



A Microscopic and Molecular Survey of *Giardia duodenalis* in Lambs in Siirt, Türkiye

Özgür Yaşar ÇELİK^{1,a,✉}, Burçak ASLAN ÇELİK^{2,b}, Adnan AYAN^{3,c}, Özlem ORUNÇ KILINÇ^{4,d}, Kerem ERCAN^{5,e}, Muhammed Ahmed SELÇUK^{6,f}, Özge OKTAY AYAN^{7,g}

¹Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, TÜRKİYE

²Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, TÜRKİYE

³Department of Genetics, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, TÜRKİYE

⁴Özalp Vocational School, Van Yüzüncü Yıl University, Van, TÜRKİYE

⁵Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, TÜRKİYE

⁶Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, TÜRKİYE

⁷Department of Parasitology, Van Yüzüncü Yıl University, Faculty of Medicine, Van, TÜRKİYE

^aORCID: 0000-0001-6365-2688; ^bORCID: 0000-0002-0130-970X; ^cORCID: 0000-0002-6564-3416; ^dORCID: 0000-0001-6233-7109

^eORCID: 0000-0003-4914-8578; ^fORCID: 0000-0003-1769-4558; ^gORCID: 0000-0003-2577-3774

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Abstract

Giardia duodenalis is a parasite that causes gastrointestinal diseases and is widespread worldwide. This parasite, which causes infections especially in lambs, is highly prevalent in farm animals. In this study, the prevalence and risk factors of *G. duodenalis* in lambs were investigated. Lamb fecal samples were collected during the study and microscopic and molecular examinations were performed using Nested PCR method. While *Giardia* sp. cysts were detected in 28 (23.33%) of 120 samples, the DNA of the parasite was detected in 35 (29.17%) of 120 samples by molecular examination. At the end of the study, the prevalence of *G. duodenalis* in lambs was recorded between 23.33% and 29.17%. The highest prevalence rate among age groups was found in 16-30 days old lambs, while the highest positivity rate among locations was found in Şirvan district and the lowest positivity rate was found in the center. According to gender, the disease prevalence was higher in females than in males. However, there was no statistically significant difference between location, gender and age groups ($p>0.05$). In conclusion, *G. duodenalis* infestations are commonly found in lambs in Siirt province. The results of this study emphasize that control measures and hygiene practices should be implemented to prevent and control *G. duodenalis* infestations in lambs.

Key Words: *Giardia duodenalis*, lamb, PCR, Siirt, Türkiye

Türkiye'nin Siirt İlindeki Kuzularda *Giardia duodenalis*'in Mikroskopik ve Moleküler Yöntemlerle Araştırılması

Öz

Giardia duodenalis, dünya genelinde yaygın olarak görülen ve gastrointestinal hastalıklara neden olan bir parazittir. Özellikle kuzularda enfeksiyonlara neden olan bu parazit, çiftlik hayvanlarında yüksek oranda görülmektedir. Bu çalışmada, kuzularda *G. duodenalis* yaygınlığı ve risk faktörleri incelenmiştir. Araştırma süresince kuzu dışkı örnekleri toplanmış, mikroskopik ve Nested PCR yöntemi kullanılarak moleküler inceleme yapılmıştır. Toplam 120 örneğin 28 (%23.33)'inde mikroskopik inceleme sonucu *Giardia* sp. kistleri tespit edilirken, moleküler inceleme sonucu 120 örneğin 35 (%29.17)'inde parazitin DNA'sı tespit edilmiştir. Araştırma sonunda kuzularda *G. duodenalis* yaygınlığı %23.33 - %29.17 arasında kaydedilmiştir. Yaş grupları arasında en yüksek prevalans oranı 16-30 günlük kuzularda tespit edilirken, lokasyonlar arasında en yüksek pozitiflik oranı Şirvan ilçesinde, en düşük pozitiflik ise Merkezde bulunmuştur. Cinsiyete göre dişilerde erkeklere göre hastalık prevalansının daha yüksek olduğu kaydedilmiştir. Ancak, lokasyon, cinsiyet ve yaş grupları arasında istatistiksel olarak anlamlı bir fark görülmemiştir ($p>0.05$). Sonuç olarak, *G. duodenalis* enfestasyonları Siirt ili kuzularında yaygın olarak bulunmaktadır. Bu çalışmanın sonuçları, kuzularda *G. duodenalis* enfestasyonlarını önlemek ve kontrol etmek için kontrol önlemlerinin ve hijyen uygulamalarının uygulanması gerektiğini vurgulamaktadır.

Anahtar Kelimeler: *Giardia duodenalis*, kuzu, PZR, Siirt, Türkiye

INTRODUCTION

Giardia duodenalis (syn. *Intestinalis*, *lamblia*) is an enteric protozoan with flagella that infects people and a wide range of domestic and wild animals, causing severe diarrhea, especially in young and immune-compromised individuals (1-6).

Giardia duodenalis has recently emerged as an important parasite in domestic ruminants with unexpectedly high levels of infection (3,7). Since a high number of cysts are shed with feces due to the disease, both young animals and the environment are significantly contaminated (1). It is

stated that even though ruminants are exposed to *Giardia* species shortly after birth, infections are mostly seen towards the end of the neonatal period (5).

The disease causes malabsorption as the disease agent damages the intestinal lumen (1). When present in small numbers giardia agents do not cause clinically symptomatic disease, but in the presence of large numbers of the agent or immunologically deficient individuals, diarrhea commonly occurs (4). It has been reported that the disease can cause dehydration, weight loss, depression, poor condition, lethargy, tenesmus, abdominal bloating, brown diarrhea, hypothermia, and death in the later period, in lambs (3-5,7). The clinical chart may vary depending on the resistance of the disease agent to environmental factors, the infectious dose, the age of the animal, and the conditions of care (5).

This study aimed to microscopically and molecularly determine the *Giardia duodenalis* prevalence in lambs in Siirt province.

MATERIALS AND METHODS

The Study Area and Animal Material

This study was carried out in the Siirt province, which is located in the Southeastern Anatolia Region of Turkey. The study material consisted of 120 fecal samples from lambs of different sexes and up to 8 weeks of age randomly selected from various farms.

Sample Collection and Preparation

Fecal samples were taken from the rectum of each lamb with disposable latex gloves, placed in specimen containers, and labeled. The gender, age, and location information of the animals were recorded.

Microscopic examination

The samples were examined under a microscope (Leica DM500, Switzerland) at 40x magnification using the native examination method after they were transported to the laboratory.

DNA extraction

The kit protocol was followed for DNA extraction from the fecal sample using the QIAamp DNA Stool Mini Kit (Qiagen, Germany). The obtained DNAs were stored at -20°C for further analysis.

PCR Amplification

G7 F5'- AAGCCCAGACCTCACCCGAGTGC-3' forward and G759R 5'- GAGGCCCGCCCTGGATCTTCGAGACGAC-3', reverse primers defined by Cacciò et al. (8) were used for the amplification of the 753 bp β -giardin gene region. 1.5 mM MgCl₂, 10 pmol forward and reverse primer, 200 μ M dNTPs, 1.5 mM MgCl₂, 10X PCR buffer, 1U Taq Polymerase, Nuclease Free Water, and 100 ng DNA were used with a final volume of 25 μ L in the master mix. The reaction conditions are as follows; pre-denaturation at 95°C for 15 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at

60°C for 30 seconds, and elongation at 72°C for 1 minute, then final elongation at 72°C for 7 minutes. In the second step of Nested PCR, the primers (BG1F 5'- GAACGAGATCGAGGTCG-3' forward and BG2R 5'-CTCGACGAGTTCGTGTT-3' reverse), were used for amplification of the 511 bp β -giardin gene region as defined by Lalle et al. (9). 1.5 mM MgCl₂, 10 pmol forward and reverse primer, 200 μ M dNTPs, 1.5 mM MgCl₂, 10X PCR buffer, 1U Taq Polymerase, Nuclease Free Water, and 100 ng DNA were used with a final volume of 25 μ L in the master mix. The reaction conditions were as follows: pre-denaturation at 95°C for 15 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 1 minute, then final elongation at 72°C for 7 minutes. Images were obtained on 1.5% agarose gel by staining the resulting PCR products with RedSafe™ Nucleic Acid Staining Solution.

Statistical Analysis

Statistical analyses were conducted using commercial software (SPSS 16.0, IBM Inc., Chicago, Illinois). Data were analyzed using a chi-square test. P < 0.05 were considered significant.

Ethical Approval

Ethical approval for the present study was obtained from the Siirt University Local Ethics Committee for Animal Experiments with an ethical approval number of 20230106.

RESULTS

As a result, of the total 120 samples, 28 (23.33%) were found positive for *Giardia* sp. cysts using microscopic examination, while 35 (29.17%) samples were found positive for 511 bp-specific bands using Nested PCR (Figure 1).

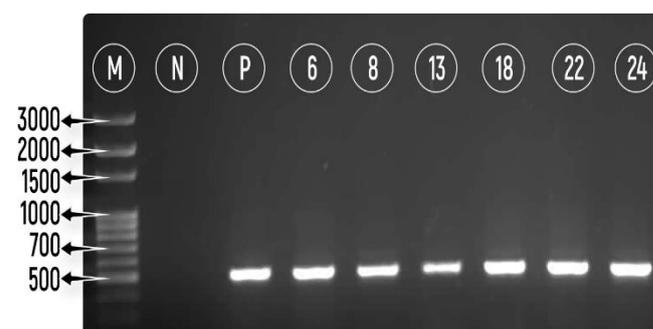


Figure 1. Amplification of *Giardia duodenalis* β -giardin gene region using nested-PCR. Lanes M: Marker, N: Negative control, P: positive control, 6, 8, 13, 18, 22, and 24 represent *Giardia duodenalis* positive samples (511 bp).

In terms of age, the highest positivity was detected in the 16-30-day-old group, while in terms of location, the highest positivity was found in the Şirvan and in terms of gender higher prevalence was found in females than in males. (Table 1). There were no statistically significant differences between location, gender, and age groups ($p > 0.05$).

Table 1. Distribution of results by sex, age, and locations

Parameters	(n)	Positive		P
		(n)	(%)	
Sex				
Female	67	22	32.84	NS
Male	53	13	24.53	
Age (Days)				
0-15	33	7	21.21	NS
16-30	59	22	37.29	
31-60	28	6	21.43	
Location				
Şirvan	31	12	38.71	NS
Baykan	28	8	28.57	
Merkez	25	5	20.00	
Kurtalan	23	7	30.43	
Aydınlı	13	3	23.08	
Overall	120	35	29.17	

NS: Non-significant

DISCUSSION AND CONCLUSION

Giardia duodenalis is among the most important causes of diarrhea in humans and farm animals and can lead to cross-contamination between humans and animals (1).

The prevalence varies according to countries and regions. In studies carried out on lambs worldwide the following rates of positivity were reported in corresponding locations: 42% in Spain (2), 2.6% in Ethiopia (10), 25.5% in Belgium (11), 4% in America (12), 57% in Canada (13), 26.8% in Norway (14), 11.1% in Australia (6) and 1.5% in Italy (15).

In studies on lambs in Turkey, 1.14% prevalence was reported in the Marmara Region (16), 36.6% in Burdur (4), 16.45%, and 20.25% by microscopic examination and PCR respectively in Kırıkkale (17), 36% and 42% by microscopic and Nested-PCR methods in Van, respectively (18), and 48.48% in a different study conducted in Van (3). *Giardia* sp. infection has been detected in a sheep in each of Erzurum and Erzincan provinces (19).

In other studies, 4.1% prevalence in calves and 1.4% in cattle in Sivas was reported (20), while 18.8% prevalence in dogs was reported in Aydın (21). 68.6% prevalence in cats in the Central Anatolia Region (22), 64.7% in calves, and 36.3% in goats in Van (18) were also reported. A prevalence of 20% was reported in samples taken from water sources in Isparta (23). In addition, a calf in Diyarbakir (24), 16 goats in Aydın (25), and a cow in Sivas (26) have been reported positive.

In this study, the prevalence of *Giardia duodenalis* in lambs detected 23.33% and 29.17% by microscopic and Nested-PCR method, respectively. These values are lower than the results of some researchers (3,4,22), are similar to some (17), and are higher than some researchers' studies (16,20,21). Geographical locations, different climates, sample sizes, animal species, age of the animal, and methods employed in research can be counted among the reasons for the differences observed between studies.

It is stated that although the diarrhea cases associated with giardiasis in lambs are more common in the age groups of 2 to 4 weeks, there seems to be no obvious age limitation (5). In a study carried out in Van, 1-60 days-old lambs were divided into three groups based on their age, and the highest

positivity was detected in the second group (3). The observation of the highest positivity in lambs aged 16-30 days in this study supports the studies performed by Ozdal et al. (3). In addition, the lack of a statistically significant difference between the groups also supports researcher (5).

In the study conducted by Wegayehu et al. (10), a higher prevalence was reported in females compared to males, but no statistically significant difference was detected. This finding is similar to the results of this study, which also detected a higher prevalence in females compared to males.

The disease is quite important since it both causes economic losses and has a zoonotic feature. As a result of this study, the prevalence of the disease in Siirt province was revealed. It is necessary to take into account the *G. duodenalis* while treating patients and taking necessary protective measures against diarrhea in farms. In addition, it was concluded that it would be useful to determine the prevalence of assemblages with zoonotic potential by investigating the assemblages of *G. duodenalis* in lambs in the region.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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✉ **Corresponding Author:**

Özgür Yaşar ÇELİK
Department of Internal Medicine, Faculty of Veterinary
Medicine, Siirt University, Siirt, TÜRKİYE
E-posta: oyc@siirt.edu.tr