates.

In the present study, the frequency of *G. duodenalis* infection was 9.34% in the cattle. In comparison with studies conducted in other countries, this frequency rate was lower than the infection rates (22.00 to 60.00%) in the dairy cattle. Variations in the prevalence of giardia were probably due to differences in management, climate and study design. In the present study, the high prevalence of infection was observed in calves than older ones. The result was along with the previous reports in other countries. It could be attributed to the development of acquired immunity that may protect animals against disease.

In this study, *G. duodenalis* was detected in 52.94% (9/17) of diarrheic cattle and 6.11% (14/229) of non-diarrheic cattle. Similar to previous study done in this area, a significant association was observed between the presence of giardia cysts and occurrence of diarrhea. It has been shown that 28.00% of diarrheic calves in East Azarbaijan province, Iran were infected with *G. duodenalis*.  

| Risk factor | Gender | Age | Stool consistency |
|------------|--------|-----|------------------|
| Total      | Male   | Female | calves (< 1) | cattle (≥1) | diarrheic | non-diarrheic |
| Infection rate | 117 | 129 | 92 | 154 | 17 | 229 |

* indicates statistically significant difference compared with the corresponding risk factor at $p < 0.05$.  

**Table 1. Prevalence of Giardia duodenalis infections by different risk factors in the cattle of Urmia, Iran (n = 246).**
In the present study, RFLP-PCR with NlaI restriction enzyme was used to identify G. duodenalis genotypes. Based on our results, the majority (82.60%) of samples (19/23) were belonged to G. duodenalis assemblage E. This finding was in agreement with previous reports in Australia, the United States, Canada, New Zealand and Brazil.\textsuperscript{24,28,30,32,33}

In this study, zoonotic genotype AI was detected in approximately 17.39% of the positive animals (4/23). Our findings are in agreement with previous studies.\textsuperscript{4,7} Besides, our study indicated that the cattle are likely to be potential reservoir of zoonotic G. duodenalis in Iran.

It is the first genotypic assessment of G. duodenalis in cattle of Iran. Based on our results, the G. duodenalis genotype E and A were determined in cattle, in Urmia. The presence of assemblage A indicates that cattle can be a potential source of zoonotic G. duodenalis cysts. Further studies in other endemic regions in Iran are required to evaluate the zoonotic importance of giardia in cattle.

Acknowledgments

The authors would like to sincerely thank the Faculty of Veterinary Medicine and Urmia University Research Council for the approval and support of this research.

Conflicts of interest

The authors declare that there is no conflict of interest.

References

1. Thompson RCA. The zoonotic significance and molecular epidemiology of Giardia and giardiasis. Vet Parasitol 2004; 126: 15-35.
2. Becher KA, Robertson ID, Fraser DM, et al. Molecular epidemiology of Giardia and Cryptosporidium infections in dairy calves originating from three sources in Western Australia. Vet Parasitol 2004; 123:1-9.
3. Trout JM, Santín M, Greiner E, et al. Prevalence of Giardia duodenalis genotypes in pre-weaned dairy calves. Vet Parasitol 2004; 124: 179-186.
4. Appelbee AJ, Frederick LM, Heitman TL, et al. Prevalence and genotyping of Giardia duodenalis from beef calves in Alberta, Canada. Vet Parasitol 2003; 112: 289-294.
5. Barwick RS, Mohammed HO, White ME, et al. Factors associated with the likelihood of Giardia spp. and Cryptosporidium spp. in soils from dairy farms. J Dairy Sc 2003; 86:784-791.
6. Lalle M, Pozio G, Capelli F, et al. Genetic heterogeneity at the giardin locus among human and animal isolates of Giardia duodenalis and identification of potentially, zoonotic subgenotypes. Inter J Parasitol 2005; 35: 207-213.
7. O’Handley RM, Olson ME, Fraser D, et al. Prevalence and genotypic characterization of Giardia in dairy calves from Western Australia and Western Canada. Vet Parasitol 2000; 90: 193-200.
8. Thompson RCA. Giardiasis as a re-emerging infectious disease and its zoonotic potential. Inter J Parasitol 2000; 30: 1259-1267.
9. Thompson RCA, Hopkins RA, Homan WL. Nomenclature and genetic groupings of Giardia infecting mammals. Parasitol Today 2000; 16: 210–218.
10. Hunter PR, Thompson RC. The zoonotic transmission of Giardia and Cryptosporidium. Inter J for Parasitol 2005; 5: 1181-1190.
11. Monis PT, Andrews RH, Mayrhofer G, et al. Genetic diversity within the morphological species Giardia intestinalis and its relationship to host origin. Infect Genet Evol 2003; 3:29-38.
12. Monis PT, Thompson RCA. Cryptosporidium and Giardia zoonoses: Fact or fiction? Infect Genet Evol 2003; 3: 233-244.
13. Fallah E, Nahavandi k, Jamali R, et al. Molecular identification of Giardia duodenalis isolates from human and animal reservoirs by PCR-RFLP. Int J Biol Sci 2008; 2:172-184.
14. Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol 2004; 4: 125-130.
15. Itagaki T, Kinoshita S, Aoki M, et al. Genotyping of Giardia intestinalis from domesticated wild animals in Japan using glutamate dehydrogenase gene sequencing. Vet Parasitol 2005; 133(4): 283-287.
16. Xiao L, Fayer R. Molecular characterisation of species and genotypes of Cryptosporidium and Giardia and assessment of zoonotic transmission. Inter J Parasitol 2008; 38(11): 1239-1255.
17. Welenga CM, Thompson RCA. Comparative evaluation of Giardia duodenalis sequence data. Parasitol 2007; 134(12): 1795-1821.
18. Plutzer J, Ongerth J, Karanis P. Giardia taxonomy, phylogeny and epidemiology: Facts and open questions. Inter J Hyg Environ Health 2010; 213(5): 321-333.
19. Hazrati Tappeh K, Manafi G, Ashgarzadeh M, et al. Incidence of Giardia lamblia subspecies by PCR-RFLP in Stool specimens of hospitalized children at Urmia Mutahhari hospital, West Azerbaijan province, Iran. Iran J Parasitol 2014; 9(4): 541-547.
20. Luchtel DL, Lawrence WP, Dewalle FB. Electron microscopy of Giardia lamblia cysts. Appl Environ Microbiol 1980; 40(4): 821-832.
21. Bertrand I, Albertini L, Schwartzbrod J. Comparison of two target genes for detection and genotyping of Giardia lamblia in human feces by PCR and PCR-restriction fragment length polymorphism. J Clin
22. Rayani M, Zamsy Unyah N, Hatam G. Molecular identification of *Giardia duodenalis* isolates from Fars province, Iran. Iran J Parasitol 2014; 9(1): 70-78.

23. Olson, ME, O’Handley RM, Ralston BJ, et al. Update a *Cryptosporidium* and *Giardia* infections in cattle. Trends Parasitol 2004; 20: 185-191.

24. Coklin T, Farber J, Parrington L, et al. Prevalence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. Vet Parasitol 2007; 150: 297-305.

25. Hunt CL, Ionas G, Brown TJ, Prevalence and strain differentiation of *Giardia intestinalis* in calves in the Manawatu and Waikato regions of North Island, New Zealand. Vet Parasitol 2000; 91: 7-13.

26. Trout JM, Santín M, Greiner E, et al. Prevalence and genotypes of *Giardia duodenalis* in adult dairy cows. Vet Parasitol 2007; 147: 205-209.

27. Huetink REC, van der Giessen JWB, Noordhuizen JPTM, et al. Epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* on a dairy farm. Vet Parasitol 2001; 102: 53-67.

28. Mendonça C, Almeida A, Castro A, et al. Molecular characterization of *Cryptosporidium* and *Giardia* isolates from cattle from Portugal. Vet Parasitol 2007; 147: 47-50.

29. Silva FM, Lopes RS, Araújo JP. Genetic characterisation of *Giardia duodenalis* in dairy cattle in Brazil. Folia Parasitol 2012; 59 (1): 15-20.

30. Trout JM, Santín M, Greiner E, et al. Prevalence and genotypes of *Giardia duodenalis* in post-weaned dairy calves. Vet Parasitol 2005; 130: 177-183.

31. Trout JM, Santín M, Greiner EC, et al. Prevalence and genotypes of *Giardia duodenalis* in 1–2 year old dairy cattle. Vet Parasitol 2006; 140: 217-222.

32. Dalir Naghadeh B, Tavasoli M, Hafeinia AR. Study of epidemiologic measures of association between *Giardia* spp. infections with occurrence of diarrhea in calves [Persian]. J Vet Res 2008; 62(6): 363-366.

33. Olson ME, Thorlakson CL, Desellers L, et al. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet Parasitol 1997; 68: 375-381.

34. O’Handley RM, Ceri H, Anette C, et al. Passive immunity and serological immune response in dairy calves associated with natural *Giardia duodenalis* infections. Vet Parasitol 2003; 113: 89-98.

35. Davoudi Y, Garedaghi Y, Safarmashaei S. Epidemiological study of Giardiasis in diarrheic calves in East-Azerbaijan province, Iran. J Anim Vet Adv 2011; 10(19): 2508-2510.