

Changes in Faecal Egg Counts at Different Hours of the Day and Relationship between Faecal Egg Count and Parasite Burden in Sheep Naturally Infected with *Dicrocoelium dendriticum*

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Abstract: The present study was carried out to determine the variations in the number of eggs excreted in the faeces at different hours of the day and the correlation between faecal egg counts and fluke burden in sheep naturally infected with *Dicrocoelium dendriticum*. For this purpose faecal samples were taken from 14 sheep at 1-h intervals from 07⁰⁰ to 19⁰⁰. Faecal samples were examined by modified Benedek sedimentation method and mean egg counts per gram of faeces (EPG) for each hour (average of group) were calculated. In general, egg counts were found higher in faecal samples taken in the afternoon than those from the morning. Although the highest EPG value was observed at 17⁰⁰ (61.3 ± 16.9), no statistical difference was found among the egg counts at different hours. In order to detect the fluke burden 7 sheep were necropsied after faecal sampling. The number of *D. dendriticum* recovered at necropsy of each animal varied between 200 and 759. While a positive correlation was observed between faecal egg count and total fluke count ($r = 0.786$, $P < 0.01$), no statistically significant correlation was found between gall bladder fluke count and total fluke count.

Key Words: *Dicrocoelium dendriticum*, egg count, fluke burden, sheep

***Dicrocoelium dendriticum* ile Doğal Enfekte Koyunlarda Günün Farklı Saatlerinde Dışkı Yumurta Sayısındaki Değişiklikler ve Dışkı Yumurta Sayısı ile Parazit Yükü Arasındaki İlişki**

Özet: Bu çalışma *Dicrocoelium dendriticum* ile doğal enfekte koyunlarda günün farklı saatlerinde dışkı ile atılan yumurta sayısındaki değişikliklerin ve dışkı yumurta sayısı ile parazit yükü arasındaki ilişkinin belirlenmesi için yapılmıştır. Bu amaçla 14 koyundan saat 07⁰⁰'den 19⁰⁰'a kadar birer saat aralıklarla dışkı alınmıştır. Dışkı örnekleri modifiye Benedek sedimentasyon yöntemi ile incelenmiş ve gram dışkıdaki yumurta sayıları (EPG) her saat için grup ortalaması alınarak hesaplanmıştır. Genel olarak öğleden sonra alınan dışkı örneklerindeki yumurta sayısı öğleden önce alınan dışkı örneklerindeki göre daha yüksek bulunmuştur. En yüksek EPG değeri (61,3 ± 16,9) saat 17⁰⁰'da gözlenmesine rağmen istatistiki olarak değişik saatlerdeki yumurta sayıları arasında bir fark bulunmamıştır. Parazit yükünü belirlemek amacıyla dışkı örneklerinin alınmasından sonra 7 koyuna nekropsi yapılmıştır. Nekropside her bir hayvandan toplanan *D. dendriticum* sayısı 200 ile 759 arasında değişmiştir. Dışkıdaki yumurta sayısı ile toplam parazit sayısı arasında pozitif bir korelasyon ($r = 0,786$, $P < 0,01$) gözlenirken, safra kesesindeki parazit sayısı ile toplam parazit sayısı arasında istatistiki olarak önemli bir korelasyon saptanmamıştır.

Anahtar Sözcükler: *Dicrocoelium dendriticum*, yumurta sayısı, parazit yükü, koyun

Introduction

Dicrocoeliosis is a widespread disease of grazing ruminants caused by *Dicrocoelium dendriticum*. This trematode lives in the bile ducts and gall bladder of domestic and wild ruminants. It occasionally affects rabbits, pigs, dogs, horses and humans (1-3).

The simplest and most commonly used technique for the intra vitam diagnosis of dicrocoeliosis is the

examination of faecal samples for the characteristic eggs (2,4). However, the egg output rate can be influenced by several factors (2,5) and the number of eggs passed into the faeces varies from day to day (6,7). It was found that the excretion of *D. dendriticum* eggs by experimentally infected lambs was higher in the afternoon than in the morning (1). Such differences can influence the results of epidemiological and anthelmintic efficacy studies.

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Nevertheless, there is no detailed study on the dynamics of egg excretion of *D. dendriticum* throughout the day, especially in the afternoon hours.

On the other hand, although *Dicrocoelium* egg counts were used to assess the fluke burden in sheep, there are controversial data on the relationship between *Dicrocoelium* egg output and parasite burden in sheep (1,4,8-10). Kopp (8) stated that there was no correlation between faecal egg counts and fluke numbers. Campo et al. (1), Rehbein et al. (4) and Rojo-Vazquez et al. (10) found a positive correlation between faecal egg and fluke counts in infected sheep.

Therefore, this study aimed to determine: (i) the variability of egg output at different hours of the day, and (ii) the relationship between faecal egg count and total fluke count; gall bladder fluke count and total fluke count in natural *D. dendriticum* infections in sheep.

Materials and Methods

Animals

The animals, aged 4 to 7 years, originated from a flock in the vicinity of Bursa, marked with ear tags and fed with hay. Fourteen animals showing the highest *D. dendriticum* egg numbers were selected from 80 naturally infected crossbred ewes (Karacabey Merino breed) for the study.

Faecal samples and laboratory procedures

Faecal samples were directly taken from each animal's rectum at 1-h intervals from 07⁰⁰ to 19⁰⁰. Samples from 2, 4, 3 and 4 animals could not be taken at 08⁰⁰, 11⁰⁰, 14⁰⁰ and 19⁰⁰ hours, respectively. The faeces were placed into labelled polythene bags, refrigerated until transported to the laboratory and stored in -20 °C until examined. The modified Benedek sedimentation method was used to process each faecal sample (11). All eggs in the sediment were counted and the results were expressed as eggs per gram (EPG). The mean (average of group) number of eggs for each hour was determined. The mean egg counts were also calculated for each animal. To exclude inter-researcher variation, samples were examined microscopically by the same researcher.

Examination of liver and gall bladder at slaughter

In order to detect fluke burden 7 sheep showing the highest EPG were purchased and slaughtered after faecal

sampling. After slaughter, the liver was removed and dissected to obtain and count the flukes in the bile ducts. The number of flukes in the gall bladder was counted separately.

Statistical analysis

Changes in the mean EPG values at different hours were analysed using one-way analysis of variance. The relationship between faecal egg count, total fluke count and gall bladder fluke count was analysed using Pearson's correlation coefficient followed by linear regression analysis of these parameters. All data were analysed using Minitab statistical package for Windows Release 13.2.

Results

Mean (\pm SE) EPG values at different hours of the day are shown in the Figure. The excretion of *D. dendriticum* eggs fluctuated widely throughout the day, not only for the group means but also for the individuals (data not shown). The egg excretion was generally low during the whole study period, and the minimum and maximum EPG values were 0 and 176, respectively. While the highest mean EPG was observed at 17⁰⁰ (61.3 ± 16.9), the lowest was detected at 9⁰⁰ (11.8 ± 3.7). However, no statistical difference was found among the egg output at different hours. In general, an increase was observed in the number of EPG excreted in the afternoon compared to morning ones (Figure).

Total number of flukes (liver and gall bladder) obtained in each sheep at necropsy varied between 200 and 759. Approximately 1% of the total fluke burden was recovered from the gall bladder, with the remaining from the bile ducts of the liver. Meanwhile, mean EPG of necropsied animals varied between 12.2 and 74.6.

The results of the correlation analysis of different parameters are presented in the Table. A positive relationship can be seen via the correlation coefficient ($r = 0.786$, $P < 0.01$) between the number of eggs excreted by each sheep and that of total fluke counts recovered. With the increase in the total fluke counts, an increase was also observed in the number of the gall bladder flukes, but this relationship was not statistically significant ($r = 0.568$, $P > 0.05$). No statistically significant correlation was found between faecal egg counts and gall bladder fluke counts ($r = 0.278$, $P > 0.05$).

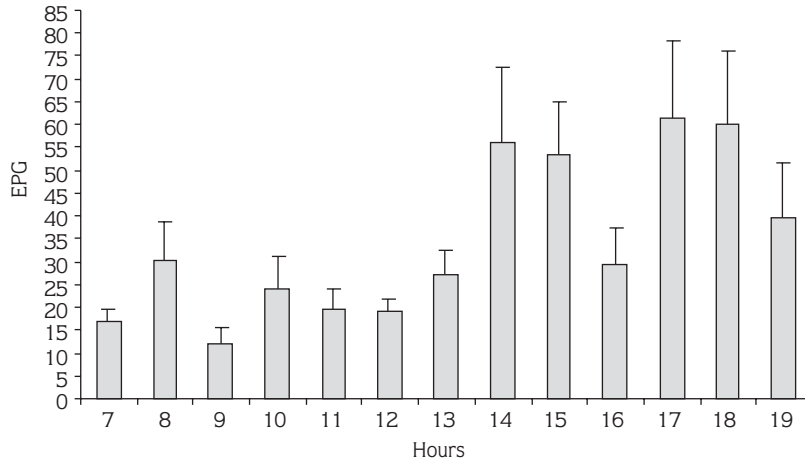


Figure. Mean (± SE) EPG values at different hours of the day.

Table. Relationships between faecal egg, total fluke, gall bladder fluke and liver fluke counts.

	Pearson's correlation coefficient		
	TFC	LFC	GBFC
EPG	0.786*	0.785*	0.278
GBFC	0.568	0.550	
LFC	0.999*		

EPG: Number of eggs per gram of faeces

TFC: Total fluke count

LFC: Liver fluke count

GBFC: Gall bladder fluke count

*: P < 0.01

The relationships between (i) faecal egg count and the total fluke count, and (ii) gall bladder fluke count and the total fluke count were described by regression equations as follows:

Regression equation

Coefficient of determination

$$\text{TFC} = 21.8 \text{ EPG}^{(P < 0.05)} + 89.9^{(P = 0.107)}$$

0.618

$$\text{TFC} = 24 \text{ GBFC}^{(P = 0.217)} + 257^{(P = 0.105)}$$

0.349

TFC: Total fluke count

GBFC: Gall bladder fluke count

To estimate the total fluke count from the faecal egg counts the regression equation was found to have considerable high coefficients of determination. However, there was no significance for the constant term of the

equation ($P = 0.107$). Because a reliable linear regression equation could not be established for this relation, the number of total flukes could not be deducted exactly from faecal egg counts.

Regression analysis of gall bladder fluke count and total fluke count showed that the determination coefficient was low. Furthermore, there was no significance for the variable term ($P = 0.217$) or the constant term ($P = 0.105$).

Discussion

We were not able to find a detailed study on the egg excretion of *D. dendriticum* throughout the day with 1-h intervals in naturally infected sheep. Kokott (12) reported that there was no particularly pronounced *Dicrocoelium* egg output at a certain time of the day, while Campo et al. (1) stated that the elimination of *D. dendriticum* eggs by experimentally infected lambs was higher in the afternoon than in the morning. In agreement with the latter finding, we found also higher egg counts in the afternoon than in the morning, but no statistical difference was found at different hours. As the egg counts were relatively low during our study period, a significant level might be reached when considering egg counts higher than those found in the present study. We also found highly variable egg counts within periods of several hours not only for the group means but also for the individuals. In sheep, the gall bladder serves as a reservoir for the continuously produced bile, which is emptied into the duodenum at discontinuous intervals during a day (13,14). This may explain the variability of faecal egg counts at different hours in *D. dendriticum* infections in sheep. However, it is not clear why the egg excretion tends to increase in the afternoon hours.

Kopp (8) studied the relationship between the number of eggs and parasite burden and reported that there was no correlation between egg and fluke counts. In contrast to Kopp (8), positive correlations were found between these parameters both in naturally infected sheep (4,9,10) and in experimentally infected sheep (1). Rojo-Vazquez et al. (10) pointed out that the correlation between the number of parasites and the number of eggs was most reliable in heavy infections (>1000 flukes). In this study, a positive correlation was found between faecal egg counts and the number of total flukes.

However, no statistically significant correlation could be found between gall bladder fluke counts and total fluke counts or between faecal egg counts and gall bladder fluke counts.

Different authors attempted to establish a relationship between faecal egg and total fluke counts with a linear regression equation (4,9,10). Calamel and Giauffret (9) claimed that mean number of adult flukes could be deducted, for a group of adequate size, from the mean number of eggs. In contrast, Rehbein et al. (4) pointed out that the regression equations for estimating the fluke numbers from faecal egg counts have shown considerable weakness. Rojo-Vazquez et al. (10) conducted 3-12 faecal examinations per sheep before the necropsy to stabilise the individual egg excretion and found that a double logarithmic regression equation fitted best to describe the relationship between faecal egg and total fluke counts. In our study, although a positive correlation was found between faecal egg counts and total fluke counts, a reliable linear regression equation could not be established for estimating the fluke numbers from faecal egg counts. This could be due to the fact that firstly the fluke numbers per sheep were low, secondly the number of animals used in the study was small and thirdly the egg output was highly variable. Thus, in order to firmly establish the type of relationship between faecal egg counts and fluke burden, it is recommended to carry out further studies using more animals infected with a higher infection rate.

In conclusion, the excretion of *D. dendriticum* eggs fluctuated widely throughout the day and the excretion of eggs seems to be higher in the afternoon hours. Therefore, the variations demonstrated here should be taken into consideration in future studies when assessing the results of coprological diagnosis in dicrocoeliosis, and if possible faecal samples should be taken at the same hours in the afternoon. Otherwise, faecal examinations carried out at different times can lead to unexpected results being drawn. Consequently, the use of a regression equation for estimating fluke (*D. dendriticum*) counts from faecal egg counts is of limited value.

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