

Study on the Prevalence and Pathological Features of Lung Worm of Sheep Inbahir Dar, Ethiopia

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Abstract: A cross-sectional study was conducted on ovine lungworms from November, 2010 to April, 2011 slaughtered in the municipal abattoir and private Hotels in Bahir Dar. The objectives of the study were to appreciate lesions, to determine the prevalence of lungworms infection, to identify the species of lungworms and to describe risk factors. Breeds of sheep under study were Farta and Washera. Gross lesions were determined by postmortem examinations and microscopic lesions were appreciated after hematoxylin eosin stain. The prevalence of lungworms was determined again using postmortem examination of adult parasites found in the lungs. The species of lungworms were identified after microscopic examination. Grossly, the most important lesions were foamy and bloody stained exudates mixed with adult parasites, multifocal patches, firm consistency of lung were detected. Histopathologically, diffused broncho and interstitial pneumonia, alveolitis and desquamation have frequently detected. Eosinophilic inflammatory cell infiltrations around trapped adult parasites, larvae and eggs were also common. Species of lung worms identified were *Dictyocaulus filaria* (49.21%), *Muellerius capillaris* (2.34%) and *Protostrongylus rufescens* (1.56%). The overall prevalence was found to be 57.55% (221/384). In all age groups, *D. filaria* had the highest prevalence than the other lungworms. The prevalence in Washera and Farta breeds' was 62.5% and 57.22% respectively. In conclusion ovine lungworms were prevalent in sheep originated from Adet, Fogera, Estie, Bahir Dar and Merawi and caused significant damage on the lung and produces characteristic gross and microscopic lesions.

Key words: Bahir Dar • Histopathology • Ovine Lungworm • Risk Factor • Sheep

INTRODUCTION

In Ethiopia, there are eighteen indigenous breeds of sheep. The diverse groups of sheep populations in the central and western highlands (>2000 meters above sea level) include Menz, Legagora, Tukur (also called Lasta), Arsi-Bale and Dangala (also called Washera, Agew) fat tailed. These are collectively referred as Abyssinian sheep or Ethiopian Highland sheep. Another category is the thin-tailed Horro and Farta sheep in the humid highlands (1400–2000 meters above sea level [1].

In spite of huge sheep population, Ethiopia has benefited little due to wide spread diseases, under developed infrastructures coupled with poor genetic performance, inadequate nutrition, management, poor husbandry and shortage of trained man power [2, 3]. Respiratory diseases are great economic concern for

sheep producers in the central highlands of Ethiopia. Example in North Showa, outbreaks of respiratory diseases occur frequently which has been killing significant numbers of sheep. Potential respiratory diseases of sheep include lungworms, pasteurellosis and Maedi-Visna [4].

The diseases of respiratory system are the leading cause of morbidity and mortality in sheep and thus major sources of economic losses [5], among these lungworms are the important factors. These include *Dictyocaulus filaria* (*D. filaria*), *Muellerius capillaris* (*M. capillaris*) and (*Protostrongylus rufescens*). Although mixed infections can occur; *D. filaria* predominates in most outbreaks of diseases [6, 7]. The signs of lungworm infection (verminous pneumonia), range from moderate coughing with slightly increased respiratory rates to severe persistent coughing [6]. Unthriftiness, dyspnea, nasal

discharge, weight loss, in case of associated bronchopneumonia, also fever and death [6, 8, 9] are important clinical signs. Infection of ovine verminous pneumonia is often recognized at slaughter house meat inspection by gross pulmonary lesions. *In vivo* diagnosis by isolation of L₁ larvae from fresh faeces through baermanization [8].

Several studies have been conducted to know the prevalence and economic significance of ovine lungworm infections in Ethiopia and indicated varied infection prevalence. Among these, Alemu *et al.* [10] in northeast Ethiopia 24.4% and in western Ethiopia 53.5%, Teffera [11] in Kombolcha 15%, Netsanet [12] in Debre Birhan 73.25%, Mekonen *et al.* [13] in Gondar town 37.74%. In Bahir Dar, different researches were under taken in related to ovine lungworm infection these were by Sisay [14] and Muluken [15] 13% and 18.5% respectively. But there was no study carried out on gross and histopathological lesions of ovine lungworm infection together with identification of their species. Therefore, the objectives of this study were; to determine pathological and histopathological lesions due to lungworm infections, to identify the species of the lungworms and to determine the present prevalence of lungworms infection and associated risk factors.

MATERIALS AND METHODS

Study Areas: The study was carried out from November, 2010 to April, 2011 in Bahir Dar municipal abattoir and Bahir Dar Hotels. Animals were brought mainly from Estie, Adet, Merawi, in and around Bahir Dar and Fogera.

Study Animals: Study Population consisted of apparently healthy slaughtered sheep at Bahir Dar municipal abattoir and Bahir Dar private hotels (Azwa, Blue Nile and Papyrus) which were selected using lottery method (randomly). Sheep slaughtered in Bahir Dar were originated from different parts of the Amhara National Regional state, mainly from Estie, Fogera, Adet, Merawi and Bahir Dar area.

Study Design: Across sectional study was under taken to collect sample from Bahir Dar municipal abattoir and private hotels. A total of 54 samples were collected in a week from the municipal abattoir and hotels. Information like origin, breed, sex, body condition score and date of slaughtered, clinical manifestations were recorded through surveys. Ear tags were given and history was recorded accordingly.

After slaughtering the lungs were collected aseptically from each ovine with the same code given at ear tag in to large plastic bucket and transported immediately to pathology department of the Bahir Dar Animal Health Investigation and Diagnostic Laboratory, for gross pathological lesion descriptions and identification of parasites. Samples for histopathology (3-4mm thickness) were kept in 10% neutral buffered formalin from apparently normal and abnormal parts [16, 17] and transported to university of Gondar, faculty of veterinary medicine, pathology and clinical pathology laboratory. Ages of sheep were estimated according to Getenby [18]. Body condition score of animals were made according to Girma [19]. Estimation of the number of lungworms per lung (infestation) after postmortem examination was made according to Chartier and Reche [20] and Kassai [8].

Sample Size Determination: The sample size was determined based on the expected prevalence rate of the disease in the study area which was 20.3% [21]. The formula applied to calculate the sample size for simple random sampling method and the study was considered 95% CI and 5% absolute precision [22].

Study Methodology: Ante mortem inspection of sheep was conducted before they were slaughtered by using standard inspection procedure [23]. The respiratory tract was examined in systematic manner according to McGavin and Zachary [5]. This was started from trachea through smooth tracheal muscle (bridges gap with in a ring [24].

Identification of Parasites: Lungworms collected with forceps and placed in 10% neutral buffered formalin and transported to the laboratory were identified using guidelines stated by Kassai [8] and Taylor *et al.* [25]. The abnormal microscopic findings were then recorded [22].

Data Management and Analysis: The data collected were entered and managed in MS Excel software programs. Then analyzed by using SPSS Statistical software version 17.00. Chi-square (χ^2) test was used to measure the association between the prevalence of the parasites and breeds of sheep, age, sex, origin and body condition score. In all analysis the confidence level was held at 95% CI and the desired absolute precision was set 5%. In this case P-value less than 0.05 were considered significant where as P-value greater than 0.05 considered non-significant.

RESULTS

Ante Mortem Examination: The ante mortem examination of 384 sheep revealed that 72.91% (280/384) had shown respiratory mild to severe clinical signs. These were watery and mucoid nasal discharges, pronounced coughing; labored respiration. Among 280 sheep which have shown clinical signs 150 were positive for medium and high degree of lungworm infestations. In addition to respiratory signs rough hair coat and emaciation were evident.

Prevalence of Ovine Lungworm Infection: Of the total of 384 apparently healthy slaughtered sheep at Bahir Dar municipal abattoir and Hotels the overall prevalence of ovine lungworm infection was found 57.55% (221/384). Breed wise, they were Washerabreeds 62.75% (15/24) which showed higher prevalence rate than Farta breeds 57.22% (206/360). There was no statistical significance between breeds of sheep and lungworm infection ($P > 0.05$) as shown in Table 1. Numerous larvated eggs examined during microscopic examination of feces (Figure 1e).

The influence of body condition for lungworm infection revealed that there was no statistical significant association among the three body condition scores ($\chi^2 = 3.324$, $P > 0.05$) which were 66.7% (2/3), 60.90% (148/243), 51.44% (71/138) in very thin, thin and moderate

body conditions respectively as it has been calculated from Table 1. An assessment for sex as a risk factor was also made and the accounted prevalence in females was 57.77% (130/225) and in males 57.23% (91/159). Almost similar prevalence rate was depicted in both sexes. And there was no any statistical significance difference between sex groups in causing ovine lungworm infection ($\chi^2 = 0.011$, $P > 0.05$) as shown in Table 1. The prevalence of ovine lungworm infection in less than one year of age category was 37.5% (3/8), one to three years of age 57.1% (205/359) and greater than three years age was 76.5% (13/17). However, there was no statistical significance association between ovine lungworm infection with the age groups ($\chi^2 = 3.387$, $P > 0.05$).

The occurrence of different species of ovine lungworms in different age groups of study animals revealed that *D. filaria* was found the most prevalent across the three age categories. These are 76.47% (13/17), 48.46% (174/359) and 25% (2/8) in greater than three, one to three and less than one year of age respectively. The detail explanation is indicated in Table 2 below.

In age group less than one year prevalence of *M. capillaris* and *P. rufescens* was observed 0% and mixed infection prevalence was relatively high at age one to three years 4.46% (16/359). There is no any statistical significance difference in ovine lungworm species of parasites and age categorized for study ($\chi^2 = 8.743$, $P > 0.05$). The higher level of prevalence of lung worm

Table 1: Prevalence of ovine lungworm infection in relation to breed, body condition and sex of sheep.

Parameters	Result		Total	Chi-square (χ^2)	P-Value
	Negative	Positive			
Breed					
Ferta	154	206	360	0.257	0.613
Washera	9	15	24		
Body condition score					
Very thin	1	2	3	3.324	0.190
Thin	95	148	243		
Moderate	67	71	138		
Sex					
Male	68	91	159	0.011	
Female	95	130	225		
Total			384		

Table 2: Variation in age group affects susceptibility of sheep to different species of lungworms

Age(years)	Species of lungworms identified					Total
	Negative	<i>D. filaria</i>	<i>M. capillaris</i>	<i>P. rufescens</i>	Mixed infection	
Less than one	5	2	0	0	1	8
One to three	154	174	9	6	16	359
Greater than three	4	13	0	0	0	17
Total	163	189	9	6	17	384

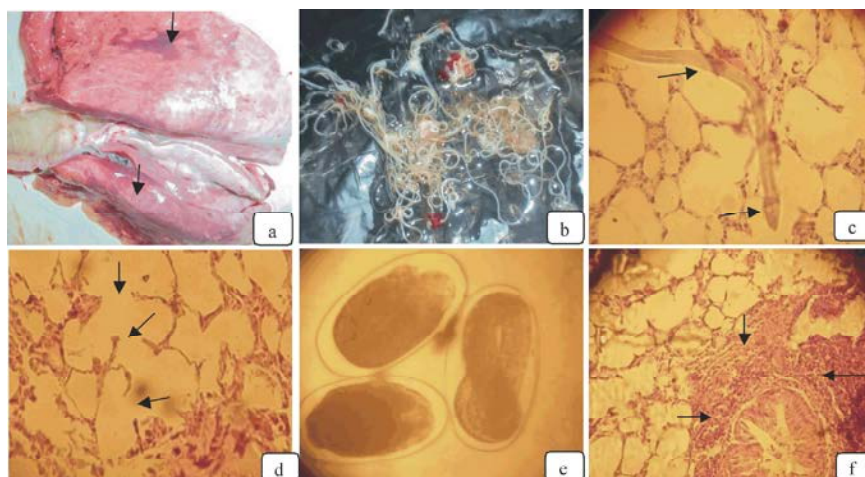


Fig. 1: Gross lung lesions of congestion due to blockage of blood vessels by numerous adult lungworms (a); adult lungworms collected during postmortem inspection of infected lungs (b); a during histopathological examinations of lungs, there was segments of lungworms in the sectioned tissues (c); Most of the infected alveoli were found large in size and with openings in to a common space (figure 1d); larva in the eggs of lungworms collected from fecal samples (e); Lumen of bronchi and bronchiole were occluded by mass of fibrous tissue surrounded by cuboidal epithelial cells and infiltrated with inflammatory cells (f).

infection was observed in sheep originated from Adet 75% (12/16), Estie 57.62% (189/328), Bahirzuria 57.14% (4/7), Fogera 52.14% (12/23) and Merawi 40% (4/10). However, there was no significance difference between lungworm infection and the geographical locations ($\chi^2 = 3.528$, $P > 0.05$).

Macroscopic and Microscopic Examinations: Gross lesions observed during postmortem examination were localized on caudal lobes of the lung and disseminated mainly within dorsal side. These were atelectasis, emphysema, multifocal patches, firm consistency and dark red to dark grey hepatization on dorsal diaphragmatic lobes, mucus and foamy exudates tinged with blood in the bronchi and bronchioles with few congested parts (Figure 1a). Long whitish colored worms were observed in moderate and heavy infestations (Figure 1b).

Histopathological examinations of all infected lungs revealed basically similar lesions consistent with the gross diagnosis of ovine verminous pneumonia; regardless of degree of infestation. Infected lungs revealed changes in the alveoli and alveolar septa, progressive specific lesions in the lung parenchyma and infiltrations of leukocytes in the interstitial part where parasites passed through, around adult worms, eggs and larvae of trapped lungworms (Figure 1c). Most of the infected alveoli were found large in size and with openings in to a common space (Figure 1d),

emphysematous lung, desquamated epithelial cells in the bronchi and the bronchioles, necrosis and diffused inflammatory cells. Thickening of bronchus and faint colored fluid inside the bronchi was also observed.

Hyperplastic epithelial cells surrounded by smooth muscle cells were also distinguished. Lungs infected with small lungworms (*M. capillaris* and *P. rufescens*, alveoli lumen have seen with larvae, eggs and desquamated epithelial cells. Inflammatory cell infiltration in bronchi and bronchioles (bronchopneumonia) and thickening of alveolar septa (interstitial pneumonia) was also evident. There were also hyperplastic smooth muscle cells devoid of epithelial cells and atelectatic alveolar septa. Sometimes the lumen of bronchi and bronchiole were occluded by mass of fibrous tissue surrounded by cuboidal epithelial cells and infiltrated with inflammatory cells (Figure 1f). Parasitic granulomas with calcified center infiltrated with eosinophils and neutrophils were common. Generally, from the histopathologic lesion we have seen that the severity of lesions was increased with degree of infestation and the most prominent inflammatory cell was the eosinophils.

DISCUSSION

In this study, we have identified three species of bronchopulmonary nematodes in sheep coming to Bahir Dar for slaughter with an overall infection rate of

Dictyocaulus filaria (49.21%), *Muellerius capilaris* (2.34%) and *Protostrongylus rufescens* (1.56%) by necropsy examinations. However; the overall lungworm infection responsible verminous pneumonia was 57.55%.

The overall prevalence of lungworm infection in this study (57.55%) was in agreement with report of Alemu *et al.* [10] in northeast Ethiopia 53.6%. However, our finding is higher from previous studies of Mekonen *et al.* [13] in and around Gondar town and Tewodros [21] in and around Bahir Dar, Mengestom [26] in Tigray (Atbsi) who reported 31.74%, 21.5%, 21.5% respectively. Higher prevalence figure was reported by Netsanet [12] in DebreBirhan which is 73.25%. The difference in the prevalence of the current study and with that of other studies in previous years might be due to the methodology used for determination of prevalence of lungworms infection and /or the difference in the geography of study areas which may favors the survival of the larvae of lungworms or intermediate host.

According to Kassai [8] postmortem examination diagnosis is very confirmatory and fecal examination technique through baermanization is less sensitive due fecal debris and other artifacts that the larvae could not be detected alone as easily as in the postmortem techniques. The other probable reason attributed for such difference could be the stage of parasites during fecal sampling. For example, in the prepatent and postpatent phases during hypobiosis it is impossible to detect the parasite larvae by fecal examination [27]. The third possible reason could be the sample size researchers used to determine prevalence.

Dictyocaulus filaria was the most prevalent in all age groups of sheep. This result agrees with previous report of Netsanet [12] around Debre Birhan and Uqubazai [28] in Hamassen Awraja but this disagree with report of Sisay [14] in Bahir Dar and Mezgebu [29] in Addis Ababa who reported *M. capillaris* was the most prevalent causes of verminous pneumonia. This could be associated with the different in life cycles. *Dictyocaulus filaria* has a direct life cycle and takes less time to reach infective stage and after ingestion adults would appear in the lung after five weeks [30]. Compared with *D. filaria*, transmission of *P. rufescens* and *M. capillaris* is epidemiologically complex event involving host, parasite and intermediate hosts. In addition, low prevalence of *P. rufescens* and *M. capillaris* in the area may be due the sampling season which is winter (dry in Ethiopia) which is not suitable to snails, intermediate host, of these species to which larvae develop to infective stage.

Breed, Age, sex, body condition score and origin of animals have been found to contribute in the variation of the prevalence rate of the current study. High level of prevalence was observed in Washerabreed also called Dangala (62.5%) compared to Fartabreeds (57.2%) of indigenous sheep. And no typical research was conducted on Washera breeds, but Menze breeds of sheep have similar characteristics with Washera as Habtamu [31] reported similar results. This different prevalence may be associated with the level of immunity; Fartabreeds are found adapted to feed shortage areas. In association to this, they graze deep and may pick up more parasites from the ground so as to acquire resistance than Washera breeds which adapted to good environment and graze at surface level. Thus they could consume less contaminated feed matter with larvae and may therefore have lowered acquired resistance and have higher prevalence than Farta breeds [32].

Adult sheep were found more affected to lung worms than the other age groups. The highest prevalence 76.5% was observed in sheep greater than three years of age followed by 57.1% and 37.5% prevalence in age groups one to three and less than one years of age respectively. This finding is however donot agree with Muluken [15] and Tigest [33] findings in North and South Gondar zones and Tewodros [21] in and around Bahir Dar. They have reported that as age increases susceptibility to lungworm decreases. This difference in prevalence of lungworm might be associated with methods in which researchers' grouped animals according to age as well as methods of determining age. But the increased prevalence in our study might be associated with the fact that with apparent ability of the host to develop acquired immunity. Therefore, adults could be carrier of infection and highest prevalence may be attributed to higher prevalence in adults.

Female and male animals appear similarly affected with lungworms even though very small difference applies 57.8% and 57.2% respectively. And this small difference exists between two sexes groups have been found statistically insignificant. This finding agrees with the result of Brook [3] who reported insignificance difference ($P > 0.05$) between male and female sheep. However, it contradicts with the result obtained by Sisay [14] who reported significance difference ($P < 0.05$) of lungworm infection between male and female sheep. This slight difference of prevalence may be associated from variation in sample size and the higher prevalence in female could be accredited to some of the physiological factors

example, preparturient relaxation of resistance which could enable them to expel the worm [34]. Additionally the way that male and female may be treated in terms of nutrition may also attribute for such difference. Males are kept for different purposes including fattening (to be sold) and breeding therefore males were given more attention by farmers and this condition could lessen the impact and prevalence of infection.

In the present study different levels of prevalence were observed in animals which have different body conditioned scores. Very thin body condition score showed highest prevalence (66.67%) this disagrees with the prevalence studied by Tewodros [21] in and around Bahir Dar, who reported on poor conditioned 33.3%. This difference in prevalence may be associated with the difference in determining the body condition score and the sample size. The increased prevalence in our study in very thin body condition could be from the fact that poor nourished animals would be less competent in getting ride off infection, but it is not unusual for well feed animal to surrender the disease provided at the right environmental conditions are made available [9].

The prevalence of ovine lungworm infection in different origins of sheep was also compared. The higher prevalence 75% was observed in sheep originated from Adet followed by Estie 57.77%. This was in agreement with the previous study at highland areas by Tigest [33] in Debarq and Habtamu [31] in DebreBirhan who reported high level of prevalence 56% and 47% respectively. This might be due to the fact that altitude is attributable to climatic parameters important for the survival and development of larvae (cool and humid environment) [35].

Pathological features of lung with ovine verminous pneumonia were studied in present study. So characteristic gross and histopathologic lesions associated with lungworms were described. Important gross lesions detected during postmortem examination include atelectasis, which have been seen frequently along with dorsal and caudal aspect of the lung. This coincides with McGavin and Zachary [5]. Microscopically, alveoli contained scarcely recognizable slits which all lying parallel; this result also correlate with Fgubb *et al.* [36]. Atelectasis of lung is associated with air way obstruction by parasites and exudates leading to pulmonary collapse [37] to which alveoli are the smallest most easily compressed parts of the lung. Dark red to dark grey colored lesions were detected which account more than 50% of observed lesions, which agrees with Christopher [38]. This lesion might be associated with

ischemia (from parasitic damage of alveolar capillaries, it might also associate with increased blood flow towards infected lung and inflammatory cells aggregation. Emphysematous lesions characterized grossly by very large, dark red colored and puffed by air on palpation was another lesion frequently seen. Microscopically, we had observed too large alveoli with many openings. This finding agreed with Mansfield *et al.* [39].

In macroscopic observation adult worms were found in the bronchi and bronchioles stained with little foamy to bloody stained exudates. Microscopically, adult lungworms, larvae and eggs were observed in sectioned tissues. Associated with them, there was eosinophilic bronchitis and bronchiolitis. The epithelium of the bronchi was thickened, hyperplastic and there were aggregations of inflammatory cells around the bronchi and bronchioles. Similar findings were described by Panuska [40]. These lesions might be associated with the fact that from parasitic infection mucus and cells became highly prominent and mucus producing epithelial cells increased in size and thus epithelial hyperplasia and thickening was found in response to lungworm infections [36].

CONCLUSION

Dictyocaulus filaria, *M. capilaris* and *P. rufescence* were important lungworms identified in the study. Of these, *D. filaria* had the highest prevalence among species identified regardless of age groups, sex, breed, body condition and origin of sheep. These species of lungworms were responsible for verminous bronchitis and pneumonia.

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