



ISSN: 2456-2912
VET 2016; 1(2): 47-52
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www.veterinarypaper.com
Received: 11-07-2016
Accepted: 12-08-2016

Shumye Fentahun
Faculty of Veterinary Medicine,
University of Gondar, Ethiopia

Rahmeto Abebe
Department of Clinical Studies,
Faculty of Veterinary Medicine

Samrawit Melkamu
College of Veterinary Medicine,
Samara University, Russia

Mulat Asrat
School of Veterinary Medicine,
Wollo University, Ethiopia

Study on lungworm infection in small ruminants: Prevalence and risk factors in and around Gondar Town, Northwest, Ethiopia

Shumye Fentahun, Rahmeto Abebe, Samrawit Melkamu and Mulat Asrat

Abstract

A cross-sectional study was conducted from November 2011 to April 2012 to estimate the prevalence of lung worm infection in small ruminants, to identify lung worm species and the potential risk factors in and around Gondar town, North West Ethiopia. Fecal and post mortem examination were conducted in 601 (112 goats and 489 sheep) and 73 (15 goats and 58 sheep), respectively. The overall prevalence of 43.76% and 46.57% was found by fecal and post mortem examination respectively. There was a perfect agreement between fecal and post mortem examination ($Kappa=0.82$). When the prevalence was calculated by considering different risk factors, the lung worm prevalence was significant ($p<0.05$) between sex with the high prevalence of female (46.77%) than males (34.87%) and different peasant association, but it was not significant ($p>0.05$) between species of animals, among different age groups, body condition score, month, management system and housing condition of the animal. The prevalence of lung worm was high in *Dictyocaulus filaria* among the three lung worm species and *Protostrongylus rufescens* has the lowest one, there was a significant difference ($p<0.05$) among them. In conclusion, this study indicated that lung worm has a high prevalence in the study area which implicate the need of control and prevention intervention.

Keywords: lung worm; prevalence; small ruminants; Gondar

Introduction

Ethiopia has numerous livestock resource with a total contribution of 15% Gross domestic product and 33% of the agricultural output. Current estimate of shows that there are 41.5 million heads of cattle, 28.2 million of sheep and goats, 5.8 million of equine species, a million camels and over 42 million of poultry (DACA, 2006) [9]. sheep and goats provide as much as 30% the meat and milk consumed in sub-Saharan Africa and are found on small holding through the continent (ILCA, 1990) [18].

Health livestock represents one of mans most valuable renewable resource. They provide high quality edible protein, fiber of all types, leaser and arrange of useful by products, as well as providing an important ways of generating and storing wealth. Supplying motive power and fuel in developing countries. However, the demands on such live stock resource are extreme. Not only do live stock production system Have to cope with the demands of the ever increasing human population, super imposed on this is an even greater and legitimate requirement for them to contribute to improving the level of human nutrition by making live stock products more freely and cheaply available. This needs accomplished in situations were the land available to raise live stock being progressively reduced by the greater imperatives and priorities associated with housing and cropping (Waller, 1997) [35].

Helminthes parasites of ruminants are ubiquitous in many tropical and sub-tropical environments of the world providing nearly perfect condition for their survival and development. Among them lung worm which inhabit the lower respiratory system impose significant economic impact (Hansen and perry, 1996) [14]. Dictyocaulidae and /or certain Metastrongylidae are kwon to exist in East Africa (Ethiopia, Kenya and Tanzania) and South Africa (Shah-fischer and Say, 1989) [31]. Parasitic or Verminous pneumonia of sheep and goats most commonly are caused by infection with *Dictyocaulus filaria*, *Muellerius capillaris*, or *Protostrongylus rufescens*. In contrast to the acute viral and bacterial pneumonias, which result in bronchopneumonia affecting the anterior ventral portion of the lung (Kahn *et al.*, 2005) [20].

Correspondence
Shumye Fentahun
Faculty of Veterinary Medicine,
University of Gondar, Ethiopia

Dictyocaulus filaria is the most important lung worm of sheep and is commonly associated with chronic syndrome of coughing and unthriftiness with usually affects lambs and kids (Urquhart *et al.*, 1996) [38]. *Muellerius capillaris* is a common lung worm of sheep and goats but infestation rarely leads clinical diseases (Jones *et al.*, 1996) [19]. According to Pugh, 2002 [26]; *D. filaria* is the most pathogen. *M. capillaris* is the most common and the least pathogenic, and *P. rufescens* is intermediate in pathogenicity. Infestation with *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens* are all encountered in lambs 4-6 months of age are most severely affected but sheep of all ages are susceptible to lung worm infestation (Blood *et al.*, 1983) [6].

Dictyocaulus filaria have direct life cycle where as *Protostrongylus* and *Muellerius* have in direct life cycles and rely on variety of snails and slugs to serve as intermediate hosts (Kahn *et al.*, 2005) [20]. The life cycle of helminthes parasite of lungs are completed through the passage of their eggs or larvae up on the air way to the pharyngeal region, from which they are swallowed and passed to the out side environment in the faeces. Because of their dependence on fecal transmission, parasitism of lungs and air ways is often diagnosed by microscopic examination of faeces. The baermann technique can used to recover the larvae of lung worm from faeces (Hendrix, 1998) [15]. Pasture provide the link between the free living and parasitic phase of nematode parasite of grazing livestock. At different level of growth, pastures species may facilitate, or impede survival of free living population, the establishment of parasite burden and lessen or intensify the effect parasites in livestock (Waller, 1997) [35].

There for the objective of the study are (1).To estimate the prevalence of lung worms (2).To identify the potential risk factors(3).To identify the species of lung worms (*D. filaria*, *M. capillaris* and *P. rufescens*).

Materials and Method

Study area

The study was conducted in and around Gondar town, which is the center of north Gondar zone in Amhara regional state. The town is located at the North-west part of Ethiopia at a distance of about of 748 km from the capital of Ethiopia. The average altitude of the area is 1967m above sea level and the latitude and longitude of the area is 12.4°N and 27.3°E, respectively. The topography of Gondar is generally marked by the presence of numerous mountains, plateaus, hilly and sloppy area and rivers and the main topographic category of the area is Weina degas (mid land). The average rain fall of the area is estimated about 1000 mm and the short rains occur during March, April, and May, while the long rainy season extends from June to September. The average maximum and minimum daily temperature is 22-30.7 °C and 12.3-17 °C, respectively (NGZ ARDO, 2009) [24].

Study animal

The study animals were randomly selected small ruminants (sheep and goats) in the study area, which are kept under the traditional husbandry system. Both sexes and all age groups were included during sampling. Generally the proportion of males to females was small in any flock and the number of goats is small in the area. These animals originated from peasant association in and around Gondar town.

Study Design and Examination Method

A cross sectional study was carried to determine the prevalence of lung worm infection in small ruminants, to

identify lung worm species and the potential risk factors in and around Gondar town from October, 2011 to April, 2012. The study was conducted based on Fecal and post mortem examination. Species of animals, age groups, body condition score, month, management system and housing condition of the animal were considered as potential risk factors

Sample size and sampling procedure

The sampling size for the study was determined by using simple random sampling technique (Thrusfield, 2005) [35]. During calculation of the sample size, an expected prevalence of 37% was taken based information from a previous study at Dessie and Kombolcha (Regassa *et al.*, 2009) [29]. The following formula was used to calculate the sample size:

$$n = \frac{1.96^2(p(1-p))}{d^2}$$

Where,

n= sample size

p= Expected prevalence

d= Desired level of precision (5%)

$$\text{Therefore, } n = \frac{1.96^2(0.37(1-0.37))}{(0.05)^2} = 358$$

The sample size calculated was 358 but in order to increase the accuracy of the study the number of the animals was increased to 601 (112 goats and 489 sheep).

Study methodology

Coprolological examination: Fecal samples were taken directly from the rectum of randomly selected animals using disposable glove. Then the samples were placed in labeled plastic sampling bottles separately and taken to the laboratory in cool box. In the laboratory, the fecal sample was screened for the presence of lung worm larvae (L_1) by using the modified Baermann technique (Kassi, 1999; Zajac and Comboy, 2006) [21, 39]. The procedure was as follows: 10gram of feces was placed in a piece of double-layer cheese cloth, which was gathered around the sample so that it was fully enclosed. Use a rubber band to fasten the cloth, passé through the rubber band by two applicator sticks, which rest the edge of glass suspended the sample. Dip the feces with nylon in to conical glass filled with Luke warm water. Allow the feces to stand at 3-4 hours and discard the feces with nylon collect the material at the bottom of hollow stem Petri dish, examine with 10x objective lens and then transfer to microscopic slide to identify the species by using pipette. The species of lung worm was identified based on the morphological features given for each species (Soulsby, 1982; Taylor *et al.*, 2007) [33, 34]. When collecting faecal samples, information regarding the species of the animals, sex, age, body condition, feeding system, housing condition, and date of the sampling and origin of the animal were properly recorded on the provided format.

Postmortem examination: In addition to coprolological examination, postmortem examination was carried out in a total of 73 animals (15 goats and 58 sheep) slaughtered at Restaurants to investigate the presence of adult lung worms. In the mean time the age and species of the slaughtered animals were recorded. Procedures: lungs from slaughtered animals were palpated for protostrongylidae nodules. If the

nodules were present the area was trimmed of and worms extracted from the tissue by gently compressing small non calcified nodules or a part of large nodules between two glass slides and carefully teasing the worm away from the tissue using thumb forceps. Air passage was opened starting from the trachea down to the small bronchi with fine blunt pointed scissors to detect the presence of adult Dictyocaulidae (Kassai, 1999) [21].

The sensitivity and specificity of the modified Baermann technique

In this study a trial was made to evaluate the ability of the modified Baerman technique to detect lungworm infected and non infected animals correctly in relation to the gold standard postmortem examination method. For this the same 73 sheep and goats were subjected to both coprological and postmortem examinations. The results of the two tests were placed in a two by two contingency table and then comparisons were made. The sensitivity of the modified Baerman technique was calculated as the proportion of the number of animals tested positive by both tests divided by the total number of animals in which lungworms were detected in postmortem examination (a/a+c). Specificity was calculated as the proportion of the number of animals tested negative by both examinations divided by the total number of animals in which lungworms were not detected in postmortem examination (d/b+d) (Thrusfield, 2005) [35].

Data analysis

The data collected from the study animals during both coprological and post mortem examinations were recorded on specially designed formats. The data were then coded and entered to computer Microsoft excel spreadsheet. All the statistical analyses were performed using STATA version 9 software. The prevalence was computed as the number of animals affected by the lungworms divided by the total number of animals tested multiplied by 100%. The association of the prevalence of lung worm infection with potential risk factors such as species, age, sex, BCS, month of sampling, feeding system, housing condition and PA was analyzed by using chi-square (X²) test.

Results

Coprological examination

Out of the total 601 small ruminants (489 sheep and 112 goats) examined by modified Baermann technique, 263

(43.79%) animals were found to be infected with lung worms. The prevalence of lungworm infection was not significantly (P>0.05) different between goats (44.64%) and sheep (43.55%) (Table 1).

Table 1: The prevalence of lung worm infection in sheep and goats

Species	No Animals examined	No positive	Prevalence (%)	X ²	P-value
Goats	112	50	44.64		
Sheep	489	213	43.55	0.0436	0.835
Total	601	263	43.76		

The results of the analysis of the prevalence of lung worm infection with the sex, age and BCS of sheep and goats are presented in Table 2. Accordingly, the infection was found to be significantly (P<0.05) associated with the sex of the animals. The prevalence was higher in females (46.8%) than males (34.9%). Age and BCS were not significantly associated with the prevalence of lung worm infection (P>0.05 in both cases).

Table 2: Prevalence of lung worm infection in small ruminants based on sex, age and BCS

Factor	No. animals examined	No. animals positive	Prevalence (%)	X ²	P
Sex					
Male	152	53	34.9		
Female	449	213	46.8	6.54	0.011
Age					
<1 yr	152	63	41.4		
1-3 yrs	324	148	45.7		
>3 yrs	125	52	41.6	1.05	0.591
BCS					
Very thin	27	10	37.0		
Thin	79	39	49.4		
Moderate	346	150	43.4		
Fat	149	64	43.0	1.57	0.667

This study showed a significant (P = 0.001) variation in the prevalence of lungworm infection between the different PAs from which animals were sampled. The highest prevalence was observed in Debarka (56.75%) followed by Weleka (51.75%) and the lowest was observed at Tseda PA (30.30%). However, the occurrence of lung worm infection was not significantly (P>0.05) affected by the month of sampling (Table 3).

Table 3: Prevalence of lung worm infection in small ruminants displayed based on PA and month of study

Factor	No Animals examined	No. animals Positive	Prevalence (%)	X ²	P-value
PA					
• Debarka	111	63	56.76		
• Fersbet	166	64	38.55		
• G.town	111	47	42.34		
• Tseda	99	30	30.30		
• Weleka	114	59	51.75	19.78	0.001
Month					
• November	81	39	48.14		
• December	168	84	50		
• January	137	57	41.61		
• February	117	52	44.44		
• March	98	31	31.63	9.4288	0.051

A test conducted to see the association between the prevalence of lung worm infection and the feeding and housing condition of the animals showed no significant

association with both factors (P>0.05 for each factor) (Table 4).

Table 4: The prevalence of lung worm infection in small ruminants displayed based on feeding and housing systems

Factor	No. animals examined	No. positive	Prevalence (%)	X ²	P
Feeding system					
• Free ranging	566	243	42.93		
• Free ranging with supplementary feed	35	20	57.14	2.70	0.100
Housing condition					
• In door	103	54	52.43		
• Out door	498	209	41.97	3.79	0.051

Postmortem examination

Postmortem examination of lungworms was performed on 73 small ruminants slaughtered at restaurants of which 34(46.57%) animals were found to be infected with different species of lung worms. As in coprological examination, there was no significant ($P>0.05$) difference in prevalence between sheep and goats, although it tends to be higher in goats (Table 5).

Table 5: Prevalence of lung worm in sheep and goats based on postmortem examination

Species	No Animals examined	No positive	Prevalence (%)	X ²	P-value
Goats	15	8	53.33		
Sheep	58	26	44.83	0.346	0.556
Total	73	34	46.57		

Unlike the coprological examination, there was a significant ($P<0.05$) association between prevalence of lung worm and age of the animals. The prevalence was considerably higher in young animals <1yr (75%) than adult animals (38.6%) (Table 6).

Table 6: Prevalence of lung worms in sheep and goats based on age in postmortem examination

Age	No Animals examined	No animals positive	Prevalence (%)	X ²	p-value
<1year	16	12	75		
1-3year	57	22	38.6	6.65	0.01

Species identification

In coprological examination, three species of lung worms' namely *D. filaria*, *M. capillaries* and *P. rufescens* were identified in both sheep and goats. In sheep, *D. filaria* was the most prevalent (52.1%) while *P. rufescens* was the least (14.6%). However in goats, *M. capillaries* was the dominant species (56%) and *P. rufescens* was the least (2%). There was a significant variation in the proportions of *M. capillaries* and *P. rufescens* between sheep and goats but no significant difference was observed for *D. filaria* (Table 7).

Table 7: Species of lung worm identified in sheep and goats in coprological examination

Species of lung worm	Proportion (%)			Test for differences between sheep and goats	
	Overall	Sheep	Goats	Z	P-value
<i>D. filarial</i>	50.2	52.1	42.0	1.13	0.259
<i>M. capillaries</i>	37.6	33.3	56.0	2.82	0.005
<i>P. rufescens</i>	12.2	14.6	2.0	2.21	0.027

In postmortem examination the same three species of lungworms were identified as in coprological examination. Similar to coprological examination, *D. filaria* and *M. capillaris* were the dominant species observed in sheep and

goats, respectively while *P. rufescens* was the least prevalent in both species of animals (Table7).

Table 8: Lung worm species identified in sheep and goats in postmortem examination

Species of lung worm	Proportion (%)			Test for differences between sheep and goats	
	Overall	Sheep	Goats	Z	P
<i>D. filaria</i>	44.1	46.2	37.3	0.004	0.97
<i>M. capillaris</i>	41.2	38.5	50	0.17	0.867
<i>P. rufescens</i>	14.7	15.4	12.5	0.37	0.713

Evaluation of the sensitivity and specificity of the modified Baermann technique

In this study a trial was made to evaluate the sensitivity and specificity of the modified Baerman technique to detect the larvae (L1) of lungworms in the faeces in relation to the gold standard postmortem examination. According based on the given formula, the sensitivity and specificity of the modified Baermann technique computed as 79% and 100% respectively (Table 8)

Table 9: Evaluation for test agreement between coprological and post mortem examinations

Fecal examination	Postmortem examination		Total
	Lung worm (+ve)	Lung worm(-ve)	
Larvae (+ve)	27	0	27
Larvae (-ve)	7	39	46
Total	34	39	73

Sensitivity = $a/a+c = 27/34 = 0.79 = 79\%$

Specificity = $d/b+d = 39/39 = 1 = 100\%$

Discussion

The present study revealed an over all prevalence of 43.76% lungworm infection in small ruminants in and around Gondar town by coprological examination. This finding is relatively lower than the prevalence reported by some previous studies: 53.6% in six districts of Wello (Alemu *et al.*, 2006) [2], 49% in and around Debre Birhan (Habtamu, 2010) [13] and 58% in Assela (Wondowoson, 1992) [36]. However it is higher than the 36.7% report from Dessie and Kombolcha districts (Regassa *et al.*, 2009) [29] and 33.83% from Gondar town (Addis *et al.*, 2011). The reason for such variation may be the different agro ecological conditions of the country, animal husbandry system and the competence of the investigator to detect the larvae of the parasite in the faeces. The observed prevalence is slightly lower than the prevalence obtained from postmortem examination (46.57%); however the sample size used for the latter was very small.

In both coprological and postmortem examinations, there was no significant variation ($P>0.05$) in the prevalence of lungworm infection between goats and sheep, although it tended to be higher in goats. When the feeding behavior of the two species is considered, naturally goats are browsers and

thus less exposed to helminthes than sheep, which are used to grazing close to the ground and consequently more exposed to the infective larvae on pasture. However due to deterioration of browsing plants associated with expansion of agricultural activities and deforestation, nowadays it is common to see goats grazing on communal pastures along with sheep and therefore both species are equally exposed to lungworm infection. The present finding is contrary to previous studies which reported a significantly higher prevalence in goats than sheep (Alemu *et al.*, 2006; Addis *et al.*, 2011)^[2] or in sheep than goats (Regassa *et al.*, 2010).

In the present study three species of lungworms were identified in both sheep and goats in both coprological and postmortem examinations; however with different proportions. *D. filaria* and *M. capillaries* were the most prevalent species identified in sheep and goats, respectively while *P. rufescens* was the least dominant in both species of animals. This finding is inconsistent with previous studied (Alemu *et al.*, 2006; Regassa *et al.*, 2010)^[2] where *M. capillaries* was reported to be the most dominant species in both sheep and goats. However it is quite in agreement with the result of Addis *et al.* (2010)^[1]. The marked difference in the proportion between *D. filaria* and the other two species (*M. capillaris* and *P. rufescens*) is associated with the differences in life cycle of these lung worms. *D. filaria*, has a direct life cycle and also takes less time to reach the infective stage and after ingestion, the larvae can appear in the feces in a few weeks (Soulsby, 1982)^[33]. Compared with *D. filaria*, the transmission of *P. rufescens* and *M. capillaris* is epidemiologically complex vent involving the animal host, parasite and intermediate host. Further more, the development of 1st stage to infective stage larvae in the snail takes 12 to 14 days and the prepatent period in the final host reaches 30 to 40 days. The probabilities of infection, transmission and re-infection would therefore be much lower compared with *D. filaria* (Urquhart *et al.*, 1996)^[38].

Similar to a previous study (Alemu *et al.*, 2006)^[2], the present study indicated that females animals were significantly ($p < 0.05$) more susceptible to lung worm infection than males. However, the study carried out by Regassa *et al.*, (2010) showed no significant variation. There are two possible explanations for the observation of higher prevalence in females than males. The first is that the resistance of infection in females might have been abrogated at time of parturition and early lactation. This periparturient relaxation of resistance in females results in inability to expel adult worms (Craig, 1998). The other possible reason could be the dominance of the numbers of females over males. The total number of female and male animals sampled in the current study was 449 (74.7%) and 152 (25.3%), respectively. The number of females is almost three times that of males. This big variation in sample size between females and males might have caused such difference in prevalence. Therefore, further study is required to verify this fact.

Age of the animals was not significantly ($P > 0.05$) associated with the prevalence of lung worm infection. Almost an equal level of infection was observed between young (41.4%) and older (41.6%) animals and the prevalence in the adult age group (45.7%) was slightly higher than that of the other age groups. This finding is inconsistent with that of previous studies (Alemu *et al.*, 2006; Regassa *et al.*, 2010)^[2] where the infection tended to increase with increasing of age. Contrary to all these reports, Addis *et al.* (2010)^[1] have reported a decrease in prevalence with increasing of age.

In contrast to some studies which reported significant association of lungworm infection with poor BCS (Sisay,

2008)^[32], the prevalence of lung worm infection in the present study was not significantly ($P > 0.05$) associated with the BCS of the animals. Out of the total 601 animals examined only 106 (17.6%) scored thin to very thin BCS while the majority (82.4%) of animals sampled scored moderate to fat BCS. The small sample size of thin and very thin animals compared to the larger size of moderate and fat animals in the present study could be a possible reason for the absence of association. It was reported that animals with low body condition scores appear to be less compatible in getting ride-off lung worm infection than animals that have high body condition score (Kimberling, 1988)^[22].

In this study the prevalence of lung worm infection was significantly ($P = 0.001$) associated with the peasant association from which samples were taken. Such variation may be the result of altitudinal differences between the PAs, accessibility to anthelmintic medication and the level of awareness of the owner. The monthly dynamics of lung worm infection during the study period showed that the prevalence was relatively higher in November and December, then declined from January through March; however, the monthly variation was not significant ($P > 0.050$). This finding is inconsistent with that of others (Alemu *et al.*, 2006; Regassa *et al.*, 2010)^[2] who reported a significant monthly variation with a sharp decline from November to March associated with the progress of the dry period.

The test made to evaluate the diagnostic capability of the modified Baermann technique has revealed that the sensitivity and specificity of the method to be 79% and 100%, respectively. This shows that when the Barman technique is applied to test sheep and goats for lungworm infection it misses only 21 infected animals out of 100 but it never misses a non infected animal.

Conclusion and Recommendation

The current study showed 43.76% and 46.57% of prevalence of lung worm infection in small ruminants in coprological and postmortem examination, respectively. All the three important lung worm species: *D. filaria*, *M. capillaris* and *P. rufescens* were identified in both sheep and goats. The observation of such a level infection in a season known to be dry in Ethiopia suggests the existence of suitable ecological conditions in the study area for the survival and perpetuation of the parasite throughout the year. Furthermore, the observed high prevalence coupled with the presence of all the important lungworm species reminds that the parasite could be a threat for small ruminant production in the area.

This study has signified that sex (being female) of the animals and peasant associations are the two important risk factors associated with the occurrence of lungworm infection in small ruminants in the study area. However, the association of infection with sex requires further verification as the number of male animals involved in the study is very small compared to females. Furthermore, the absence of association between lungworm infection and species of animal, age, body condition and month of the year should be confirmed through a further research.

Based on the above facts the following recommendations are forwarded

- Two strategic treatments using broad spectrum or specific anthelmintics, one at the beginning of dry season and the other at the end of long rainy season, should be applied to reduce the high level of infection in the area,

- Grazing of small ruminants in wet areas should be avoided, that is a favorable habitat for the development of the lung worm's larvae,
- The snail and slug intermediates host creep up on plants in early morning and evening there fore, animals should not be allowed to graze in such times.
- It is quite difficult to establish the seasonality of the infection on the basis of the facts and figures presented by this study. There fore, an additional investigation is required on the prevalence and intensity of this parasite during the rest of the years.

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