



RESEARCH ARTICLE

Prevalence of *Eimeria* species in sheep in Eskişehir province

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Received:26.04.2020, Accepted: 05.11.2020

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Eskişehir ilinde koyunlarda *Eimeria* türlerinin yaygınlığı

Eurasian J Vet Sci, 2020, 36, 4, 261-266

DOI: 10.15312/EurasianJVetSci.2020.307

Öz

Amaç: Çalışma koyun yetiştiriciliğinin yaygın olarak yapıldığı Eskişehir ilinde, koyunlarda bulunan *Eimeria* türlerinin yaygınlığını araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: Araştırma Eskişehir iline bağlı Seyitgazi, İnönü, Mihalgazi ile Mahmudiye ilçelerinde yetiştirilen 300 merinos ırkı koyun üzerinde yürütülmüştür. Her bir hayvanın rektumundan tekniğine uygun olarak alınan dışkı numuneleri Fulleborn'un doymuş tuzlu su metodu ile incelenmiştir. Pozitif çıkan dışkı numunelerinde gram dışkıdaki *Eimeria* oocist miktarı (OPG) Modified McMaster yöntemi ile belirlenmiştir. Daha sonra, her bir pozitif örnek ayrı petri kutularında üzerine %2,5'lük Potasyum dikromat ilave edildikten sonra laboratuvarında sporlanmaya bırakılmıştır. Sporlanmış oocistlerin ölçümü Nikon Eclipse 80i trinoküler araştırma mikroskopunun x100'lük büyütmesinde DS-5M-L1 dijital kamera sistemi ile mikrometrik olarak yapıldıktan sonra tür teşhisleri ilgili kaynaklardan yararlanılarak yapılmıştır. Araştırmada elde edilen verilerin istatistiksel değerlendirmesinde Ki-Kare (χ^2) testi kullanılmıştır.

Bulgular: Araştırma sonucunda muayene edilen 300 adet dışkının 61 (%20.33)'inde *Eimeria* oocistlerine rastlanmıştır. *Eimeria ovinoidalis* (%57,38), *E. ahsata* (%45,90), *E. bakuensis* (%42,62), *E. parva* (%42,62), *E. crandallis* (%11,48), *E. granulosa* (%4,92), *E. faurei* (%3,28), *E. intricata* (%3,28) ve *E. weybridgensis* (%3,28) olmak üzere dokuz *Eimeria* türü teşhis edilmiştir. Enfeksiyon oranı genç hayvanlarda, yaşlı hayvanlardan daha yüksek bulunmuştur ($p<0.05$). Enfekte hayvanlarda 2-5 türle enfeksiyon yaygın olarak gözlenmiştir. Muayene edilen hayvanlarda klinik bulgulara rastlanmamıştır.

Öneri: Bu çalışmada, patojen türlerin yaygın olarak bulunması Koccidiosis'in Eskişehir ili koyunlarında risk oluşturabileceği, hastalıktan korunma ve kontrol yöntemleri konusunda yetiştiricilerin bilinçlendirilmesi ile hastalığın neden olacağı ekonomik kayıpların azaltılabileceği kanaatine varılmıştır.

Anahtar kelimeler: Coccidiosis, *Eimeria*, koyun, Eskişehir

Abstract

Aim: The study was carried out to investigate the occurrence of *Eimeria* species in sheep in Eskişehir province, Turkey, where sheep breeding is common.

Materials and Methods: This study was carried out on 300 merino sheep raised in the Seyitgazi, İnönü, Mihalgazi and Mahmudiye districts of Eskişehir. Fecal samples from each sheep were brought to the laboratory and examined by Fulleborn saturated salt flotation method. *Eimeria* oocyst number per gram of feces (OPG) was determined by the Modified McMaster method in positive fecal samples. Later, each positive sample was added to 2.5% Potassium dichromate in separate Petri dishes and left to be sporulated in the laboratory. The measurement of sporulated oocysts was made micro metrically with the DS-5M-L1 digital camera system at x100 magnification of the Nikon Eclipse 80i trinocular research microscope. Species identification of *Eimeria* oocysts was made according to the morphological features reported in the literature. Chi-square (χ^2) test was used to analyze the data.

Results: *Eimeria* species were identified in 61 (20.33%) of the 300 samples of feces. In total nine different *Eimeria* species were identified including *Eimeria ovinoidalis* (57.38%), *E. ahsata* (45.90%), *E. bakuensis* (42.62%), *E. parva* (42.62%), *E. crandallis* (11.48%), *E. granulosa* (4.92%), *E. faurei* (3.28%), *E. intricata* (3.28%) and *E. weybridgensis* (3.28%). The infection rate was higher in young animals than older animals ($p<0.05$). Infection with 2-5 species was frequently detected in infected animals. No clinical findings were observed in the examined animals.

Conclusion: The high prevalence of pathogenic species shows that Coccidiosis is a risk for sheep raised in Eskişehir province. Potential economic effects of Coccidiosis can be reduced by informing breeders about disease prevention and control methods.

Keywords: Coccidiosis, *Eimeria*, sheep, Eskişehir





Introduction

Coccidiosis is one of the most important parasitic diseases in sheep breeding countries. Infection is commonly reported in lambs that are 4-7 weeks old and exposed to stress. Although the infection is usually subclinical in older animals, these animals are essential in terms of being a source of infection for newborns (Jawasreh et al 2013). In sheep, *Eimeria ahsata*, *E. bakuensis*, *E. crandallis*, *E. faurei*, *E. granulosa*, *E. gonzalezi*, *E. gilruthi*, *E. intricata*, *E. marsica*, *E. ovinoidalis*, *E. pallida*, *E. parva*, *E. weybridgensis*, and *E. punctata* have all been reported as causative species of Coccidiosis (Platzer et al 2005). Among these, *E. ovinoidalis*, *E. bakuensis*, and *E. ahsata* are the most pathogenic species (Khan et al 2011). Clinical Coccidiosis is encountered in sheep breeding, especially during the weaning period, causing diarrhea and developmental retardation (Alzieu et al 1999). It has been reported that meat yield decreased by 23.7%, wool yield decreased by 28.7% and milk yield decreased by 36% in infected animals (Mimioğlu et al 1969). Fitzgerald (1980) reported that this disease caused an estimated annual production loss of 140 million dollars in sheep in America. Environmental factors and the immunity of animals have an important role in the course of Coccidiosis. Crowded sheep pens increase environmental contamination with oocysts, resulting in clinical Coccidiosis (Bauer 1989). Coccidiosis prevalence was reported in the range of 29.9% to 100% in sheep in Turkey (Sayın et al 1986, Güler et al 1990, Özer 1991, Küçükerdan and Dumanlı 1992, Demir 1995, Arslan et al 1999, Gül and Değer 2002, Kaya 2004, Gül 2007, Gül and Günyaktı 2016, Akyüz et al 2019).

This study describes the prevalence of Coccidiosis and the distribution of responsible *Eimeria* species in sheep for the first time in Eskişehir province, where sheep husbandry is common and economically significant.

Material and Methods

This study was carried out from November 2018 to May 2019 in the Seyitgazi, İnönü, Mihalgazi and Mahmudiye districts of Eskişehir, which have different climatic characteristics. The altitude of the Seyitgazi district is 1040 m above sea level and it is under the influence of the Mediterranean climate. The İnönü district has a continental climate with an altitude of 840 m. The altitude of the Mihalgazi district is 214 m above sea level with a transitional climate. Mahmudiye district has a continental climate with an altitude of 888 m, and receives high rainfall throughout the year.

Sheep were divided into 3 age groups; 123 young (< 1 year old), 70 yearlings (1-2 years) and 107 adults (> 2 years). The sampled herds were composed of merino sheep raised extensively. The sheep had not been treated with any anticoccidials. Fecal samples were collected directly from the rectums

of all 300 sheep using gloves. The 300 sheep included 273 females and 27 males. Protocol information such as the age, gender and exact location of each animal was recorded. The fresh feces samples collected from each animal were examined by flotation technique using saturated salt solution, and oocysts per gram (OPG) quantified using the Modified McMaster technique. A part of each sample (3 grams) was re-suspended in 42 ml flotation fluid and filtered. After the filtration process, the suspensions (0.1 ml) were transferred to the McMaster slide chambers. The oocysts in both chambers were counted and the oocyst number per gram of feces (OPG) was calculated. *Eimeria* oocysts from positive samples were sporulated for 3-5 days at room temperature using 2.5% potassium dichromate. Sporulated oocysts were measured using the DS-5M-L1 digital camera system at the x100 magnification of the Nikon Eclipse 80i trinocular research microscope. *Eimeria* oocysts identification was based on their morphology according to the literature (Mimioğlu et al 1969, Levine 1985, Levine and Ivens 1986).

Statistical Analysis

Results were statistically analyzed using a chi-square (χ^2) test.

Results

Out of the 300 fecal samples examined, 61 (20.33%) were positive for *Eimeria* oocysts. The overall prevalence of *Eimeria* species in the four districts of Eskişehir province ranged from 2.67% to 36%. A statistically significant ($p < 0.001$) difference was found in their prevalence among the different localities. The highest prevalence (36%) was detected in sheep in Mahmudiye district (Table 1).

The prevalence was much higher in the group of young sheep than the other two age groups ($p < 0.05$) (Table 2).

Table 1. Distribution of Coccidiosis according to locality

Locality	Number of examined animals	Number of infected animals	Prevalence (%)
Mahmudiye	75	27	36.00
İnönü	75	20	26.67
Seyitgazi	75	12	16.00
Mihalgazi	75	2	2.67
Total	300	61	20.33

χ^2 : 28.541 df: 3 $p < 0.001$



Table 2. Distribution of Coccidiosis according to age and OPG values

Age	Number of examined animals	Number of infected animals	Prevalence (%)	OPG (Min. - Max.)
< 1 year	123	36	59.00	500-4650
1-2 year	70	3	4.90	200-5200
> 2 year	107	22	36.10	200-4000
Total	300	61	100	

χ^2 : 10.274 df: 1 p<0.05

Table 3. *Eimeria* species distribution in infected sheep (n: 61)

<i>Eimeria</i> species	Number of infected animals	Percentage (%)
<i>E. ovinoidalis</i>	35	57.38
<i>E. ahsata</i>	28	45.90
<i>E. bakuensis</i>	26	42.62
<i>E. parva</i>	26	42.62
<i>E. crandallis</i>	7	11.48
<i>E. granulosa</i>	3	4.92
<i>E. faurei</i>	2	3.28
<i>E. intricata</i>	2	3.28
<i>E. weybridgensis</i>	2	3.28

n: number of positive animals

Table 4. Number of animals infected with different *Eimeria* species and infection frequencies

	Number of <i>Eimeria</i> species				
	1	2	3	4	5
No. of infected sheep	16	27	12	5	1
Frequency (%)	26.23	44.26	19.67	8.20	1.64

Table 5. Distribution of infection according to gender

Host gender	Number of examined animals	Number of infected animals	Prevalence (%)
Female	273	51	83.60
Male	27	10	16.40
Total	300	61	100

χ^2 : 5.110 df: 1 p<0.05





Overall, nine *Eimeria* species were identified in the infected sheep: *Eimeria ovinoidalis* (57.38 %), *E. ahsata* (45.90%), *E. bakuensis* (42.62%), *E. parva* (42.62 %), *E. crandallis* (11.48%), *E. granulosa* (4.92%), *E. faurei* (3.28%), *E. intricata* (3.28%) and *E. weybridgensis* (3.28%). The most abundant species was *E. ovinoidalis* (Table 3).

Multiple-species infections of *Eimeria* species were detected in 45 (78.78%) of the 61 infected animals (Table 4).

The overall prevalence of *Eimeria* oocysts in females was higher (83.60%) than that in males (16.40%). There was a statistically significant difference ($p < 0.05$) in prevalence between males and females (Table 5).

Discussion

Fifteen *Eimeria* species are known to infect sheep (Long and Joyner 1984, Rommel 2000). *E. ovinoidalis*, *E. bakuensis*, *E. crandallis*, *E. weybridgensis*, *E. parva* and *E. faurei* are the predominant species in Central and Eastern Europe (Rommel 2000). Previously in Turkey, *E. ahsata*, *E. bakuensis*, *E. crandallis*, *E. faurei*, *E. intricata*, *E. marsica*, *E. ovinoidalis*, *E. pallida*, *E. parva*, *E. granulosa*, *E. weybridgensis*, *E. crandallis* and *E. punctata* species have all been recovered from sheep (Sayın et al 1986, Arslan et al 1999, Gül and Değer 2002, Kaya 2004, Gül 2007, Akyüz et al 2019). In this study, *Eimeria ovinoidalis*, *E. ahsata*, *E. bakuensis*, *E. parva*, *E. crandallis*, *E. granulosa*, *E. faurei*, *E. intricata* and *E. weybridgensis* species were reported for the first time in Eskişehir province. Also, in this study, *E. ovinoidalis* (57.38%), *E. ahsata* (45.90%), *E. bakuensis* (42.62%) and *E. parva* (42.62%) were found to be common *Eimeria* species (in the 300 animals sampled), in agreement with previous findings (Maingi and Munyua 1994, Gül and Değer 2002, Wang et al 2010).

More broadly, the prevalence of Coccidiosis in sheep in different regions and countries has been found to be in the range of 16.67-92.9% (Maingi and Munyua 1994, Yakhchali and Rezaei, 2010, Wang et al 2010, Saratsis et al 2011, Souza et al 2015, Nourollahi-Fard et al 2016, Kyriánová IA et al 2017, Mohamaden et al 2018, Alcalá-Canto et al 2020). In Turkey, the prevalence of infection was previously determined to be between 29.9% and 100% (Sayın et al 1986, Güler et al 1990, Özer 1991, Küçükercan and Dumanlı 1992, Demir 1995, Arslan et al 1999, Gül and Değer 2002, Kaya 2004, Gül 2007, Gül and Günyaktı 2016, Akyüz et al 2019). The difference in these prevalence values could be due to variations in the climate, temperatures and amounts of rainfall in different seasons, the ages and immune systems of the host animals, hygiene conditions and rearing systems (Gouet et al 1984, Catchpole and Harris 1989, Gauly et al 2004, Chartier and Paraud 2012). In this investigation, we found the highest prevalence of Coccidiosis in animals less than 1 year old, which agreed with previous findings (Maingi and Munyua 1994, Arslan et

al 1999, Wang et al 2010, Nourollahi-Fard et al 2016).

Also in this work, the prevalence of Coccidiosis in the sheep sampled was considerably higher in the Mahmudiye district where rainfall is higher than the other districts throughout the year. Taylor (2012) reported that moisture caused by rain in places with inadequate drainage may influence the prevalence of *Eimeria* sp. A warm and moist environment provides ideal conditions for oocyst sporulation.

Furthermore, in this study, mixed infections with different *Eimeria* species were seen more commonly than infections with a single *Eimeria* species. This correlates with prior findings of other researchers (Yakhchali and Rezaei 2010, Wang et al 2010, Nourollahi-Fard et al 2016). In the study of Nourollahi-Fard et al (2016) the prevalence of *Eimeria* in females was greater than that in males, which is also similar to our findings in the present study.

Conclusion

The apparent high prevalence of pathogenic species discovered in this study reveals that Coccidiosis infection is a significant risk for sheep raised in Eskişehir province. Possible economic losses due to the disease could be reduced by raising awareness of the disease amongst breeders and perhaps by providing more educational resources about general disease prevention and control methods.

Acknowledgement

This study was presented as a poster in the 4th International Congress on Advances of Veterinary Sciences and Techniques (ICAVST).

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

This work was supported by Afyon Kocatepe University Scientific Research Commission with the project number 17.Kariyer.145.

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Critical Review: Hatice Çiçek

Ethical Approval

This study was carried out with the permission of the Afyon Kocatepe University, Experimental Animals Production and Research Center Ethics Committee (Decision Number: AKU-HAYDEK 49533702/26 report.)

CITE THIS ARTICLE: Eser M, Göksu A, Çiçek H, Erez MS, et al., 2020. Prevalence of Eimeria species in sheep in Eskişehir province. *Eurasian J Vet Sci*, 36, 4, 261-266

