Studies on Sheep Lungworms in Bursa Province of Turkey: Determination of Prevalence and Relationships Between Larval Output and Parasite Burden in the Lungs

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Abstract.- This study was carried out to determine lungworm species and their prevalence, evaluate the effect of host age and breed on the infections and the correlation between faecal larvae counts and adult lung worms counts in sheep in Bursa Province, Turkey. A total of 104 sheep of different age and breed were examined for the lungworm infection. Prevalence of infection was 45.1% and 62.5% by coproscopy and necropsy, respectively. The faecal examination and postmortem showed Dictyocaulus filaria (23%; 31.7%), Cystyocaulus ocreatus (24%; 31.7%) and Protostrongylus rufescens (3.8%; 5.8%). Seven (6.7%) and six (5.7%) sheep had mixed infections with two nematode species in necropsy and faecal examinations, respectively. There was a significant difference (p<0.05) between >4 year old and <4 year old age groups. There was no significant difference in the prevalence of lungworm infections in different sheep breeds. Although there was a positive correlation between larval excretion in faeces and lung nematode counts, reliable regression equations could not be established. Therefore, the number of worms in the lungs could not be deducted exactly from faecal lungworm larvae counts.

Key Words: Bursa-Turkey, larval excretion, lungworm, parasite burden.

INTRODUCTION

Lung nematodes are frequently found in sheep and it is an important problem for sheep breeders throughout the world. Protostrongylidae and Dictyocaulidae nematodes cause lungworm infections in sheep. Protostrongylidae species occur in the alveoli, bronchioles and parenchyma of the lungs of various species of mammals. Dictyocaulidae species are located in respiratory passages of the lungs (Soulsby, 1986; Umur et al., 2006). These parasites cause respiratory problems such as bronchopneumonia and death in young sheep. Infections contribute to low productivity (reduced weight gain, retarded growth, decreased milk production) and to the economic losses (Burger, 1992; Soulsby, 1986; Umur et al., 2006). Dictyocaulus filaria, Cystyocaulus ocreatus, Muellerius capillaris, Protostrongylus rufescens and Neostrongylus linearis are known to exist in Turkey (Dik et al., 1993). But no detailed investigation was done to determine lung nematodes of sheep in the Bursa Province of Turkey so far.

MATERIALS AND METHODS

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Study area and animals
Study area (Bursa) is located in southeast of the Marmara Sea (40°E, 28–30°N). The study was carried out over a period of one year (between March 2003 and July 2004) and 104 sheep slaughtered in Bursa Meat and Fish Plant were examined for lung nematodes and larvae. The breeds of sheep in the study were Kıvırcık which is the predominating sheep breed of the Marmara region in Turkey and crossbred. Animals are divided into 2 age groups. One group is less than 4 years old, the other group is more than 4 years old. The age of sheep was estimated by stage of dentition and by anemnesing the owners of the animals.

Faecal examination
Prior to slaughter individual faecal samples were taken from all animals included in the study. Lungworm larvae were separated from 5 g of faeces using the Baermann funnel (MAFF, 1986). Larvae were identified based on morphological characteristics (Guralp, 1981).

Post-mortem examination
After faecal sampling all sheep were slaughtered and detailed necropsies were carried out. The respiratory tract was recovered from each animal and taken to the laboratory for examination of parasitic nodules. All suspected nodules were examined in detail by dissection. The trachea and main bronchi were opened with a pair of scissors, searched for the presence of adult worms and all visual parasites were collected. The lungs were cut to pieces 1-2 cm. diameter and placed in physiologic saline solution in an incubator at 37°C for two hours. The lung pieces were removed and remaining fluids were poured through a screen. Parasites were collected under the stereo microscope. Identification of adult parasites was done by direct microscopy (Umur et al., 2006).

Statistical analyses
Prevalence data were analysed by chi-square test. The relationship between faecal larvae counts and lungworm counts was analysed using Pearson’s correlation coefficient followed by linear regression analysis. Probability (p) values < 0.05 were considered to indicate significant differences among groups. All data were analysed using Minitab 13.2 statistical software (Minitab Inc., 2000).

RESULTS

The overall prevalence of lungworm infection was 62.5% and 45.1% by necropsy and faecal examinations, respectively. Both methods showed that C. ocreatus and D. filaria were the most prevalent species (Table I). According to the postmortem examinations the prevalence of lungworms species in sheep lungs were as follows C. ocreatus (26.9 + 4.8 = 31.7%), D. filaria (25 + 4.8 + 1.9 = 31.7%), and P. rufescens (3.9 + 1.9 = 5.8%). Mixed infections were seen only in seven animals in which five of them included D. filaria and C. ocreatus and other two included D. filaria and P. rufescens (Table I).

<table>
<thead>
<tr>
<th>Lungworm</th>
<th>Faecal examination</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Cystocaulus ocreatus</td>
<td>20</td>
<td>19.2</td>
</tr>
<tr>
<td>Dictyocaulus filaria</td>
<td>18</td>
<td>17.3</td>
</tr>
<tr>
<td>Protostrongylus rufescens</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>C. ocreatus + D. filaria</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td>D. filaria + P. rufescens</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Overall prevalence</td>
<td>47</td>
<td>45.1</td>
</tr>
</tbody>
</table>

In faecal examination, the following species with their prevalences, were determined: C. ocreatus (19.2 + 4.80 = 24%), D. filaria (17.3 + 4.8 + 0.9 = 23%) and P. rufescens (2.9+0.9=3.8%). Statistical analysis indicated that there was a significant difference in the overall prevalence of infection between the age categories (p < 0.05). As shown in Table II, the highest was in >4 year old age group (53.2%), (69.3%) and the lowest in <4 year old age group (33.3%), (50%) by faecal examinations and necropsy, respectively. On the other side, although higher prevalence was observed in Kıvırcık sheep compared to crossbreds there was no significant difference between the age classes (Table II).

The results of the correlation analysis between larvae and total nematode counts are
presented in the Table III. Significant correlation (r=0.618, P<0.05) was determined between the number of excreted \textit{C. ocreatus} larvae and that of \textit{C. ocreatus} counts recovered. On the other hand, increase of the total \textit{D. filaria} and \textit{P. rufescens} counts, an increase was also observed in the number of excreted larvae by each sheep, but this relationship was not statistically significant. The results of the regression analysis between faecal larvae counts and the total lung nematode counts are given in Table III.

Table II.- Prevalence (%) of lungworm infections in sheep relative to host age and breed.

<table>
<thead>
<tr>
<th>Category</th>
<th>Faecal examination</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4 old</td>
<td>42</td>
<td>14  33.3\textsuperscript{a}</td>
</tr>
<tr>
<td>&gt;4 old</td>
<td>62</td>
<td>33  53.2\textsuperscript{b}</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kivircik</td>
<td>10</td>
<td>6  60\textsuperscript{a,s}</td>
</tr>
<tr>
<td>Crossbred</td>
<td>94</td>
<td>41  43.6\textsuperscript{a,s}</td>
</tr>
</tbody>
</table>

\textsuperscript{a, b} values with different letters in each category are significantly different, p<0.05; n.s.: not significant

Most compatible relationship had been found between total lungworm counts and the faecal larvae counts having statistically significant in all terms of equations for \textit{C. ocreatus}. Although regression equations describing the relation of parasite burden and larvae counts for the \textit{D. filaria} had considerable high coefficient of determination, there was no significance for the variable term of equations. Regression analysis of \textit{P. rufescens} showed that the determination coefficient was low. Furthermore, there was no significance for the variable term.

**DISCUSSION**

Prevalence of lungworm infections in sheep varied depending on climate conditions and management practices (Burger, 1992; Soulsby, 1986; Umur \textit{et al.}, 2006). In this study, we have identified three species of bronchopulmonary nematodes in sheep in Bursa province with an overall infection rate of 62.5% and 45.1% by necropsy and faecal examinations, respectively. These results agree with those of other researchers, who also reported the prevalence of infection as 53.17% in Ankara (Doganay \textit{et al.}, 1989), 47.2% in Istanbul (Bagci and Bıyıkoglu, 2003) and 50.5% in Kars (Umur and Arslan, 1998) provinces with faecal examination. Lungworm infection was observed as 55.3% in Kars (Umur and Arslan, 1998), 42.9% in Elazığ (Tasan \textit{et al.}, 1997) provinces and 19.85% in Trakya Region (Gargili, 1995) with necropsy. In a study conducted in South Marmara Region, Oncel (2000) reported that the prevalence of lungworm infection was 33.8% and 38% based on faecal examination and necropsy findings, respectively. The overall prevalence of lungworm infections reported in our study was higher than the prevalence rate reported by Oncel (2000) in South Marmara Region which had similar climatic conditions as those in our study area. These differences were probably linked to feeding habitat of sheep in different localities. On the other hand, the highest proportion of young animals in their study may be the causing factor of this situation. The lower prevalence of lungworms infection for young animals could be due to lower exposure to infective larvae of parasites.

The prevalence of lungworm infection by faecal examination was lower (45.1%) than the results obtained by necropsy (62.5%). This finding is in agreement with the observations of Alema \textit{et al.} (2006), Oncel (2000) and Yildiz (2006). The stage of parasites can lead to this situation. Because in the prepatent or postpatent phases it is very difficult to detect these nematodes by coproscopic methods. Another explanation would be that egg production may be inhibited by the immune reaction of the host (Alemu \textit{et al.}, 2006).

Umur and Arslan (1998) stated that \textit{C. ocreatus} and \textit{D. filaria} were the most prevalent species in Turkey. In this study, \textit{C. ocreatus} and \textit{D. filaria} were the most commonly reported lungworms. On the other hand, our results confirmed the findings of other studies performed in some part of the world (Alemu \textit{et al.}, 2006; Gorski \textit{et al.}, 2004; Rehbein \textit{et al.}, 1998, Tarazona, 1984).

Alemu \textit{et al.} (2006) found that the prevalence of \textit{M. capillaris} and \textit{P. rufescens} infections were higher over 4 years of age. Thomson and Orita (1988) reported that an increase in the prevalence of
 Martha and the squirrel  

protostrongylid infection with an increase in the age of the sheep. In the present study the prevalence of lungworms infection was higher in the >4 year age animals (p<0.05). These findings are in agreement with the study of Alemu et al. (2006) and Thomson and Orita (1988). They found that the highest infection rates with lungworm were observed in older than youngsters.

Alemu et al. (2006) and Sissay (1996) showed that female animals are more susceptible to lungworms infection than males. However, we could not analyse the effect of sex in our study as all of the animals were females.

There are very limited number of studies on the effect of animal breeds on the prevalence of lungworms infections in sheep. Gorski et al. (2004) studied to estimate the prevalence of lungworms in nine breeds of sheep naturally infected with internal parasites. They reported that lungworm infections were found in Kamieniecka, Pomorska and Mountain Sheep with the prevalence from 2.5% (Kamieniecka) to 12.5% (Pomorska). Getachew (1989) reported that significantly greater number of D. filaria worms were recovered from the lungs of the Ethiopian sheep than from the crossbreds in experimental infections. In this study, although higher prevalence was observed in Kıvırcık sheep compared to crossbred there was no significant difference between the breeds.

Some studies have been done on factors effecting the larval excretion in lungworm infections (Diez-Banos et al., 1994; Pelletier et al., 2005). These authors stated that various factors such as season, sex and reproductive efficiency of host could effect on larvae output. However, there are very limited number of studies on the relationship between larval excretion and parasite burden in sheep (Getachew, 1989). In the present study, a correlation has been detected between the number of larvae and the number of nematodes in lungs. Especially, this correlation was strong for C. ocreatus. However, the regression equations calculated for estimating numbers of lungworm from faecal larvae counts have shown weaknesses. While regression equation for the D. filaria had considerable high coefficient of determination, there was no significance for the variable term of regression. In contrast to our findings Getachew (1989) found that faecal larval output do not reflect the number of adult parasites and there was no direct relationship between LPG and the number of adult worms in the lungs.

As a conclusion, the prevalence of bronchopulmonary nematodes are considerably high in sheep in Bursa Province, Turkey. Although a correlation was found between larval excretion and nematode counts in lungs, because of reliable linear regression equation could not be established, the number of lungworms could not be deducted exactly from faecal larvae counts.

REFERENCES


(Received 11 December 2007, revised 22 May 2008)