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Comparative Study on Lungworm Infection in Clinically Diagnosed Respiratory Signs and Apparently Healthy Sheep and Cattle in Tiyo District, Central Ethiopia

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Abstract: Cross sectional study with purposive sampling was conducted from November 2016 to May 2017 in Oromia regional state, Tiyo district. The objective of the study was to estimate proportion of lungworm infection in sheep and cattle with respiratory clinical signs and apparently healthy ones, and identify the species of the respiratory helminthes circulating in the area and assess possible risk factors of lungworm infection in sheep and cattle in the study area. About 216 fecal samples (102 cattle and 114 sheep) were collected from different age groups, sex and husbandry system after categorizing animals into two groups (those with clinically diagnosed respiratory signs and apparently healthy). Individual samples were processed in the laboratory by using modified Baerman technique and larvae were identified according to recommended procedure. On overall, out of 216 animals examined, about 90(53)(46.27%) sheep and 37(34.3%) cattle) were found infected with lungworm. From these infected animals 29 cattle (50.88%) and 41 sheep (69.49%) were with respiratory clinical signs. Cattle and sheep with clinically diagnosed respiratory signs were having 3.2 and 5.26 times lungworm infection than apparently healthy ones, respectively. The species of lungworm involved in cattle in all positive animals was D. Viviparous. The proportions Ovine lungworm infection by D. filaria, M. capillaris, P. rufescens and mixed infection were found to be 43.9%, 14.0%, 5.3% and 14.0%, respectively. Age, body condition and management system showed statistically significant association with lungworm infection (p < 0.05). Thus, lungworm infection is one of major causes of respiratory disease complex (RDC) in Tiyo district; requiring emphasis and further investigation to reduce its incidence and assess its impact on the economy.

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Key words: Lungworm, Risk factors, Tiyo

1. Introduction

In Ethiopia, agriculture is the mainstay of the country and also the major resource of employment and income. About 85% of the population live in the rural area, and are primarily engaged in agriculture and related activities. Thus, agriculture directly or indirectly forms an important component of the livelihood of more than 60 million people in the country [1].

Ethiopia is a leading African country in livestock population and ranks 9 in the world [2]. The livestock subsector accounts for about 40% of the agricultural Gross domestic product (GDP) and 20% of the total GDP [3] without considering the contribution of livestock in terms of draught power, manure and transport services. Excluding exports of live animals and other products, leathery and leather products alone contribute 18% of the total exports [4]. An estimate indicates, the country is a home for about 54 million cattle, 25.5 million sheep and 24.06 million goats. From the total cattle population, 98.95% are local breeds and the remaining are hybrid and exotic breeds [5]. However, the economic gains from these animals remain insignificant when it is compared to their huge number [2]. This low productivity is a reflection of disease, limited genetic potential and husbandry standard. The morbidity of animals is generally estimated to be in the range of 8-10% of national cattle herd per annum and 14-16% and 11-13% of national sheep and goat flock per annum respectively with average live weight loss of 70kg for cattle and 6kg for sheep and goat. The national value of this direct loss is estimated to be of 550 million Ethiopian birr [6].

Concurrent infection of respiratory tract by virus, bacteria and lungworm has been described and such disease conditions are commonly known as respiratory disease complex (RDC), indicating the difficulty to attribute to only one etiology [7, 8]. In the cool central highlands of Ethiopia, respiratory disease complex has been identified as leading, irregular and insufficient vaccination program for disease such as Pasteurellosis and Pest des Petites Ruminant (PPR). Lack of strategic mass drenching against lungworms and occurrence of viral infections may play sufficient roles in the presence of respiratory disease complex in Ethiopia [9].

Helminthes parasites of ruminants are ubiquitous, with many tropical and sub-tropical environments of the world providing nearly perfect conditions for their survival and development [10]. Lungworms are widely distributed throughout the world, but are particularly common in countries with temperate climates, and in the highlands of tropical and sub-tropical countries [11]. The species of importance in ruminants belong to two different families; the Dictyocaulidae and the Metastrongylidae. The Dictyocaulidae include Dictyocaulus viviparus in cattle and buffaloes, and Dictyocaulus filaria in sheep and goats. These worms are 5-10 cm long and live in the trachea and bronchi. The Metastrongylidae are represented by at least three species in small ruminants. Protostrongylus rufescens is a small worm (1.5-3.5 cm) found in the bronchioles, Muellerius capillaris (1.2-2.5 cm) which is located in

the alveoli, and Cystocaulus ocreatus (2-5 cm) is found in the terminal bronchioles. An infection of the lower respiratory tract by any of these nematodes of species may result in bronchitis or pneumonia or both [12]. Dictvocaulus, Mullerius and Protostrongvlus are the most important and widely distributed respiratory tract helminthes of sheep and goats. Among these parasites, Dictvocaulus filaria is mostly the cause of verminous pneumonia [6]. However, the incidence of parasitic diseases including respiratory helminthosis varies greatly from place to place depending on the relative importance of the factors [10]. Even if there are some studies regarding respiratory helminthosis of small ruminants in study area there are very few studies on Bovine lungworm infection and association of lungworm infection with respiratory clinical disease. Therefore, the objectives of this research include the following:

> To estimate the proportion of lungworm infection in sheep and cattle with clinically diagnosed respiratory signs and apparently healthy animals in Tiyo district.

 \succ To identify lungworm species those are involved in the study area, and associated risk factors.

Materials and Methods **2.1.** Study area



Figure 1: Map of the study area. Source: Quantum GIS software

The study was conducted from November 2016 to May 2017 in Oromia regional state in Arsi zone around Tivo district. Tivo woreda is located in Arsi zone situated at 6° 79' and 8°49'N latitude and 38°41' and $40^{\circ}44$ ` E longitude in central Ethiopia at a distance of 175 Km south east of the capital city Addis Ababa. It has an area of 2,118,675 hectares, of which 39.7%, 29.1% and 27.5% is highland, lowland and mid altitude, respectively. The altitude of the area is ranging between 500 (Awash and Wabe valley) and 4245 (mount Kaka) meters above sea level. The annual temperature varies between 10°c and 25°c. The average annual rainfall ranges between 901mm and 1200mm with some spatial and temporal variability in quantities and distribution. Its pattern is of bimodal type with 60% occurring long rainy season extending from June to September and the short rainy season from December to February. The other two seasons are the cool dry season extending from October to November and the major dry season from March to May [13].

2.2. Study animals

The study animals were sheep's and cattle's in Tiyo district of Oromia regional state maintained under different husbandry system such as traditional and small holder extensive management production system, intensive and semi-intensive system. Study animals were considered irrespective of age, sex, breed, body condition score, deworming history, husbandry system and health status.

2.2.1. Case definition

Apparently healthy cattle and sheep: are animals that have regular breathing pattern and normal both on inspiration and expiration, no nasal discharge and no other respiratory signs.

Clinically diseased cattle and sheep: are those animals that show respiratory signs such as an irregular breathing pattern and grunting on expiration, coughing, serous and mucoid nasal discharge.

2.3. Study design

Across sectional study with purposive sampling method was conducted from December 2016 to May 2017 to predict proportion of lungworm infection in clinically diseased and apparently healthy cattle and sheep. Faecal samples were collected from purposely selected 126 sheep and cattle under extensive, intensive and semi intensive husbandry systems to examine first stage larvae (L1) using modified Baermann technique.

2.4. Sample size determination

To determine sample size and estimate the difference between proportions [14] formula was used. Hence the expected proportion of cattle and sheep recovered with parasitic load in apparently healthy cattle and sheep (APHC, APHS) is set at 50% (P1) while for clinically diagnosed cattle and sheep (CDC,

CDS) expected proportion is estimated at 80% (P2) recovery. The power of study is set at 80%, 95% confidence interval and significance level of

P <0.05 was used.

i.e. N >
$$\frac{(Z_1 - \alpha/2 + Z_1 - \beta)^2 [P_1(1 - P_1) + (P_2(1 - P_2)]}{(P_1 - P_2)^2}$$

Where $Z_1 - \alpha = 50\%$ significance level

 Z_1 - $\beta = 80\%$ power level

 P_1 = Expected proportion in apparently healthy cattle and sheep

 P_2 = Expected proportion of clinically diagnosed cattle and sheep

N1> $\frac{7.89x [0.5(1-0.5) + 0.8(1-0.8)]}{(0.5-0.8)^2}$

 N_1 > 36, hence N_2 >36; the total sample required is at least 36 + 36 = 72 sheep and 72 cattle (144 animals in total). For the study total of 102 cattle and 114 sheep (both sides =216 animals) were included from four districts of study area.

2.5. Sampling strategy

During sampling each study animal was individually identified based on respiratory signs up on auscultation, history and visual observation. Those with respiratory signs were considered as clinically diseased and without respiratory signs were considered as apparently healthy before sampling procedure. During sample collection various potential risk factors including sex, age, husbandry system, deworming history and body condition where recorded.

2.6. Sample collection, processing and examination

Fecal samples were collected per-rectum from fresh deposits using plastic gloves in clean plastic bags, labeled and kept in icebox and transported to Parasitology laboratory of AAU College of veterinary and agriculture. After arrival, individual samples were processed by modified Baermann technique described by Glovirina [15] and Hansen et al., [10]. About 10 gram of fecal sample was weighed and wrapped with double layered gauze suspended in a beaker with luke warm water for 24 hours. After removing sample packet from beaker, few milliliter of fluid from bottom of beaker was filled in test tube, and centrifuged at 1000rpm for 2 minutes after which the sediment was checked in the petridish for the presence of larvae. For further species identification, a drop of fluid from petridish was transferred to a microscope slide, on which a drop of iodine was added to fix larvae. Then cover slip was gently placed over the drop and examined under microscope with magnification power of 10X. All larvae were identified morphologically as described by previous workers (16, 17).

2.7. Data analysis

The data collected during sampling and laboratory findings were entered and stored in MS-excel and the statistical software STATA version 13

(18) was used to analyze the data. Descriptive statistics was used to determine the percentage and frequency lungworm infection by dividing the total number of positive animals to the total number of examined animals during study time. Chi square statistics and odds ratio (OR) were used to compare the association between variables. In order to consider a result to be statistically significant, 95% CI and P-value< 0.05 were taken.

3. Results

3.1. Lungworm infection in cattle

Out of total 102 cattle examined 36.27% (37/102) were positive for lungworm infection. From these positive animals about 29 (78.37%) were with respiratory clinical signs while other 8 (21.62%) where apparently healthy (Table 1). The result revealed that animals with respiratory clinical signs are 3.2 times more likely to have lungworm infection than apparently healthy ones. The lungworm species involved in cattle was *Dictyocaulus filaria* (figure 3.D).

Table 1. Pro	oportion	of lungworm	infection	in	cattle based	on res	piratory	clinical	signs

Respiratory signs	Number of animals examined	No. Positive	OR (95% CI)
Clinically diseased	57	29(50.88%)	1(Reference)
Apparently healthy	45	8(17.78%)	0.31(0.093 - 1.05)
Total	102	37(36.27%)	







Body condition



Management system

Figure 2. Lungworm infection rate in cattle based on different risk factors

An attempt was made to know the influence of different factors such as sex, age, body condition, and

Positives in percennt

management system on the lungworm infection rate in cattle (Figure2). Based on sex, female animals were found to have higher infection rate (38.98%) than males (32.56%). Based on age, young animals were highly infected (50%) with lungworm when compared with adults (27.42%). Lungworm infection in cattle with poor body condition was (85.71%) as compared with those of medium (31.51%), and good (13.33%) body conditions. Lungworm infection in cattle was found to be high in extensive management system (53.23%) than semi – intensive (7.69%) and intensive management system (11.11%). Age, body condition and management system showed statistically

significant association with lungworm infection at p<0.05. However, the difference in sex was statically insignificant (p>0.05) (Figure2).

3.2. Lungworm infection in sheep

Out of a total of 114 sheep examined, 53 (46.49%) were positive to lungworm infection. From these infected animals, about 41 (74.54%) were with respiratory clinical symptoms wile 12 (20.33%) were apparently healthy. According to this finding sheep with respiratory clinical signs were 5.26 times more likely to have lungworm infection than apparently healthy ones (Table 2).

Table 2. Proportion of lungworm infection	in sheep based o	on respiratory clinica	l signs
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Respiratory signs	Number of animals examined	Positive	Negative	OR (95%CI)
Pneumonic	55	41(74.54%)	14(25.45%)	1(Reference)
Apparently healthy	59	12(20.33%)	47(79.66%)	0.191 (0.055 -0.663)
Total	114	53(46.9%)	61(53.50%)	

Lungworm species recovered from sheep where *Dictyocaulus filaria*, *Mullerius capillaries and Protostrongylus rufescens*. *D. filaria* was dominant lungworm species (43.86%) in the study area followed

by *M. capillaries* (14.04%), mixed infection (14.04%), and *P. rufescens* (5.26%) being the least detected species in the study area (Table3).

Table 3. Lungworm species isolated from sheep in study area

Lungworm species	Number of animals examined	Number of positives (%)
Dictyocaulus filaria	114	50(48.86%)
Mullerius capillaries	114	16(14.04%)
Protostrongylus rufescens	114	6(5.26%)
Mixed infection	114	16(14.04%)
Total	114	53(46.49%)

An attempt was made to know the influence of different factors such as sex, age, and body condition and management system on the ovine lungworm infection rate (Figure 4). Based on sex, female animals were found to have higher infection rate (48.84%) than males (45.07%). Based on age, young animals were highly infected (65.91%) with lungworm when compared with adults (34.29%). Lungworm infection in sheep with poor body condition was (100%) as compared with those of medium (48%) and good (12%) body conditions Lungworm infection in sheep kept in different management system (extensive and semi intensive) was found to be high in extensive system (59.3%) and semi-intensive (9.09%). Age, body condition and management system showed statistically significant association with lungworm infection at p<0.05. However, the difference in sex was statically insignificant (p>0.05).







Figure 4. Lungworm infection rate in sheep based on different risk factors

4. Discussions

The result in this study revealed that over all infection rate of lung in cattle was 36.3% which is very high compared to finding of Zerhun and Tesfaye [19] at Addis Ababa municipal abattoir where the prevalence was found to be 1.5% and Mahmood *et al.*, [20] in Pakistan with prevalence of 4.76%. This could be due to cattle slaughtered in abattoir were purchased from different market places of the country, mostly from low land areas and because the climate altitude and rainfall are known to influence the abundance of lungworm [21, 22].

The proportion of lungworm infection in cattle with respiratory clinical signs was 3.22 times higher (50.7%) than without respiratory signs (17.8%). This high recovery rate in pneumonic animals could be accredited to that *Dictyocaulus viviparous* is the major causative agent of parasitic bronchitis in animals is ingested as larvae and matures into adult worms after penetration of the intestinal wall and via the lymph nodes and blood circulation to the lungs. In lungs, the pathological changes occur due to the influx and activation of eosinophils and mast cells that result in restriction of the airways, resulting in edema, emphysema and collapse of the alveoli [23].

Over all infection rate of ovine lungworm in the Tiyo district during study time was (46.5%). This was lower when compared with previous finding of Wondosen (58.8%) in the same study area [24], Netsanet (73.35%) in Debre Birhan [25], and Bekele (59.4%) in Arsi (26). The present finding was also higher than previous study done by Asaye (20.3%) in Baherdar [27], Beyene (34.9%) in Ambo [28] and Brook (27.8%) in Asella [29]. Such variation in the infection rate could be due to difference in study design, sampling method and size of sample. The difference might also be attributed to the variation in altitude, rain fall, humidity and temperature in different areas of the country. However, the level of prevalence is almost comparable with previous studies of Sisay (44.7%) in Baherdar [30], Basazinew (48.3%) around Dessie zuria [31] and Alemu (53.6%) in North Eastern Ethiopia [32].

In this study, sheep with respiratory clinical signs were highly infected (69.5%) with lungworm than apparently healthy (21.8%). Sheep with respiratory clinical signs were 5.26 times more likely to have lungworm infection than apparently healthy ones. This finding agrees with the previous study of Eyob [33] who reported higher prevalence of lungworm infection in the animal showing clinical signs (86.8%) than apparently normal (57.1%). Such difference could be due to the stage of the parasite in the patent phase, the adults in the segmental and lobar bronchi cause bronchitis, with eosinophils, plasma cells, and lymphocytes in the bronchial wall; a cellular exudate,

frothy mucus, and adult nematodes are found in the lumen [34]. Another possible reason is that there is post patent parasitic bronchitis in 25% of heavily infected and then recovered animals [35].

The lungworm species identified from sheep in the study area were *D. filaria* (43.86%), *M.capillaris* (14.04%), *P. rufescens* (5.26%) and mixed infection (14.04%). The present finding is comparable with the previous report of Ibrahim [36] in Mekelle and Mekonen *et al* [37] in Gondar town. But it disagrees with the report of Sissay [30] in Bahirdar (39.3%) and Mezgebu [38] in Addis Ababa who reported *M. capilaris* as the most prevalent. This high proportion of *D. filaria* could be due to different transmission dynamics of *P. rufescens* and *M. capillaries*, and hosts susceptibilities for the *P. rufescens* and *M. capillaries*. In addition, dry season is not suitable to intermediate host snail species [16] and *D. filaria* reaches the infective stage within less time [17].

An attempt was made to assess the association of different factors with level of lungworm infection. The proportion of ovine lungworm infection was higher in female (48.8%) than male (45.1%). However, difference was not statistically significant (p>0.05). This result contradicts with the earlier study of Alemu [32] and Birtukan [39] who reported significant variation in the infection rate of lungworms in males and females. However, the result agrees with that of Sisay [30] who reported insignificant difference of lungworm infections concerned on sheep sex. This high proportion in female could be related to some of the physiological facts meaning the pre-parturient relaxation of resistance can result in the inability to expel the worm Craig [40]. In addition, farmers kept male animals for different purposes like fattening, breeding and as a result males were dewormed more frequently than females.

The proportion of lungworm infection in animals with poor body condition was found to be higher when compared to with animals of medium and good body conditions (p < 0.05). The result of the present study agrees with the previous report of Asaye [27], Alemu [32], Muluken [41] and Mengestom [42]. The possible reason for this result might be associated with the nutritional management of animals. Poor body condition occurs as a result of lack of feed or nutritional management. This may lead to lack of resistance to infection and contribute for increased prevalence rate in poorly conditioned animals. Furthermore, considerable weight loss is associated with infection as a result of D. filaria [43]. With regard to age, lungworm infection was significantly higher in young animals than adult. This finding agrees with data reported by Radostitis et al [44], Urquhart et al [16] and Mekonen et al [37] who reported that young sheep were found to be infected more than adults. This might be associated with the naturally acquired immunity against infection slowly develops with age, and *D. filaria* infection decrease with increasing age of the animal.

In this study, proportion of lungworm infection was found higher in animals kept under extensive management system than those under semi-intensive husbandry system which revealed statistically significant association with management system (P<0.05). This finding agrees with previous reports of Sissay [1996] and Mekonen *et al* [37], this could possibly be associated with receiving an inadequate keeping and poor conditions of sheep in extensive management system, animals under well nourishment and watering led to less risk of helminthes [45].

5. Conclusion

The result of current study revealed that lungworm was one of the major parasites of sheep and cattle in Tiyo district. It was significantly high in sheep and cattle with clinically diagnosed respiratory signs compared to apparently healthy ones. In the study area, *D. filaria* was found the most dominant ovine lungworm species followed by *M.capillaris*, mixed infection and *P. rufescens*.

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8/25/2020

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