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ORIGINAL ARTICLE

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Determination of *Giardia duodenalis* genotypes in sheep and goat from Iran

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Abstract *Giardia duodenalis* is an important zoonotic intestinal protozoan worldwide So far, seven assemblages have recognized for *G. duodenalis* (A–G) and there are the firm findings which assemblages A and B have zoonotic potential and assemblage E in livestock. In the presented work, the *G. duodenalis* isolate were determined genetically by the single PCR *ssu-rRNA* and nested PCR of triose-phosphate isomerase (*tpi*) genes in asymptomatic and symptomatic sheep and goats from Ahvaz, south west of Iran. The results revealed that only assemblage E, livestock-associated *G. duodenalis* was present in sheep and goat isolates. The results also presented 19.8 and 15.9 % prevalence of *G. duodenalis* infection sheep and goats under 12 month age, respectively. There was a significant relationship between formless stool and existence of isolates. We suggest although *G. duodenalis* is prevalent in sheep and goats but, these animals have no zoonotic risk for giardiasis in Ahvaz, Iran, but this parasite may play a role on enteric disorder of sheep and goats.

Keywords *Giardia duodenalis* · Genotyping · *ssu-rRNA* · *tpi*, Sheep · Goat · Iran

Introduction

Giardia duodenalis (synonyms: *G. intestinalis* and *G. lamblia*) is one of the most common intestinal protozoan parasite that infects humans and wide range of animals and has a worldwide distribution (Buret et al. 1990; Yoder et al. 2010).This parasite produces resistant cysts, which are infective when excreted in feces. Transmission of the *G. duodenalis* occurs directly (fecal/oral) by cyst contaminated food or water (Adam 2001; Porter et al.1990; Coklin et al. 2007). Infected humans reveal symptoms from the range of asymptomatic with shedding the infective cysts in environment to acute or chronic disease (Gardner and Hill 2001) maybe due to host factors or strain variations (Sousa et al. 2006). Predominant sign in these variable symptoms is diarrhea (Buret et al. 1990). Ruminants which infected with *G. duodenalis* are mostly asymptomatic, but subclinical signs such as reduction in growth rate, impairment in feed conversion efficiency and persistent diarrhea are observed occasionally (Lalle et al. 2005).

The genetic characterization of *G. duodenalis* on the basis of studying the conserved genes loci shows that, seven assemblages have currently recognized for this parasite (A–G) (Monis et al. 2003). Assemblages A and B have been detected in humans and in a wide range of other mammalian hosts. Thus, these two genotypes have the highest zoonotic potential risk for public health (Xiao and Fayer 2008), whereas, Assemblages C and D has found only in dog isolates and assemblage E in livestock (cattle, sheep and goats), assemblage F in cat and assemblage G in rat isolates (Caccio and Ryan 2008).

Specifying of *G. duodenalis* genotypes has performed after substantial sequence differences found in the genes such as ss-rRNA, glutamate dehydrogenase (gdh), triosephosphateisomerase (tpi), and β-giardin (bg) genes (Wielinga and Thompson 2007; Plutzer et al. 2010).

In Iran, although infection with *G. duodenalis* has been reported in some livestock animals in some regions, but there are few epidemiological data on prevalence of this

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parasite in sheep and goats and so far, there is not any data neither on *G. duodenalis* genotypes in sheep nor in goats. Therefore, the main objective of the current study was to determine the genotype of *G. duodenalis* and the prevalence of the parasite with emphasis to clinical symptoms in sheep and goats of Ahvaz, south west of Iran.

Materials and methods

Fecal samples

During the period of May 2011 until February 2012, fecal samples collected from in sheep and goat farms under 12 month age of Ahvaz city, south west of Iran. Stools were collected from 86 sheep and 94 goats and stored at 4 °C. After preparing the fecal smears on slides and logol staining, cysts of *G. duodenalis* have been detected by light microscopy. After that, *Giardia* cysts were purified by centrifugal sedimentation in sucrose solution from positive samples. Supernatant collected and after washing by PBS, phosphate-buffered saline, pH 7.4, stored at 20 °C before DNA extraction.

Extraction of genomic DNA

G. duodenalis cysts were re-suspended in 400 µl of lyses buffer from stool DNA extraction kit DNA (AccuPrep® stool) and lysed by six cycles freeze and thaw from 30 min on −70 °C to 30 min on +70 °C. Genomic DNA was extracted using mentioned kit according to manufacturer illustrations.

Polymerase chain reaction and sequencing

Selected genes for polymerase chain reaction were 292-bp *ssu-rRNA* (Appelbee et al. 2003) and 605-bp and 530-bp *tpi* (Sulaiman et al. 2003). Approach taken in this study was modified as follows.

Amplification of the *ssu-rRNA* sequences of genomic DNA was carried out in 50 µl reaction volumes containing 1–5 µl of DNA template, 2.0 mM $MgCl_2$, 200 mM of each dNTP, 5 % DMSO, 12.5 pmol of each forward and reverse primer, 0.5 Unit of *Taq* DNA polymerase.The cycling protocol included an initial hot start (94 °C for 3 min), 40 PCR cycles of 94 °C for 30 s, 58.8 °C for 30 s, 72 °C for 60 sand final extension was 72 °C for 7 min.

For 605-bp *tpi*, we employed a 50 mL reaction volume containing 1–5 µl of DNA template, 3.0 mM of $MgCl_2$, 200 mM of each dNTP, 100 pmol of each oligonucleotide primer and 1.25 Unit of Taq DNA polymerase. The cycling protocol included an initial cycle of 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 50.5 °C for 30 s, 72 °C for 60 s and a final extension of 72 °C for 30 min. Nested PCR was applied for 530 bp *tpi*. For this purpose, the same procedure was utilized with specific primers for amplification of 1 µl of PCR products of 605-bp *tpi*.

The sizes of the DNA amplicons were determined by a 1.5 % agarose gel electrophoresis stained with ethidium bromide. To verify the results, 10 µl of each PCR product was electrophoresed in a 1.5 % agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. The PCR products were identified by size using a 100 base pair ladder.

Sequencing

PCR products of ss-rRNA and tpi (530 bp) were purified using pfu enzyme (FERMENTASE®). Purified products were sequenced with the same PCR primers used for the original amplification in 10 ml reactions by Biosystems DNA Analyzers Sequencing (BIONEER® Korea). To determine the *Giardia* genotype of in the samples, each sequence for all two gene fragments was independently compared to GenBank sequences of *Giardia* genotypes.

Results and discussion

In the present study, the overall prevalence of *G. duodenalis* in sheep under 12 months age was 19.8 % (17 of 86), whereas the prevalence of *G. duodenalis* in goats under 12 month age was 15.9 % (15 of 94). The prevalence obtained in this study is point prevalence. Several longitudinal studies revealed cumulative infection rates of higher number of animals within a flock have been found to excrete *Giardia* (Ralston et al. 2003). Positive feces samples were form less in sheep (7/17) and goats (5/15), Symptom of diarrhea was observed in any of the samples. The weight loss was seen in sheep (2/17) and (1/17) goats of *Giardia* positive samples (Table 1).

Outbreak of giardiasis in sheep and goats is mostly without clinical sign and mortality but, In an outbreak of giardiasis on a sheep farm, *Giardia*-infected lambs had malabsorption, decreased weight gain, and reduced feed efficiency (Aloisio et al. 2006). Also In experimentally infected with *Giardia* cysts in lam, the infection was associated with delays to reach slaughter weight and decreased carcass weight (O'Handley and Olson 2006).

Table 1 Prevalence, clinical signs and genotyping in sheep and goats infected with *Giardia duodenalis*

	Prevalence (%)	Clinical sign formless stool (%)	Genotyping
Sheep	19.8	41.2	Assemblage E
Goat	15.9	33.33	Assemblage E

According to BLAST analysis (NCBI GenBank database), Sequence analysis both *tpi* (accession no AF069559) and *ssr-RNA* (accession no AF113902) showed that 100 % of the farms samples from sheep and goats had only one genotype and identified as assemblage E.

Infection with *G. duodenalis* is wide spread in both animals and humans and to understand the epidemiology of the infection or to implement control measures, it is important to identify the animals that can act as a source of human infection. The risk posed by animals can be better understood by the molecular characterization of cysts for better identification genotyping. In this study, ss-rRNA and tpi were used. The primers used in the PCR assay described here were specific for *G. duodenalis* hoofed Animal and humman genotypes or DNA from other eukaryotes and prokaryotes was used (Appellbe et al. 2003; Sulaiman et al. 2003). The sequencing of the fragments ss-rRNA and tpi were detected in all samples of Assemblage E. Sequence analysis of ss-rRNA locus in this study correlates with the tpi. The tpi- and ss-rRNA-based genotyping tool are useful in epidemiologic investigations of giardiasis in hosts and good phylogenetic marker for analysis of the molecular genotyping of *G. duodenalis* parasites.

Assemblage E of *G. duodenalis*is predominant genotype in ruminants, but maybe this animals harbor zoonotic assemblages. Although this group of animals infected with assemblage A and B, which have zoonotic potential. Genotype of *Giardia* isolates of sheep and goats that collected by Public and Veterinary Health Institutions from the European Countries by ss-rRNA, tpi, bg and gdh, were assemblage E (79 %) assemblage A (18 %) and assemblage B (2.3 %) (Sprong et al. 2009). Goat fecal specimens were positive for only livestock-associated *G. duodenalis* assemblage E in Spain (Ruiz et al. 2008). Molecular characterization of *Giardia* by ss-rRNA in sheep in Maryland showed, the prevalence of *Giardia* was higher in ewes than in lambs and all lambs infected with assemblage E (Santin et al. 2007). Genotype E was the most common species identified in both pre- and post-weaned sheep in Australia A much higher prevalence of *Giardia* was identified in post-weaned lambs and sheep (>8 weeks old) (Yang et al. 2009). Lebbad et al. (2010) reported that 61 % of *Giardia* infection in Sweden sheep was assemblage E.

For sheep and goats, studies have all identified a predominance of assemblage E and assemblage A identified infrequently. Assemblage B has rarely been found in sheep (Xiao and Fayer 2008).

Molecular epidemiological studies of giardiasis showed that assemblage A and assemblage B infect humans. It is commonly believed that, assemblage E could infect livestock but the recent identification of unusual host, such as humans in Egypt (Foronda et al. 2008). The results of many studies indicate that, Assemblage E only in livestock and have not the ability to transmit the infection to humans.

Conclusion

The current study provides the first definitive genotypic assessment of *Giardia* spp. isolated from sheep and goats in Iran and it is safe to say that not anthroponotic transmission of these isolates of *Giardia* between humans and sheep and goats. Outbreaks of giardiasis in sheep and goats are mostly without any clinical signs and or mortality, but we saw the relationship between the *Giardia* and some symptoms although these findings should be validated in studies including other larger number of samples and determination genotype of other animals.

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