

Endoparasites Determined by Fecal Examination in Sheep in Erzurum Province

Erzurum İlinde Yetiştirilen Koyunlarda Dışkı Bakışı ile Tespit Edilen Parazitler

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ABSTRACT

Objective: The aim of the current study was to determine the presence and prevalence of *Eimeria* and helminth species in sheep raised in Erzurum province by using fecal examination.

Methods: Faecal samples were collected from a total of 784 sheep raised in Aziziye, Yakutiye and Palandöken districts between February-March 2019. The samples were examined by Fulleborn's flotation, Benedect sedimentation, and Baermann-Wetzel methods.

Results: *Eimeria* spp. and helminths were found in 49.36% (387/784) and 74.11% (581/784) of the samples, respectively. Identified *Eimeria* species were as follows: *E. parva* (59.68%), *E. ovina* (51.67%), *E. faurei* (47.80%), *E. ahsata* (39.27%), *E. granulosa* (36.62%), *E. punctata* (28.42%), *E. pallida* (26.09%), *E. ovinoidalis* (18.34%), *E. crandallis* (16.79%), *E. intricata* (15.76%), *E. weybridgetensis* (11.36%) and *E. marsica* (6.20%). Helminth species identified at genus/species level were *Dicrocoelium* spp. (33.91%), *Fasciola* spp. (5.68%), *Paramphistomum* spp. (2.58%), *Moniezia* spp. (5.85%), Trichostrongylid type egg (49.05%), *Marshallagia* spp. (38.73%), *Nematodirus* spp. (20.98%), *Trichuris* spp. (14.46%), *Protostrongylus* spp. (18.42%), *Dictyocaulus filaria* (2.41%) and *Muellerius capillaris* (1.38%).

Conclusion: Parasitic diseases cause important economic losses in livestock industry. In following years, it is aimed to plan prevention and control strategies for the parasites detected in this area in line with the data of this study and to share this data with the animal breeders.

Keywords: Erzurum, sheep, flotation, sedimentation, Baermann-Wetzel

ÖZ

Amaç: Bu çalışmanın amacı, Erzurum ilinde yetiştirilen koyunlarda *Eimeria* ve helmint türlerinin varlığını ve yaygınlığını dışkı bakışı ile ortaya koymaktır.

Yöntemler: Dışkı örnekleri, Şubat-Mart 2019 tarihlerinde Aziziye, Yakutiye ve Palandöken ilçelerinde yetiştirilen farklı yaş ve cinsiyetteki 784 koyundan toplandı. Toplanan dışkı örnekleri Fulleborn flotasyon, Benedek sedimentasyon ve Baermann-Wetzel metotları ile incelendi.

Bulgular: İncelenen örneklerde, *Eimeria* oocystleri %49,36 (387/784), helmint etkenleri ise %74,11 (581/784) oranlarında saptandı. *Eimeria* spp. pozitif örneklerde; *E. parva* (%59,68), *E. ovina* (%51,67), *E. faurei* (%47,80), *E. ahsata* (%39,27), *E. granulosa* (%36,62), *E. punctata* (%28,42), *E. pallida* (%26,09), *E. ovinoidalis* (%18,34), *E. crandallis* (%16,79), *E. intricata* (%15,76), *E. weybridgetensis* (%11,36) ve *E. marsica* (%6,20) türleri belirlendi. Cins/tür bazında tanımlanan helmint etkenleri şunlardır: *Dicrocoelium* spp. (%33,91), *Fasciola* spp. (%5,68), *Paramphistomum* spp. (%2,58), *Moniezia* spp. (%5,85), Trichostrongylid tip yumurta (%49,05), *Marshallagia* spp. (%38,73), *Nematodirus* spp. (%20,98), *Trichuris* spp. (%14,46), *Protostrongylus* spp. (%18,42), *Dictyocaulus filaria* (%2,41) ve *Muellerius capillaris* (%1,38).

Sonuç: Parazitler enfeksiyonlar, hayvan yetiştiriciliğinde önemli ekonomik kayıplara neden olmaktadır. İlerleyen dönemlerde, bu çalışmanın verileri doğrultusunda bu bölge hayvanlarında saptanan parazitlere yönelik koruma ve kontrol çalışmalarının planlanması ve hayvan yetiştiricileri ile bu bilgilerin paylaşılması düşünülmektedir.

Anahtar Kelimeler: Erzurum, koyun, flotasyon, sedimentasyon, Baermann-Wetzel



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INTRODUCTION

Sheep raising is economically important in the world, as well as in the Erzurum region and Turkey. It is one of the primary sources of animal protein needed for human nutrition. Due to the subtropical climatic conditions of Turkey, inappropriate sheep husbandry systems and parasitic drug resistance, sheep are at high risk to parasitic infections (1,2). Gastrointestinal (GI) parasites are the serious causative species of both clinical and subclinical diseases in sheep which lead to growth retardation, weight loss, mortality, decrease in milk and wool productivity, thus causing tremendous economic losses (2). Therefore, it is very important to investigate the presence and prevalence of disease species, application of appropriate preventive measures and treatment with effective anti-parasitic drugs will aid in the improvement of animal health and economic gain. Based on necropsy and coprological examinations conducted in sheep in different continents and regions of the world, reports showed that the prevalence rate of *Eimeria* and helminth infections ranges between 4.2-92.9% (3-7) and 0.2-100% (4,8-10), respectively. The results of the studies carried out on sheep faecal samples in Turkey, the following 37.3-100% *Eimeria* spp. (11-16) and 0.39-100% helminth (16-18) prevalence were reported. In past years, some studies of sheep parasites presented in Erzurum region (19-21) but contemporarily no comprehensive study has been conducted on endoparasitic fauna of sheep, in Erzurum province. The purpose of this study was to investigate the presence and prevalence of parasitic species at sheep faecal samples by quantitative and qualitative coprological techniques in Erzurum region.

METHODS

Ethics Committee Approval

Ethical approval of the study was obtained from the Ethics Committee of Atatürk University of Veterinary Faculty (approval number: 2019/01).

Study Area

The study was conducted in Erzurum province of Turkey between February-March 2019. The province is the fourth largest city in Turkey with a surface area of 25.066 km² and located in the northeastern part. It is situated at an elevation of 1853 m above sea level with an average annual temperature of 5.8 °C (42.4 °F) and characterized by continental climatic conditions. Erzurum has large expanses of meadows and pastures with a rate of approximately 64.7%. The greenish nature of the meadows and pastures is maintained through all year by sufficient rainfall with average annual precipitation of 425 mm.

Sample Size

According to the Turkey Statistical Institute (TUIK), Erzurum has the 1.85% of Turkey's sheep population. The total sheep number in the country is approximately 35.194.972 and 649.716 of them are raised in Erzurum (22). The distribution of sheep breeds in Erzurum is 83%, 13%, 2% and 2% for Morkaraman, Akkaraman, Hemşin, and others, respectively. To determine the sample size, the data of TUIK was considered and 784 (95% confidence level, 3.5% confidence interval) sheep found to be enough to represent Erzurum's sheep population. The sample size in terms of the districts was calculated by stratified sampling method.

Sample Collection

Samples were collected from private sheep farms located in Aziziye, Yakutiye and Palandöken districts between February-March 2019. These districts have similar climatic and geographical conditions. In this study, a total of 784 (females (n): 667, males (n): 117, <1 age group (n): 186, >1 age group (n): 598, race: Morkaraman) clinically healthy sheep faecal samples were collected. The sheep found at meadow or pasture at least once, no administration of antiparasitic drug during the last 3 months and to be raised in Erzurum region were the properties of the sheep that sampled. The faecal samples were collected directly from the rectum of each animal and placed in screw-capped labeled plastic containers. The age, sex, breed, and raising address of the animals were recorded.

Laboratory Techniques

Collected faecal samples were immediately transported to the Parasitology Laboratory of Veterinary Faculty, Atatürk University. At first, faecal samples were macroscopically investigated in term of consistency, presence of blood, mucus and cestode proglottids. Later on, each of the fecal samples were processed by Fuelleborn's flotation, Benedect sedimentation, Baermann-Wetzel methods and then examined microscopically for the presence of *Eimeria* spp. oocysts, trematode and cestode eggs, nematode eggs and larvae. Evaluation and identification of the parasitic species at the genus and/or species level were performed by using light microscope (Nikon Eclipse Ci, Japan) and digital camera system (Nikon DS-Fi2, Japan). Quantitative analysis was conducted on all *Eimeria* positive faecal samples to determine oocysts per gram (OPG) by McMaster's technique (23). The severity of the *Eimeria* infection was evaluated as mild (<1.800 OPG), moderate (1.800-6.000 OPG) and severe (>6000 OPG) (24). Then, 2% K₂Cr₂O₇ was added to *Eimeria* positive samples and exposed to sporulation under laboratory conditions by stirring regularly. Identification of sporulated *Eimeria* oocysts was performed based on morphological features (25). Helminth eggs were defined based on morphological criteria of the eggs at genus/species level, and then eggs per gram (EPG) was calculated in positive samples. Infection degree was evaluated based on EPG as mild (<500), moderate (500-1000) and severe (>1000). Nematode larvae were defined at genus and/or species level according to the morphological criteria (26).

Statistical Analysis

Positive samples were identified and enumerated. The count data were subjected statistically by SPSS 20 software (SPSS Inc., Chicago, IL, USA), chi-square (χ^2) test to attain associations of sex, age, OPG and EPG at $p < 0.05$.

RESULTS

The macroscopic examination of the samples revealed that the feces were in normal consistency and not containing blood, mucus, or cestode proglottids. Out of 784 faecal samples, 683 (87.12%) were found to be positive with at least one parasite. Respectively, 49.36% (387/784) and 74.11% (581/784) sheep were infected with *Eimeria* spp. and helminth species. Whereas 102 (13.01%) sheep infected with only *Eimeria* species, 285 (36.35%) had mix infection with *Eimeria* species and helminths. Identified *Eimeria* species were as follows: *E. parva*, *E. ovina*, *E. faurei*, *E. ahsata*, *E. granulosa*, *E. punctata*, *E. pallida*, *E. ovinoidalis*,

E. crandallis, *E. intricata*, *E. weybridgensis* and *E. marsica* (Figure 1). Among the *Eimeria* species, *E. parva* has the highest prevalence of 59.68% (231/387) (Figure 2). In positive samples, 3 to 8 *Eimeria* species were determined. The infection with 8 *Eimeria* species were including *E. parva*, *E. ovina*, *E. faurei*, *E. granulosa*, *E. pallida*, *E. ovinoidalis*, *E. crandallis* and *E. marsica*.

The prevalence of infection was significantly ($p < 0.05$) higher in <1 age group than >1 age group. With regards to sex, the prevalence of infection was higher in females than in males and the difference was insignificant ($p > 0.05$) (Table 1).

OPG was calculated as 1.550 (100-12.850) on average. The intensity of infection in age groups (<1 age group: moderate and severe; >1 age group: mild) showed statistically significant differences ($p < 0.05$). The moderate and severe OPG range in females was at high rate while mild OPG range was high in males ($p > 0.05$) (Table 2).

The prevalence rates of helminth infections were as follows; 28.95% (227/784) trematode, 4.34% (34/784) cestode, 60.1% (471/784) GI nematode, and 15.94% (125/784) lungworm. At least one helminth agent was determined in 74.11% (581/784) of

the samples. Identified helminth species at genus or species level were as follows: *Dicrocoelium* spp. (25.13%, 197/784), *Fasciola* spp. (4.21%, 33/784), *Paramphistomum* spp. (1.91%, 15/784), *Moniezia* spp. (4.34%, 34/784), trichostrongylid type egg (36.35%, 285/784), *Nematodirus* spp. (15.56%, 122/784), *Marshallagia* spp. (28.7%, 225/784), *Trichuris* spp. (10.71%, 84/784), *Dictyocaulus filaria* (*D. filaria*) (1.79%, 14/784), *Protostrongylus* spp. (13.65%, 107/784) and *Muellerius capillaris* (*M. capillaris*) (1.02%, 88/784). Trichostrongylid type eggs had the highest [49.05% (285/581)] and *M. capillaris* had the lowest [1.38% (8/581)] rate in helminth positive samples (Figure 3, 4, 5).

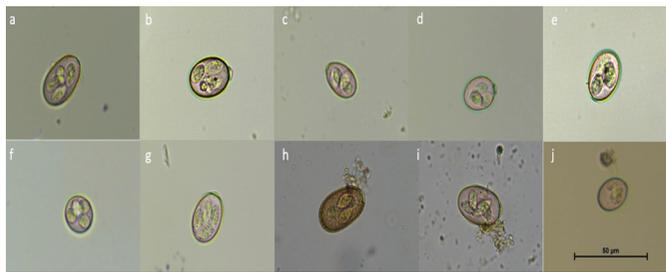


Figure 1. a: *E. faurei*, b: *E. granulosa*, c: *E. marsica*, d: *E. ovina*, e: *E. ovinoidalis*, f: *E. parva*, g: *E. weybridgensis*, h: *E. intricata*, i: *E. ahsata*, j: *E. crandallis*, Magnification: 400x; Scale bar: 50 µm

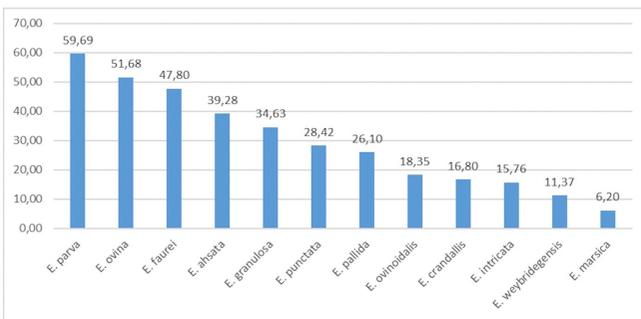


Figure 2. Number of positive animals (n)=387

Table 1. Distribution of *Eimeria* infection in age and sex group

		n/N	%	p
Sex	♀	332/667	49.78	>0.05
	♂	55/117	47.01	
Age	<1	132/186	70.97	<0.05
	>1	255/598	42.64	

n: Number of *Eimeria* positive animals, N: Total number of animals

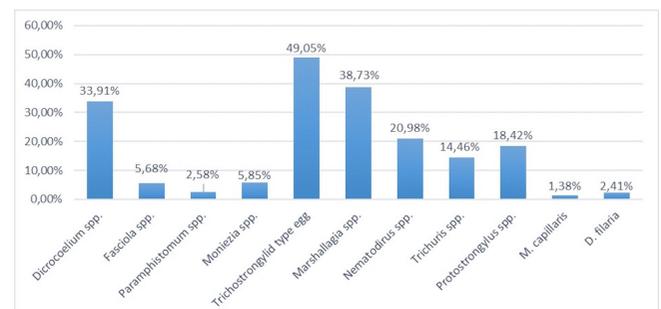


Figure 3. Number of positive animals (n)=581

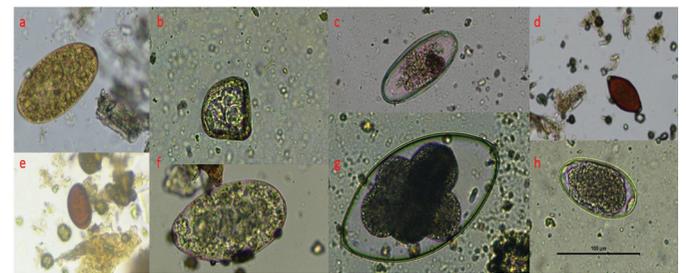


Figure 4. a: *Fasciola* spp., b: *Moniezia* spp., c: *Marshallagia* spp., d: *Trichuris* spp., e: *Dicrocoelium* spp., f: *Paramphistomum* spp., g: *Nematodirus* spp., h: Trichostrongylid type egg, Magnification: 200x, scale bar: 100 µm

Table 2. Age and sex-wise OPG range in infective sheep

		Age		p	Sex		p
		<1 N=132 n (%)	>1 N=255 n (%)		♀ N=332 n (%)	♂ N=55 n (%)	
OPG	Mild	20 (15.15)	205 (80.39)	<0.05	204 (61.45)	36 (65.45)	>0.05
	Moderate	80 (60.60)	45 (17.65)		97 (29.22)	15 (27.27)	
	Severe	32 (24.24)	5 (1.96)		31 (9.34)	4 (7.27)	

N: Number of *Eimeria* positive animals, n: Number of infected animals with related OPG range, OPG: Oocysts per gram

The helminth infection rate was determined higher in the >1 age group than <1 age group, and the dependency of positivity to the age was found insignificant ($p>0.05$). The positivity rate was found higher in females than males ($p>0.05$) (Table 3).

The presence of *Dicrocoelium* spp. and *Marshallagia* spp. was higher in females than males ($p<0.05$) (Table 4).

Single and mix parasite infection rates are shown in Table 5.

EPG was counted as 550 (100-3750) on average. The intensity of infections in age groups (<1 age group: moderate and severe; >1 age group: mild) showed statistically significant differences ($p<0.05$). The severe OPG range was higher in females, while mild and moderate OPG ranges higher in males ($p>0.05$) (Table 6).

Table 3. Prevalence of helminth species in the age and sex groups

		n/N	%	p
Sex	♀	500/667	74.96	>0.05
	♂	81/117	69.23	
Age	<1	133/186	71.51	>0.05
	>1	448/598	74.92	

n: Number of helminth positive animals, N: Total number of animals

Table 4. Prevalence of helminth species in the age and sex groups

	Sex		Age	
	♀ N=667 n (%)	♂ N=117 n (%)	<1 N=186 n (%)	>1 N=598 n (%)
<i>Dicrocoelium</i> spp.	181a (27.13)	16b (13.67)	44 (23.65)	153 (25.58)
<i>Fasciola</i> spp.	27 (4.04)	6 (5.12)	11 (5.91)	22 (3.67)
<i>Paramphistomum</i> spp.	12 (1.79)	3 (2.56)	5 (2.68)	10 (1.67)
<i>Moniezia</i> spp.	27 (4.04)	7 (5.98)	10 (5.37)	24 (4.01)
Trichostrongylid type egg	239 (35.83)	46 (38.32)	55 (29.57)	230 (38.46)
<i>Nematodirus</i> spp.	105 (15.74)	17 (14.52)	32 (17.20)	90 (15.05)
<i>Marshallagia</i> spp.	210a (31.48)	15b (12.82)	50 (26.88)	175 (29.26)
<i>Trichuris</i> spp.	69 (10.34)	15 (12.82)	20 (10.75)	64 (10.70)
<i>Dictyocaulus filaria</i>	13 (1.94)	1 (0.85)	4 (2.15)	10 (1.67)
<i>Protostrongylus</i> spp.	85 (12.74)	22 (18.80)	24 (12.90)	83 (13.87)
<i>Muellerius capillaris</i>	7 (1.04)	1 (0.85)	3 (1.61)	5 (0.83)

N: Number of sampled animals, n: Number of animals infected with the related species. Number of different lowercase letters (n) in the same line represents significant difference ($p<0.05$)



Figure 5. a: *D. filaria* (blue arrow: protruding knob of the head), b: *M. capillaris* (red arrow: the thorny structure on the tail), c: *Protostrongylus* spp. Magnification: 100-200x, scale bar: 100 μm

Table 5. Single and mix infection rates of GI parasites according to the presence of oocytes and eggs in sheep

		n/N	%
Single	Trichostrongylid type egg	58	8.49
	<i>Marshallagia</i> spp.	63	9.22
	<i>Nematodirus</i> spp.	14	2.05
	<i>Trichuris</i> spp.	11	1.61
Double	<i>Eimeria</i> spp. + Trichostrongylid type egg	72	10.54
	<i>Eimeria</i> spp. + <i>Marshallagia</i> spp.	31	4.54
	<i>Eimeria</i> spp. + <i>Nematodirus</i> spp.	7	1.02
	<i>Eimeria</i> spp. + <i>Moniezia</i> spp.	10	1.46
	<i>Eimeria</i> spp. + <i>Trichuris</i> spp.	20	2.99
	Trichostrongylid type egg + <i>Marshallagia</i> spp.	36	5.27
	Trichostrongylid type egg + <i>Nematodirus</i> spp.	5	0.73
	Trichostrongylid type egg + <i>Moniezia</i> spp.	2	0.29
	Trichostrongylid type egg + <i>Trichuris</i> spp.	9	1.32
	<i>Marshallagia</i> spp. + <i>Nematodirus</i> spp.	10	1.46
	<i>Marshallagia</i> spp. + <i>Moniezia</i> spp.	1	0.15
	<i>Marshallagia</i> spp. + <i>Trichuris</i> spp.	3	0.44
	<i>Moniezia</i> spp. + <i>Trichuris</i> spp.	1	0.15
	<i>Nematodirus</i> spp. + <i>Moniezia</i> spp.	1	0.15
<i>Nematodirus</i> spp. + <i>Trichuris</i> spp.	1	0.15	
Triple	<i>Eimeria</i> spp. + 2 GI helminth	69	10.10
	GI helminth	22	3.22
>Triple	<i>Eimeria</i> spp. + 3 GI helminth	24	3.51
	GI helminth	4	0.59

N: Number of animals infected with at least one parasite, 683, n: Number of animals infected with related species, GI= Gastrointestinal

Table 6. Age and sex-wise EPG range in infective sheep

		Age		p	Sex		p
		<1 N=98 n (%)	>1 N=373 n (%)		♀ N=407 n (%)	♂ N=64 n (%)	
EPG	Mild	16 (16.33)	251 (67.29)	<0.05	27 (55.77)	40 (62.50)	>0.05
	Moderate	67 (68.37)	114 (30.56)		160 (31.31)	21 (32.81)	
	Severe	15 (15.31)	8 (2.14)		20 (4.91)	3 (4.69)	

N: Number of GIN infected animals, n: Number of infected animals with related EPG range, EPG: Eggs per gram

DISCUSSION

The presence and prevalence of *Eimeria* and helminth species in sheep were revealed by qualitative and quantitative coprological techniques and it was observed that 87.12% of the sampled sheep was infected with at least one parasitic agent.

The prevalence of *Eimeria* infection, a common problem in all sheep breeding countries worldwide, is reported between 11.4-92.9% (3-7) in the world and 37.3-100% (11-16) in Turkey. In the previous studies conducted in sheep in different provinces of Turkey, the prevalence of *Eimeria* species was between 56% and 100% (12,13,15,16). The prevalence was 49.36% in this study which is lower than the mentioned studies. In contrast, Sayın et al. (11), reported a lower prevalence (37.26%) in their study including sheep samples from different cities in the Aegean region. *Eimeria* species have high morbidity as a result of high flock population, poor sanitation, irregular litter removal, immunosuppression connected with stress factors and concurrent infections (2,6).

The differences in the prevalence of *Eimeria* infections in regions or provinces are related to the variation in climate, husbandry and nutrition, sheepfold conditions, anticoccidial drug usage, breed, age, health and immunity status of the sheep and diagnostic methods used for the analysis.

Currently, fifteen *Eimeria* species are known to infect sheep (25). In Turkey, twelve *Eimeria* species have been identified so far (13,15) and all those species were observed in the present study. Immunity is one of the most important factors affecting the presence and prevalence of *Eimeria* infections. The host's immunity can change depending on the age and sex of the animal. In the earlier studies, findings of more prevalent *Eimeria* infections in females than males caused by some stress-factors such as pregnancy, lambing, and lactation were reported (6,27). In the present study, compatible results with those studies were obtained. Specific immunity develops against recurrent *Eimeria* infections with the advance in age of the animals. The *Eimeria* spp. infections were found higher in <1 age group than >1 age group at the present study, and this result is similar with the previous studies (6,28). The OPG ranges, moderate and severe in <1 age group while mild in >1 age group were determined. Similar result was reported by other researchers (5,27,28). In the earlier ages of the sheep when the immune system is not developed accurately, the oocyst output rates are at high levels for *Eimeria* infections. The oocyst output rates decrease with the advancing ages and re-infections. Moderate and severe OPG in females, and mild OPG in males were determined in this study. Similar result was reported by Khan et al. (27) and Yakhchali and Golami (29). High levels of OPG in females is clarified by high number of oocyst production

as a result of immune depression due to physiological stress factors such as pregnancy, lambing, and lactation (6).

The prevalence of helminths in sheep was reported between 0.2-100% around the world in the studies based on necropsy and stool examination (4,8-10). The prevalence of trematodes, cestodes, GINs, and lung nematodes were reported between 3.1-72.6% (30,31), 7.56-21% (16,32), 0.39-100% (16-18) and 7.8-34% (16,33), respectively, in Turkey. In this study, the prevalence of trematodes, cestodes, GINs, and lung nematodes were determined as 28.95%, 4.34%, 60.10%, and 15.94%, respectively. The present results were lower than the results of earlier studies of India (34,35) 77.75-90.11%; Turkey (16,17) 75-100% and Spain (8) 100%. Distinctly, lower rates than this study were stated in India (68.55%), Ethiopia (63.33%) and Germany (62.8%) (9,24,36). The differences in the prevalence of helminth infections depend on many factors including the parasite (biological features), host (genetic resistance, age, physiological status) and environmental factors (climate, elevation, husbandry system). The pasture composition, animal diversity and the rate of herds and flocks in the same pasture are very important in the transmission of helminths due to the contamination of the pasture (9,37,38). In addition, antiparasitic drug usage, sample size, and co-infections have effect on the prevalence of the parasitic infections (9,34).

Trichostrongylid type eggs were found at the highest rate (49.5%) among the helminth positive samples which can be explained by the direct life cycle of these species. These results are supported by the studies of some researchers (9,10,16,37). The helminth infections were more prevalent in females (74.96%) than males (69.23%) in this study. Similar results were reported by Khajuria et al. (36) and Belina et al. (9). In contrast, Idris et al. (24), and Yasmin et al. (39), stated that helminth infections were more prevalent in males (24,39). Similar to the *Eimeria* infection, physiological stress factors which cause immunodepression are responsible for the high prevalence of helminth infections in females (35). Age is another factor that affects the prevalence of GI parasites. In general, adults are more resistant to the parasitic infections than younger animals due to age-related changes in the host immunity. In this study, higher infection rate was observed in adults which was in line with the results of Belina et al., (2017) and Singh (2017) (9,34). On the contrary, Idris et al., (2012) and Khajuria et al., (2013) presented higher infection rate in young animals (24,36). The reasons of higher infection rate in adults can be explained by the spread on large areas during grazing, overfeeding on contaminated pastures and more exposure to stress factors. The EPG ranges were determined as severe in the

<1 age group and females, mild and moderate infection in the >1 age group and males. The variety among the age groups can be commented with the immunity resulting from recurrent infection in the meantime the variety in the sex groups can be explained by the physiological status effect similar to the OPG ranges. The EPG range was determined higher in young animals by Idris et al., (2012), and in females by Khajuria et al., (2013) similar to the present study. But, on the other hand, some studies presented higher EPG range in males (24,36).

CONCLUSION

This study reports the presence and prevalence of *Eimeria* spp, GI system helminths and lung nematodes in sheep flocks in Erzurum province of Turkey. Considering the high prevalence of these parasites in study area, it is concluded that effective control and management programs should be applied including proper usage of anthelmintics and anticoccidials to improve the health and productivity of the animals.

* Ethics

Ethics Committee Approval: Ethical approval of the study was obtained from the Ethics Committee of Atatürk University of Veterinary Faculty (approval number: 2019/01).

Informed Consent: Informed consent is not necessary as it is an animal experiment.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: E.G., M.A., R.K., S.Y., H.G., Design: E.G., M.A., R.K., S.Y., H.G., Data Collection or Processing: E.G., M.A., R.K., S.Y., H.G., Analysis or Interpretation: M.A., Literature Search: M.A., Writing: M.A., E.G.

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