

Prevalance and Molecular Characterisation of *Giardia duodenalis* Infection on Goatkids Southeast Turkey

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Abstract

Giardia duodenalis, a common intestinal protozoan, affects humans and mammals, including goats. This study investigated the prevalence and genetic characteristics of *G. duodenalis* in goat kids in southeastern Turkey. Fecal samples were collected from 112 goatkids, and microscopic and molecular analyses were conducted. A direct immunofluorescence test was used to confirm the presence of *G. duodenalis* cysts in collected fecal samples. Molecular confirmation was done with Polymerase chain reaction (PCR) using *G. duodenalis* SSU-rRNA. The secondary PCR products of all positive samples were sequenced in one direction on an automated sequencer. Nucleotide sequence analysis was performed by BLAST alignment using the National Center for Biotechnology Information database. Out of 112 fecal samples, 28.57% tested positive for *G. duodenalis*. Genotyping revealed that all positive samples belonged to assemblage E, a genotype previously identified in various domestic animals and even humans, suggesting zoonotic potential. These findings emphasize the significance of *G. duodenalis* in goatkids for both animal and human health and highlight the need for further epidemiological studies to assess its zoonotic potential in different regions of Turkey.

Keywords: Assemblages E, *Giardia duodenalis*, goatkids, Turkey

1. Introduction

Giardia duodenalis, also known as *Giardia lamblia* or *Giardia intestinalis*, is a familiar intestinal protozoan that infects humans and a diverse array of wild and domesticated mammals. Giardiasis can result in a broad range of clinical symptoms, with the severity ranging from no apparent signs to severe and acute gastrointestinal illness (Heresi and Cleary, 1997; Cai et al., 2021). The host's susceptibility to this parasite appears to be influenced by their immune system's status, making young and immunocompromised individuals particularly susceptible to infection. The main route of infection is fecal-oral transmission via contaminated food and water (Adam, 2001). The most common clinical symptoms associated with *G. duodenalis* are the excretion of malodorous diarrhea, weight loss, and a failure to thrive, often resulting in significant production losses and sometimes death (Aloisio et al., 2006; Geurden et al., 2008). *Giardia duodenalis* prevalence in goats varies from 10% to 60,2 % depending on animal age, geographical location, and diagnostic techniques worldwide (Robertson, 2009; Feng and Xiao, 2011; Akinkuotu et al., 2019). Based on molecular evidence, humans can be infected by assemblages A, B, and occasionally E of the eight genotypes or assemblages (A to H) of *G. duodenalis*. In ruminants, *G. duodenalis* assemblage E is most frequently recorded in calves, sheep, and goats. However, the “zoonotic” assemblages A and B are also common, suggesting that ruminant hosts could be significant reservoirs of *G. duodenalis* infection to humans (Geurden et al., 2008; Xiao and Feng, 2017; Ryan et al., 2019; Thompson and Ash, 2019). In the present study, we have investigated the prevalence of giardiasis in goat kids from the southeast Turkey. The isolates found have been further characterized by genotyping to evaluate the potential transmission of *G. duodenalis* from goats to humans.

2. Materials and Methods

2.1. Animal source

This study was performed on 12 dairy goat farms (Hair goats breed) located in Diyarbakır, Turkey. 112 fecal samples were collected directly from the rectums of all animals up to one month of age. The fecal samples were transported in ice-cooled containers to the laboratory.

2.2. Microscopic analysis

Fresh feces were examined for the presence of *Cryptosporidium* oocysts by Crypto/*Giardia*-Cel FITC Stain was used to demonstrate *cryptosporidium* oocysts. Fecal samples stain following kit procedure and examined by fluorescence microscope.

2.3. DNA extraction, *G. duodenalis* genotyping, and sequence analysis

Total DNA extraction was conducted with the direction of the suggestion kit by using ZR Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA). In the first PCR step, a fragment of the SSU rRNA for *Giardia* (130 bp) gene was amplified by PCR using primers previously described (Hopkins et al., 1997) and the primers GiarF and GiarR for the secondary PCR (Read et al., 2002) as previously described. First and second PCR amplifications were performed in 25 µl volumes with the final mix containing 2 µl Q solution, 10 pmol of each primer, 1,25 unit DNA polymerase, 0,2mM of each dNTP, 2,5 mM MgCl₂, 10x PCR buffer, and H₂O. First PCR reaction was heated to 96 °C for 2 min followed by 35 cycles of 96 °C for 4 s, 62 °C for 30 s., and 72 °C for 45 s, and one cycle of 72 °C for 4 min, and second PCR reaction was heated to 96 °C for 5 min followed by 35 cycles of 96 °C for 45 s, 55 °C for 30 s, and 72 °C for 45 s, and one cycle of 72 °C for 7 min. Negative and positive controls were included in all PCR sets. The secondary PCR products were sequenced in one direction on an automated sequencer (ABI PRISM 310 model; Perkin-Elmer, USA).

3.Results and Discussion

32 (28.57%) out of 112 diarrheic fecal samples were positive for *Giardia duodenalis* with immunofluorescent-antibody test. The prevalence of *Giardia duodenalis* in different farms ranged from 10% to 44.44% (Table 1). The prevalence of *Giardia duodenalis* in goat was reported to be 35.8% in Belgium (Geurden et al., 2008), 59% in Spain (Ruiz et al., 2008) 40.4% in Greece (Tzanidakis et al., 2014), 16,3% in China (Yang et al., 2023), 36,3% in Turkey (Ayan et al., 2019), 33.8% in goats in India (Utaaker et al., 2017), 12.3%

in Ghana (Squire et al., 2017), 60,2% in Nigeria (Akinkuotu et al., 2019), 11% in Brazil (Bomfim et al., 2005) *Giardia* prevalence is high compared to results from China, Ghana, Brazil while low compared to previous studies in Belgium, Spain, Greece, Turkey, Indian, Nigeria. The reason for the lower rate in this study than most other studies reported in the world may be the geographical location of the region, the low density of the goat population, and the effect of farm management.

Table 1. Prevalence and molecular characterization of *Giardia duodenalis* in goat kids in different farm

Number of the farm	Number of goat tested	<i>Giardia duodenalis</i> positive (%)	Genotype of <i>Giardia duodenalis</i>
1	10	3 (33,33)	E
2	9	4 (44,44)	E
3	9	3 (33,33)	E
4	9	4 (44,44)	E
5	9	2 (22,22)	E
6	11	2 (18,18)	E
7	8	3 (37,5)	E
8	9	3 (33,33)	E
9	9	2 (22,22)	E
10	10	2 (20)	E
11	9	3 (33,33)	E
12	10	1 (10)	E
Total	112	32 (28,57)	32(100%)

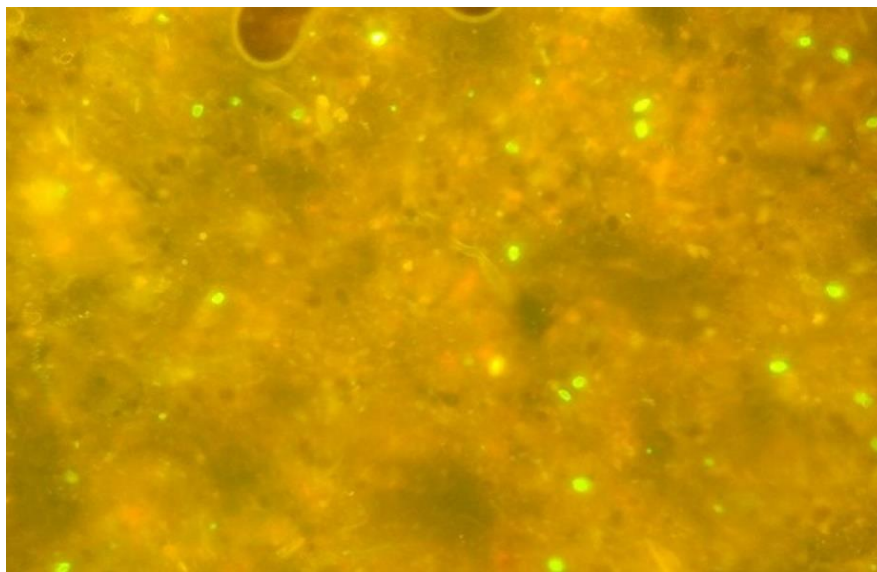


Figure 1. Fluorescence microscopy image of giardia cysts

The SSU rRNA gene fragment was amplified from the 32 samples in which *Giardia* cysts were microscopically identified (Figure 1,2). The sequences of the PCR products were analyzed, and it was determined that all samples maintained *Giardia* cysts, assemblage E. Assamblage E identified in this study were identical to those submitted under the GenBank numbers MF069070.1 and MF069058.1. Assemblage E has been found in various domestic animals, including cattle, sheep, pigs, goats, and alpacas (Ryan and Cacciò, 2013; Cai et al., 2021). Although initially believed to be only an animal-adapted genotype, its zoonotic potential was revealed by a report of this genotype in

humans (Zahedi et al., 2017). Likewise, research conducted in Belgium, Australia, Sweden, the USA, Norway, Spain, China, Iran, and Mexico have confirmed our findings that assemblage E is predominant in small ruminants. [Jafari et al., 2014; Kiani-Salmi et al., 2019; Geurden et al., 2008; Ryan et al., 2005; Yang et al., 2009; Lebbad et al., 2010, Robertson et al., 2010; Gomez-Munoz et al., 2009; Faridi et al., 2020; Di Giovanni et al., 2006; Ruiz et al., 2008). The giardia genotype detected in goatkids was only Assemblage E and other genotypes were not seen because of factors such as the age of the animals used in the research and environmental conditions hygiene conditions.

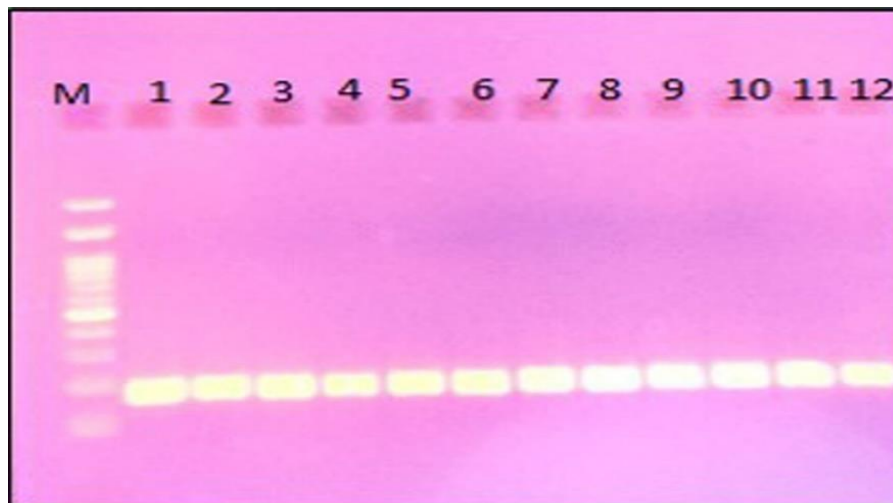


Figure 2. Electrophoretic (2% agarose) separation of SSU rRNA gene(130bp). Lane M: ladder (Marker); lane 1-12: positive sample

4. Conclusion

At the end of our study, new information has been revealed regarding the prevalence and genetic characterization of *Giardia duodenalis* in goat kids in the southeastern region of Turkey. Furthermore, the detection of the zoonotic importance of *Giardia* genotype, (assemblages E), has led us to conclude that the cysts by goat kids are not only important for animal health but also for human health. To assess the zoonotic potentials and genotype distributions of *Giardia*

duodenalis in goat, epidemiological studies are needed in different regions of Turkey.

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