Pakistan J. Zool., vol. 40(5), pp. 365-369, 2008.

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

OYA GİRİŞGİN, BAYRAM ŞENLİK, AHMET ONUR GİRİŞGİN AND VOLKAN AKYOL
Parasitology Department, Faculty of Veterinary Medicine, Uludag University, 16059 Gorukle / Bursa, Turkey

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.

* Corresponding author: *E-mail*: vakyol@uludag.edu.tr

0030-9923/2008/0005-0365 $ 8.00/0

Determination of first stage larvae in faeces is the simplest, non invasive and most commonly used technique for the intravitam diagnosis of lungworm infections (van Wyk *et al.*, 2004; Zajac *et al*., 1994). Several factors can effect the larval excretion such as season, lactation, and reproductive effort (Diez-Banos *et al.*, 1994; Pelletier *et al.*, 2005). Pelletier *et al*. (2005) reported that faecal excretion of *Protostrongylus* spp. by Bighorn sheep varied seasonally and peaked in females prior to lambing. Although larvae counts were used to assess the parasite burden in host, there is no detailed study on the relationship between lungworm larvae excretion and parasite burden in sheep.

The objectives of the present study were to investigate (i) the prevalence of lungworm infections in sheep in Bursa Province of Turkey, (ii) the effects of age and breed on the prevalence of infection and (iii) the relationship between faecal larvae excretion and parasite burden of respiratory system nematodes in sheep.

MATERIALS AND METHODS

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 prevalance 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 I.- Prevalence of single and multiple infections with lung nematodes in sheep (n=104).

Lungworm	Faecal examination		Necropsy	
	No.	%	No.	%
Cystocaulus ocreatus	20	19.2	28	26.9
Dictyocaulus filaria	18	17.3	26	25
Protostrongylus rufescens	3	2.9	4	3.9
C. ocreatus+D filaria	5	4.8	5	4.8
D. filaria+P. rufescens	1	0.9	2	1.9
Overall prevalence	47	45.1	65	62.5

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 *C. ocreatus* larvae and that of *C. ocreatus* counts recovered. On the other hand, increase of the total *D. filaria* and *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.

Category	n	Faecal examination		Necropsy	
		No.	%	No.	%
Age (Years)					
<4 old	42	14	33.3[a]	21	50[a]
>4 old	62	33	53.2[b]	43	69.3[b]
Breed					
Kivircik	10	6	60[n.s.].	8	80[n.s.]
Crossbred	94	41	43.6[n.s.].	56	59.5[n.s]

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 significants in all terms of equations for *C. ocreatus*. Although regression equations describing the relation of parasite burden and larvae counts for the *D. filaria* had considerable high coefficient of determination, there was no significance for the variable term of equations. Regression analysis of *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 *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 *et al.*, 1989), 47.2% in İstanbul (Bagcı 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 *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 prevalance 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 Alemu *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 *et al.*, 2006).

Umur and Arslan (1998) stated that *C. ocreatus* and *D. filaria* were the most prevalent species in Turkey. In this study, *C. ocreatus* and *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 *et al.*, 2006; Gorski *et al.*, 2004; Rehbein et al., 1998, Tarazona, 1984).

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

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

Table III.- Correlation coefficient, and regression equation between faecal larvae excretion and the number of nematode in lungs.

Lungworm	Correlation coefficient	P value	Regression equation	Coefficient of determination
C. ocreatus	0.618	0.002	TLC=1.52 $^{(P < 0.005)}$ + 0.0188 LPG $^{(P< 0.005)}$	0.340
D. filaria	0.416	0.097	TLC=2.18 $^{(P < 0.005)}$ + 0.0522 LPG $^{(P= 0.097)}$	0.560
P. rufescens	0.372	0.190	TLC=0.634 $^{(P < 0.05)}$ + 0.0242 LPG $^{(P= 0.190)}$	0.274

TLC, Total Lungworm count; LPG, Larvae per gram of faeces.

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 youngers.

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 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, bbecause of reliable linear regression equation could not be established, the number of lungworms could not be deducted exactly from faecal larvae counts.

REFERENCES

ALEMU, S., LEYKUN, E.G., AYELET, G. AND ZELEKE, A., 2006. Study on small ruminant lungworms in northeastern Ethiopia. *Vet. Parasitol*., **142**: 330-335.

BAGCI, O. AND BIYIKOGLU, G., 2003. The prevalence of lungworms in sheep slaughtered in different slaughterhouses in Istanbul. *T. Parazitol. Derg*., **27**: 139-143.

BURGER, H.J., 1992. Helminthen. In: *Veterinärmedizinische Parasitologie*, 4. Auflage (eds. J. Eckert, E. Kutzer, M. Rommel, H.J. Bürger and W. Körting), pp. 174-319. Parey Buchverlag, Berlin.

DIEZ-BANOS, P., MORRONDO PELAYO, P., FEIJOO PENELA, A., CARILLO ONZALEZ, B. AND LOPEZ SANDEZ, C., 1994. Relationship between the excretion

of protostrongylid larvae in sheep in North-west Spain and climatic conditions. *J. Helminthol*., **68**: 197-201.

DIK, B., SEVINC, F. AND GUNEGI, H.B., 1993. Prevalence of lungworms in sheep slaughtered in Konya Meat and Fish Plant. *Turk Vet. Hek. Derg*., **5**: 39-42.

DOGANAY, A., BURGU, A. AND TOPARLAK, M., 1989. Sheep metastrongylose in Ankara region. *Etlik Vet. Mikrob. Derg*., **6**:99-113.

GARGILI, A., 1995. *The lungworms in the kivircik sheep breed in Thrace, Turkey*. Ph.D. thesis, Ist.Univ. Sag.Bil.Enst.

GETACHEW, T., 1989. *The pathogenicity of* Dictyocaulus filaria *in Ethiopian highland and Hampshire cross sheep*. Proceedings of a conference held at Bamenda/Kameroon, 18-15 january.

GORSKI, P., NIZNIKOWSKI, R., POPIELARCZYK, D., STRZELEC, E., 2004. Natural parasitic infections in various breeds of sheep in Poland. *Arch. Tierz. Dummerstorf.*, **47**: 50-55.

GURALP, N., 1981. *Helmintology*. Ankara Univ. Vet. Fak., 2nd Ed, Publ. No: 368, pp .599.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (MAFF), 1986. *Manual of veterinary parasitological laboratory techniques.* Reference Book 418. 3rd ed. Her Majesty's Stationery Office, London.

MINITAB INC, 2000. *Statistical Software*. Minitab Release, 13.20, State College, PA, USA.

ONCEL, T., 2000. The prevalence of helminth species in sheep in Southern Region of Marmara. *T. Parazitol. Derg*., **24**: 414-419.

PELLETIER, F., ANN PAGE, K.,´E OSTIGUY, T. AND BIANCHET, M.F., 2005. Faecal counts of lungworm larvae and reproductive effort in bighorn sheep, *Ovis canadensis*. *OIKOS*, **110**: 473- 480.

REHBEIN, S., VISSER, M. AND WINTER, R., 1998. Endoparasitic infections in sheep from the Swabian Alb. *Dtsch. Tierarztl. Wochanschr*., **105**: 419-424.

SISSAY, A., 1996. *Preliminary study on the prevalence of ovine lungworm infection in and around Bahir Dar*. DVM thesis. AAUFVM, Debre Zeit, Ethiopia.

SOULSBY, E.J.L., 1986. *Helminths, arthropods and protozoa of domesticated animals*. 7 th. Ed. Bailliere Tindall London. Pp. 262- 271.

TARAZONA, J.M., 1984. *Lungworm infections in goats and sheep in Spanish conditions*. Les Maladies de la Chevre. Colloque Intern. Niort. France, 9-11 October, pp. 353-355.

TASAN, E., KOROGLU, E. AND ALTAS, M.G., 1997. Prevalence of lungworms in Elazig province, Turkey. *F.U. Saglik. Bil. Derg*., **11**: 273-276.

THOMSON, E.F. AND ORITA, G., 1988. Seasonal prevalence of protostrongylid and Dictyocaulus species of lungworms in Awassi sheep in North-west Syria. *Trop. Anim. Hlth. Prod*., **20**: 187–189.

UMUR, S., KOROGLU, E., GUCLU, F. AND TINAR, R., 2006. Nematoda. In: *Helmintology* (ed. R. Tinar), pp. 214-449. Nobel Yayin Dagitim, Ankara, pp. 214-449.

UMUR, S. AND ARSLAN, M.O., 1998. The lungworms of cattle and sheep in Kars region, Turkey. *T. Parazitol. Derg*., **22**: 88-92.

VAN WYK, J.A, CABARET, J. AND MICHAEL, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. *Vet. Parasitol*., **119**: 277-306.

YILDIZ, K., 2006. Prevalence of lungworm infection in sheep and cattle in the Kırıkkale province. *T. Parazitol. Derg*., **30**: 190-193.

ZAJAC, A.M., 1994. Faecal examination in the diagnosis of parasitism. In: *Veterinary clinical parasitology* (eds. M.W. Sloss, R.L. Kemp and A.M. Zajac), pp. 3-93. Iowa States University Press, Ames.

(Received 11 December 2007, revised 22 May 2008)