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Molecular Prevalence of *Giardia duodenalis* and Subtype Distribution (Assemblage E and B) in Calves in Siirt, Turkey

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GIARDIA duodenalis is a common intestinal protozoan parasite that infects domestic and wild mammals, birds, and humans, especially livestock, worldwide. Studies on the prevalence of Giardia duodenalis in calves in Turkey are very limited. The aim of this study was to determine the molecular prevalence and subtype distribution of Giardia duodenalis in calves in Siirt. The animal material of the study consisted of 100 calves of different breeds and sexes in Siirt province. Using a disposable latex glove, feces samples were taken from the rectum of each calf and put in separate fecal containers. Sex and age information was recorded for each sample collected. As a result, 5% and 9% positivity was detected by microscopic and nested-PCR methods, respectively. The prevalence was 9.68% in females and 7.89% in males (P>0.05). Prevalence by age groups was as follows: 11.32% in the 1-6 month group, 7.41% in the 7-12 month group, and 5% in the older than 12 months group (P>0.05). As a result of the sequence analysis of the five PCR-positive samples, 80% Assemblage E and 20% Assemblage B were detected. As a result of this study, in addition to Assemblage E, Assemblage B was detected as well, which also has zoonotic properties. This situation may pose a risk for breeders. To better understand the distribution of G. duodenalis assemblages in calves in Siirt, studies in larger herds are needed.

Keywords: Calves, Giardia duodenalis, Siirt, Turkey.

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Introduction

*Giardia duodenali*s is a common intestinal protozoon [1-4]. Historically, giardiasis has been the most commonly diagnosed waterborne disease in developed countries [5]. Today, giardiasis is considered the most common cause of diarrhea in humans with 280 million infections a year [6], and has been included in the disease initiative (Neglected Disease Initiative), which consists of diseases neglected by the World Health Organization [7].

Eight different assemblages of *G. duodenalis* (A to H) are grouped based on genomic characteristics and host specificity [8]. While Assemblage E dominates cattle, Assemblage A and B follow it in terms of prevalence [3, 4, 9-11]. Only assemblages A and B are known to be present in humans, making them potentially zoonotic [3, 10, 12].

Commonly, *G. duodenalis* infects the host's small intestine. Infected calves can shed up to 10^8 cysts per gram of feces, resulting in extensive environmental contamination [1, 10]. At two weeks of age, the number of cysts in the feces peaks, and it stays high until six weeks of age. Cyst excretion is lower in older animals, which may hinder diagnosis, especially when low-sensitivity diagnostic techniques are used [13]. This parasite is transmitted from one host to another, directly through a fecal-oral path, direct contact with infected individuals, or ingestion of food or water contaminated with stool [1-4, 10, 14, 15]. It is reported that the most important factor in the contamination of the water is the excrement of livestock [9].

While the disease is usually subclinical or asymptomatic in adult cattle, it causes decreased weight gain rate, decreased feed efficiency, lower carcass weight, prolonged time to slaughter, diarrhea, and even death in young animals [3, 6, 8, 10, 16, 17].

Studies on the prevalence of *Giardia duodenalis* in calves in Turkey are quite limited. The aim of this study was to determine the molecular prevalence and subtype distribution of *G. duodenalis* in calves in Siirt province of Turkey.

Material and Methods

The Study Location and Sample collecting

This research was carried out in Siirt province located in the Southeastern Anatolia region of Turkey. The animal material of the study consisted of 100 calves of different races and sex. Fecal samples were collected from the rectum of each calf with a disposable latex glove and placed in individual fecal containers. Sex and age information was saved for each collected sample. The samples were immediately transported to the laboratory and stored at 4°C until DNA extraction.

Microscopic analysis

The Nativ-Lugol technique was used to check all samples for the presence of *Giardia* cysts. A drop of saline solution was placed on one side of the clean slide and a drop of Lugol solution was placed on the other side. With the help of a plastic stick, rice grain sized pieces of faeces were taken from different parts of the faeces and homogenised on the slide. The coverslipped preparations were examined with the 40X objective of the microscope (Leica, Hamburg, Germany).

DNA extraction

DNA extraction in all samples was performed using a commercial kit (GeneMATRIX Stool DNA Purification Kit), according to the manufacturer's guidelines. The obtained DNAs were kept at -20°C until the further procedures.

PCR amplification

For PCR amplification, the 753 bp *β-giardin* gene region was amplified using the primers (G7 F5'-AAGCCCGACGACGACCTCACCCG-CAGTGC-3' forward and G759R 5'- GAGGC-CGCCCTGGATCTTCGAGACGAC-3' reverse) first described by researcher Caccio *et al.* [2]. Nested PCR was then performed using the primers (BG1F 5'- GAACGAGATCGAGGTCCG-3' forward and BG2R 5'-CTCGACGAGTTCGT-GTGTT-3' reverse) described by Lalle *et al*. [11]. The resulting PCR products were stained with RedSafe™ Nucleic Acid Staining Solution and (iNtRON Biotechnology, Inc, Korea) images were obtained on 1.5% agarose gel (Bioshop, Ontario, Canada).

DNA Sequence and Phylogeny

Five (A, B, C, D, E) positive PCR samples suitable for sequencing were sequenced as forward

and reverse by receiving service from a private company (BM Labosis, Ankara). The collected DNA sequences were examined in the BioEdit software one by one, aligned, and made accessible for study [19]. The NCBI Basic Local Alignment Search Tool was used to match the altered DNA sequence formats to the data sets in order to find assemblages [20]. The phylogenetic tree was created using the *β-giardin* gene sequences with access codes from the NCBI GenBank database, the DNA sequences obtained as a result of the study, and the assemblages associated with the study samples were determined.

Statistical analysis

The chi-square test was performed with SPSS V16.0 to examine the relationship between the grouped variables. P<0.05 was regarded as the significant level.

Ethical approval:

This study was approved by Siirt University Animal Experiments Local Ethics Committee (Decision number: 2020/04-03)

Results

The distribution of *Giardia duodenalis* according to the sex and age of the calves is given in Table 1. As a result of microscopic examination, *Giardia* spp. cysts were found in 5% (5/100) of samples, while specific bands of 511 bp were obtained in 9% (9/100) of samples as a result of the Nested PCR method. The prevalence was 9.68% in females and 7.89% in males (P>0.05). Prevalence by age groups was as follows: 11.32% in the 1–6-month group, 7.41% in the 7–12-month group, and 5% in the older than 12 months group (*P*>0.05).

The data sets were aligned in the BioEdit tool to generate a phylogenetic tree, and the Mega X program employed the Maximum Likelihood Method (Figure 1) [21, 22]. When the DNA sequences were compared with the database in NCBI Basic Local Alignment Search Tool, it was observed that samples A, B, C, and D overlapped with Assemblage E at a rate of 100%, while sample E overlapped with Assemblage B at a rate of 99.76% (Table 2).

Discussion

Giardia duodenalis is the most common intestinal protozoon, present in both developed and developing countries, causing giardiasis in 200 million people worldwide each year [23]. While giardiasis infections are common in young calves, prevalence can vary depending on factors such as climate, immunity, and herd management [1].

In studies conducted in many regions of the world, the prevalence of the disease was reported as 23.9%-40% in the USA [5, 12, 23], 72.4% in Germany [17], 42.4% in Vietnam [8], 49% in Norway [10], 13.3% in Egypt [4], 10% in Korea [3], 44.79% in Nepal [24], 42% in Canada [25], 14.1% in Portugal [26] and 3.63%-64.7% in Turkey [9, 27-31]. As a result of this study, a 5% prevalence was determined using the microscopic examination and 9% using the Nested PCR method. The results are similar to the results of the researchers [3, 4, 28, 30].

Eight different assemblages of *G. duodenalis* (A to H) are grouped based on genomic characteristics and host specificity [23]. In cattle, 3 assemblages, A, B, and E are widely identified. The most frequently reported variant is

TABLE 1. Distribution of G.duodenalis infection-wise sex and ages

Variable		Examined (n)	Positive		*P*
			(n)	(%)	
Sex					
	Female	62	6	9.68	0.762
	Male	38	3	7.89	
Age (Month)					
	<7	53	6	11.32	
	7-12	27	2	7.41	0.663
	>12	20	1	5.00	
Total		100	9	9.00	

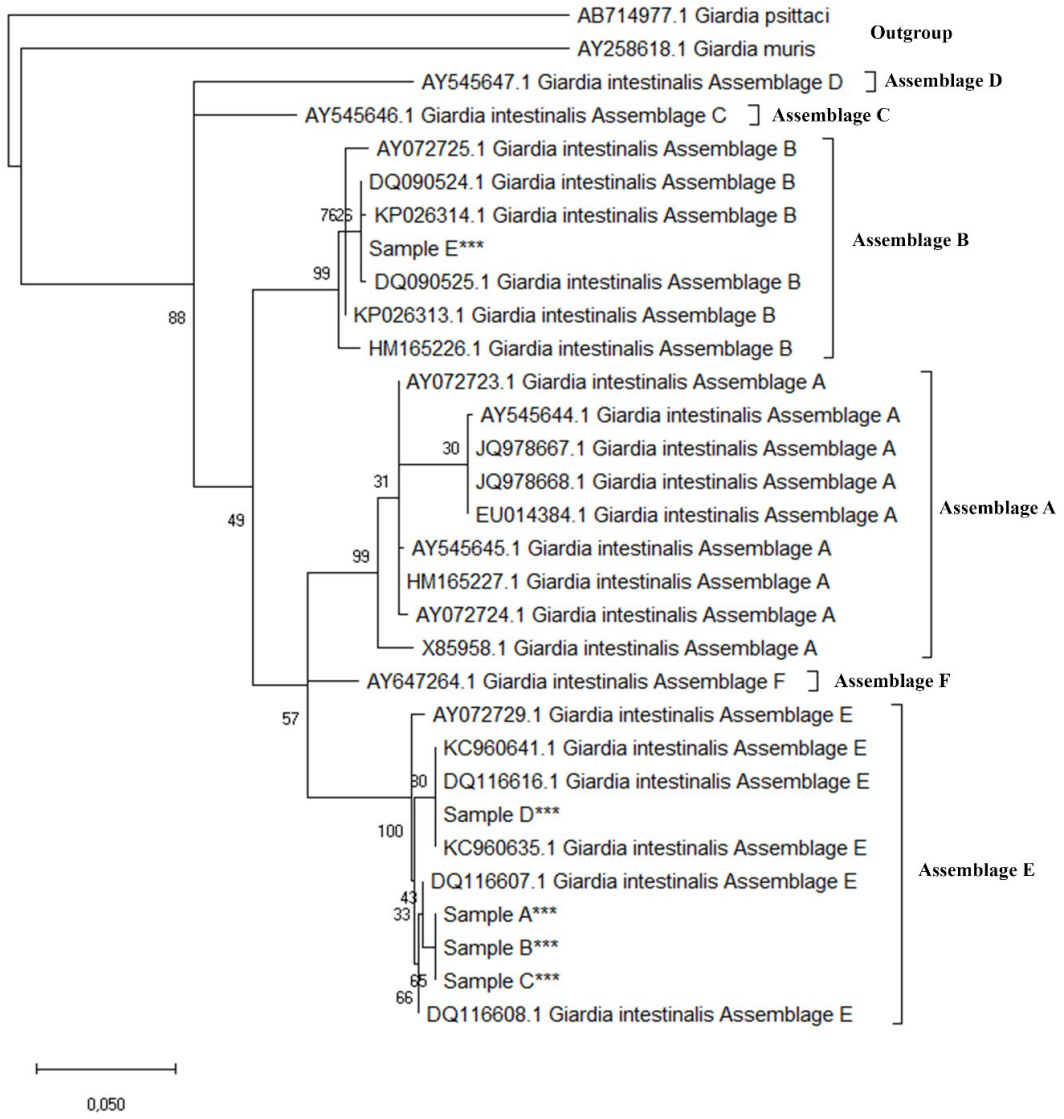

Fig. 1. Isolates of G. duodenalis are analyzed using the Maximum Likelihood method and the β-giardin gene region to determine their phylogenetic relationships. The Bootstrap values (1000 bootstrap) are depicted by numbers at the nodes. As an outgroup, Giardia psittaci and Giardia muris were utilized.

TABLE 2. Results of comparisons between research samples produced by the NCBI Basic Local Alignment Search Tool

Samples	Access codes for the most similar sample	Assemblage	Similarity ratio
A	KT922250, KT922248, MK610387	E	100%
B	KT922250, KT922248, MK610387	E	100%
C	MK610387, MK427701, MK659571	E	100%
D	MK610390, MK720232, MK452884	E	100%
E	MN457746, MG924453, LC508615	B	99.76%

Assemblage E, followed by Assemblage A [3-5, 8, 12, 17, 25].

In this study, Assemblage E and zoonotic Assemblage B were determined as a result of the analysis of five samples suitable for sequencing. As a result of the study, the rate of Assemblage E was determined to be higher (80%). This finding supports the researchers [4, 5, 8, 12, 25]. Limited studies are reporting the presence of Assemblage B in cattle. [11, 25, 26, 32]. In this study, the detection of zoonotic assemblage B in 20% of the samples supports the researchers.

The *G.duodenalis* strains obtained as a result of the sequence analysis of *β -giardin* genes were found to have 100% (KT922250, KT922248, MK610387, KT922250, KT922248, MK610387, MK610387, MK427701, MK659571, MK610390, MK720232, MK452884), and 99.76% (MN457746, MG924453, LC508615) similarity with Assemblage E and B, respectively.

Geographical region, herd size, the number of samples, age of the animal, a method applied, and season can explain the reasons for the differences observed between studies.

In a study performed by Naguib *et al.* [4], a higher prevalence in females compared to males was reported, while other researchers [24, 31] reported a higher prevalence in males. As a result of this study, the prevalence was found higher in females (9.68%) compared to males (7.89%). This result is similar to the study carried out by Naguib *et al*. [4], however, it was determined that the difference was not statistically significant (P>0.05).

The research revealed that animals under the age of six months had the highest frequency, particularly in the 2–4-month age groups. As a result of this study, the highest prevalence was detected in the age group of 1-6 months (11.32%), which is similar to the findings of the researchers [3-5, 8, 10, 24, 27, 31, 33].

Conclusion

Giardia infections cause significant economic losses by causing poor performance and even death in young animals. As a result of this study, in addition to Assemblage E, which is common in farm animals, Assemblage B was also detected in this study. This situation may pose a risk for breeders of the region, as Assemblage B is also zoonotic. To better understand the distribution of *G. duodenalis* assemblages in calves in Siirt, studies in larger herds are needed.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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