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Prevalence of Small Ruminant Lung Worm Infection in Jimma Town Dawit Weldesenbet

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Abstract: Across sectional study of lung worm infection was conducted in Jimma town from November, 2010 to march, 2011 with the aim of determining the prevalence of lung worm infection of small ruminants to assess some of the determinant factors and to identify the species of respiratory helminthes involved in the area. For this purpose fecal samples of 360 sheep and goats of all age groups and both sexes were examined by Modified Baermann technique for the extraction of L1 larvae. The finding indicated that 26.7 % (96) were found invariably infected with different species of lung worm. *Dictyocaulus filaria* was the most prevalent species accounted for 12.2% of the total positive samples. There was a significant difference (p<0.05) in the prevalence of lung worm infection with regard to sex, age and body condition of sheep while in goats, the prevalence of infection did not show statistically significant difference. Additionally the intact lungs of 113 sheep and goats from Jimma municipal abattoir were systematically opened and examined for lung worms following a standard procedures and 29.2 %(33) were harboring one or more species of lung worms. It can be summarized that small ruminant lung worm infection is problem of a significant magnitude in Jimma city. Lack of treatment and poor management and husbandry practices are likely factors responsible for the high prevalence rates observed in the area of study. Finally possible control measures of the disease are forwarded.

Key words: Small Ruminant • Lung Worm • Jimma Town • Prevalence • Sheep • Goat • *Dictyocaulus Filaria* • Baerman • *Protostrongylus Rufescens* • *Muellerius Capillarie*

INTRODUCTION

Small ruminants provide as much as 30% of meat and milk consumed in sub-Saharan Africa and is found on smallholdings throughout the continent. They are especially important in the more extreme climates of the world [1].

Ethiopia has a population of about 44 million cattles, 23 million sheep and 23 million goats; 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 generally estimated to be in the range of 8-10% of national cattle herd per annum and 14-16%, 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 estimated to be of 550 million Ethiopian birr [3].

Helminthes parasites of ruminants are ubiquitous, with many tropical and subtropical environments of the world providing nearly perfect conditions for their survival and development. Although these parasites are widely prevalent, they can be less obvious than signs of other livestock diseases. Partly for this reason, infection with helminthes parasites are among the most established that high prevalence rates of the infection with less obvious signs are associated with poor production and unthriftiness [1]. Dictyocaulidae and/or certain Metastrongylidae are known to exist in east Africa (Ethiopia, Kenya and Tanzania) and South Africa [4]. The incidence of parasitic diseases, including respiratory helminthes varies greatly from place to place depending on the relative importance of the factors. Verminous pneumonia due to various lung worm species has been reported to exist in sheep and goats particularly in the high land areas of Ethiopia [5-11]. Therefore to increase the potential of small ruminants production and to get the

maximum benefit from them prevention and control of lung worms *Dictyocaulidae* and, *Metastrongylidae* is very important. Therefore, the present study was designed:

- To determine the prevalence of lungworm infection rate in sheep and goats.
- To study some of the determinant factors of the infection.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in Jimma zone, Southwestern part of Ethiopia at Jimma university open air clinic and Jimma municipality abattoir. Jimma town, the capital of Jimma zone is located in Oromia Regional Administration, 346 km Southwest of Addis Ababa at latitude of about 7013'-8056' N and longitude of about 35052'-37037' E and at an elevation ranging from 880 m to 3360 m above sea level. The study area receives a mean annual rainfall of about 1530 millimeters which comes from the long and short rainy seasons. The annual means of minimum and maximum temperature during the study period were 14.4 and 26.7 degree Celsius respectively.

Study Design: The study was cross sectional survey involving 473 small ruminants (268 ovine and 205 caprine) of which 258 were males and 215 were females. The explanatory variables considered were age, sex and species of animal and management system. Each individual of the sampled sheep and goats was examined for the presence of lungworm through clinical or post mortem examination.

Sampling Method and Sample Size: The sampling method was simple random sampling technique to select the animals and to determine the sample size. The sample size was decided using the formula given by Thrusfield [11] with 95% confidence interval. Expected prevalence was 50% and at 5% absolute precision. According to the above formula, the calculated sample size was 384. However, in order to increase the accuracy, the number of sampled small ruminants were 473.

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

Where:

n = Required sample size

 $P_{exp} = Expected prevalence$

d = Desired absolute precision

Sample Collection and Study Methodology

Coproscopic Examination: Three hundred sixty samples were taken randomly from extensive and semi intensive farms found in Jimma town. Fecal samples were collected directly from rectum of all selected animals using disposable gloves and stored in universal bottle till examination. During sample collection the date, species, sex, age and management system were properly recorded. Each bottle was properly labeled corresponding to the animal identity.

The techniques recommended by Fraser [12] and Urquhart [13] were employed for identification of lung worm species from the collected samples. In the laboratory, following conventional method of Baerman technique for detection of lungworm larvae, 25 grams of fresh feces were weighed from each sample for the identification of L₁ larvae. Samples were enclosed with gauze fixed on to a string rod and submersed in a clean glass tube filled with warm water in a Barman apparatus. The whole apparatus was left for 2 to 3 hours and then the sediment was examined under the low power of microscope after siphoning off the sediment.

Postmortem Examination: One hundred thirty samples (65 sheep and 48 goats) were collected for post mortem examination of adult lung worms and the identification of the species involved. Lung was examined immediately after slaughter from the sampling units. The species of animal, species of lung worm and date of sampling of slaughtered animals were labeled. The air passages were opened starting from the trachea down to the bronchi with fine blunt pointed scissors to detect the parasites.

Data Analysis: The results were analyzed in relation to sex, species of animal, age, management, body condition and species of lung worms. Animals were categorized into three age groups, group I (less than 6 months), group II (6 - 24 months) and group III (greater than 24 months), the data obtained was coded for the above factors and entered into excel. Then chi-square was used to compare the prevalence rate of small ruminants' lung worm infection for possible significant difference. The differences were regarded as significant if p-value is less than 0.05 using SPSS statistical soft ware.

RESULTS

Coproscopic Examination: A total of 360 small ruminants (203 sheep and 157 goats) were examined by modified Baerman technique in Jimma town. The identification results showed 26.7 % (96/360) prevalence of lung worm

Table 1: Prevalence of lungworm in Sheep and Goats

Species	Examined	Positive	Prevalence (%)	x^2 -value	p-value
Ovine	203	52	25.60	0.263	0.609
Caprine	157	44	28.02		
Total	360	96	26.70		

Table 2: Prevalence of lungworm Infection on the Basis of Sex

Sex	Examined	Positive	Prevalence (%)	x^2 -value	p-value
Male	145	43	29.7	1.109	0.29
Female	215	53	24.7		
Total	360	96	26.7		

Table 3: Prevalence of lung Worms in Different Age Groups of Small Ruminants

Age	Examined	Positive	Prevalence (%)	x^2 -value	p-value
< 6 months	54	14	25.9	0.445	0.801
6-24 months	200	56	28.0		
>24month	106	26	24.5		
Total	360	96	26.7		

Table 4: Identification Results of lung Worm Species

Species	Examined	Positive	Prevalence (%)	x^2 -value	p-value
D. filaria	360	44	12.2	0.00	3.60
M. capilaris	360	25	6.90		
P. rufescens	360	27	7.50		
Total	360	96	26.7		

Table 5: Prevalence of lung Worm Infection under Different Management Systems

Management						
system	Examined	Positive	Prevalence (%)	x^2 -value	p-value	
Semi-intensive	91	26	28.6	0.226	0.635	
Extensive	269	70	26.0			
Total	360	96	26.7			

infection. The specific prevalence was found to be 25.6 % (52/203) and 28.02% (44/157) in sheep and goats respectively (Table 1). In this study the prevalence of lung worm infection was found to be higher in goats than sheep but this difference was not statistically significant (x^2 >0.05) (Table 1).

The investigation result revealed higher prevalence of lung worm in male animals 29.7% (43/145) than female animals 24.7% (53/215). However this difference was not statistically significant ($x^2 > 0.05$) (Table 2).

Comparison of the prevalence of lung worm infections in the different age groups showed relatively higher prevalence in age group between 6 - 24 months (28. %). The lowest prevalence was observed in animals greater than 24 months (24.5%). There was no significant difference (p>0.05) among these proportions (Table 3).

Final identification of the lung worm species were done based on the morphology of the larvae by viewing the fecal samples with a light microscope. *Dictyocaulus*

filaria was found to be the highest prevalent (12.2 %) among the different identified species. The identification result showed that there was strong significant difference among the species of lung worms in small ruminants $x^2 < 0.05$ (Table 4).

In this study the prevalence of lung worm was found to be higher in the semi-intensive system (28.6%) When compared to the extensive management system (26%) However this difference was not statistically significant ($x^2 > 0.05$) (Table 5).

Based on body condition, animals were categorized into three groups; poor, medium and good The lungworm infection rate according to the physical body condition was recorded to be 26.8% in animals with poor body condition, 25.6% in those with medium body condition and 28.3% in animals with a good body condition as shown in Table 6. Statistically, there was no significant difference in the lungworm infections in different physical body conditions (P>0.05).

Post Mortem Examination: A total of 113 small ruminants 65 sheep and 48 goats were examined for post mortem examination among which 33 (29.2 %) were positive for lung worm infection with a prevalence of 30.76% (20 / 65) and 27.08% (13 / 48) respectively.

Table 6: Prevalence of lung Worm Infection in Different Body Conditions.

BCS	Examined	Positive	Prevalence	x²-value	p-value
Poor	41	11	26.8%	0.281	0.861
Medium	199	51	25.6%		
Good	120	34	28.3%		
Total	360	96	26.7%		

Table 7: Prevalence of lung Worm Infection in Sheep and Goat during PM Examination

Species animal	Examined	Positive	Prevalence (%)
Sheep	65	20	30.76
Goat	48	13	27.08
Total	113	33	29.20

Table 8: Prevalence of lung Worm Species in Small Ruminants during Post Mortem Examination of lung

Lungworm spp.	Examined	Positive	Prevalence
Dictyocaulus filaria	113	13	11.5%
Muellerius capillaries	113	10	8.85%
Protostrongylus rufescens	113	10	8.85%
Total	113	33	29.20%

Table 9: Coproscopic and Post Mortem Examination of lung Worm.

Type of examination	Examined	Positive	Prevalence (%)
Coproscopy	360	96	26.70
Post mortem	113	33	29.20

Comparison of the Overall Prevalence of lung Worm Infection was found to be Higher in Post Mortem Examination 29.20 % than Coproscopic Examination 26.7% (Table 9).

DISCUSSION

The present study revealed the presence of nematode species parasitizing the respiratory tract of small ruminants that causes bronchitis and pneumonia with an overall infection rate of 26.7% by coproscopic examination. This level of prevalence was in agreement with previous studies conducted by Tsegaye [5] at Gaint, Brook [14] at Assela, Uqubazghi [15] at Hamassien Awraja and Dawit [16] in and around Tse-Ada-Emba who reported prevalence rates of 32.2%, 27.8 %, 27.6% and 27.7% respectively.

However, the current finding was lower than prevalences reported by Netsanet [7] in Debrebirhan, Alemu et al. [10] in North West Ethiopia Mezgebu [9] in Addis Ababa and Mihreteab and Aman [28] in Tiyo who reported prevalence of 73.25%, 53.6%, 48% and 57.1% respectively. The present report was higher than Teffera, [8] at Deseie and Kombolcha Frewengel [17] in Tigray and Sissay, [18] in Bahirdar who reported prevalence of 15.47%, 11.24% and 13% respectively. The differences in the prevalence of lung worms of small ruminants in the above studies might be associated with differences in the larval detection methods, the difference in the study areas which favors the survival of the larvae of the lung worm or the snail intermediate host in case of P. rufescens and the different sample sizes used by the researchers. It might also be associated with nutritional status, level of immunity, management practice of the animal, rain fall, humidity, temperature differences and season of examination in the respective study areas.

The specific prevalences of lung worms were 25.6 % (52of 203) and 28.02% (44 of 157) in sheep and goats respectively. The prevalence of lung worm infection was relatively higher in goats than in sheep. This finding agrees with the report of Wilson [20] and Alemu *et al.* [10] who reported goats are more susceptible to infection than sheep. Where goats appear to develop less immunity due to their grazing behavior. Goats with their browsing behavior consume uncontaminated matter with parasite larvae, so being less exposed to larvae have lower acquired resistance than sheep as reported by Wilsmore [21].

In the study area, the prevalence of lung worm in sheep was (25.6%) which shows almost two times less than the prevalence of sheep in Tiyo district (57.1%) as recorded by Mihreteab and Aman [28]. This differences may be associated with the variations in agro-ecology of both areas.

In the present study, D.filaria showed highest prevalence in relation to other species of lung worm in small ruminants followed by P. rufescens whereas M. capillaries was the least prevalent. This result agrees with the findings of Alemu et al. [10] Netsanet [7] Mihreteab and Aman [28] Nemat and Moghadam [30] and Addis et al. [29] who reported D. filaria to be the highest prevalent. In contrast to the present findings, Sissay [18] in Bahirdar and Mezgebu [9] in Addis Ababa reported that M. capillaris is the most prevalent. The possible explanation for the predominance of D. filaria in the present study area might be attributed to the difference in the life cycles of the parasites. D.filaria which has direct life cycle takes less time to reach the infective stage and after ingestion larvae can appear in feces after five weeks by Mengestom [23]. So, the probability of infection, transmission and reinfection with a season could be much higher as compared with M. capillaries and P. rufescens as reported by Abebe [24].

In the study area comparison of the overall prevalence of lung worm infection was found to be higher in post mortem examination 29.20 % than Coproscopic examination 26.7% and this is not in agreement with the study of Addis *et al.*[29] who reported higher prevalence in coproscopic than postmortem examination 33.83% and 32.6% respectively. This is may be due to geographic localities and agroecologic factors.

According to the current finding, *P. rufescens* was the second important species of lungworm parasite, which showed prevalence of 7.5% of total examined sheep and goats. This result agrees with the previous work reported by Abebe [24] and Hassen [25] in and around Assela with prevalence of 10% and 9% respectively. But the result was lower than previous studies reported by Sissay [18] in Bahirdar (39.3%) and Mezgebu [9] in Addis Ababa (54.9%) where, *M. capilaris* was the most prevalent.

On the attempt made to know the influence of sex on the over all prevalence of infection, the study clearly indicated that there is no significant difference (P>0.05) in susceptibility to infection with lungworm, hence sex dependant variation was not encountered. This result is not in agreement with the earlier study of Sissay [18] and

Sefinew [26] who reported significant variation in the infection rate with lung worms in males and females and coincides with Netsanet [7] in and around Debre Birhan and Teffera [8] in and around Dessie and Kombolcha who reported equal susceptibility to infection. These may be due to the fact that improper distribution of sample selection between the two sexes as observed by Addis *et al.* [29] or almost all female samples of sheep and goats are not in preparturient period during the study time [22].

With regard to age, generally, the highest prevalence (28%) was observed in animals of 6 - 24 month age while the lowest prevalence(24.5%) was observed in animals of age groups greater than 24 months with no statistically significant difference (p>0.05) between the prevalences of the different age groups. This report agrees with Netsanet [7]. D. filaria infection decreases with increasing age of the animal. This might be associated with the apparent developed acquired immunity of the host so that adult animals have the lowest infection and the lowest prevalence [23]. In the present study, the influences of management system on the prevalence of lung worms were observed. The obtained results clearly indicated that there was no statistically significant difference (P > 0.05) observed in susceptibility to lung worm infection between extensive and semi-intensive management systems. This finding was different from Sissay [18] in and around Bahir Dar, Sefinew [26] in Wollo and Eyobe [19] in Assela who reported that management had significant effect on susceptibility of animals to infection. The possible reason for the present finding could be due to the fact that, animals in semi intensive management system were grazed in the same pasture with extensively grazing animals; feeding moist pastures in the field until environment temperature rises like those of extensively managed small ruminants in the area.

The current study was conducted to see the influence of body condition on the prevalence of lung worm infection and revealed that there was no statistically significant association (P >0.05). The prevalence of lung worm infection by coproscopic examination was higher (28.3%) in animals of good body condition than those of poor ones (26.8%). The possible reason for this finding coincides with Mengestom [23] small ruminants loss of weight cannot only be attributed to the lung worm infection alone since Haemonchus contortus and other GIT helminthes can also be the reason, in addition failure to deworm the animals, or else lack of feed or inappropriate nutritional management could also be the possible reason.

Post mortem examination results showed that the prevalence was higher in sheep 35.76 % (20 of 65) than in goats 27.08% (13 of 48). This might be due to the difference in the areas from which animals were collected which favors the survival of lung worm larvae or the snail intermediate host in case of *P. rufescens* and different sample sizes. It might also be associated with nutritional status, management practice of the animal, rain fall, humidity and temperature differences.

Overall prevalence of lung worms in slaughtered small ruminants in Jimma town was 29.2% using post mortem examination while the prevalence of coprological finding was 26.7%. The higher prevalence in post mortem examination agrees with the finding of Teffera [8] around Dessie and Kombolcha, Frewengel [17] in and around Mekele and Eyobe [19] in Assela reported higher prevalence in postmortem than coprological. But, the present finding disagrees with the finding of Sissay [18] in Bahir Dar, Paulos [27] in Arsi (chilallo) who reported higher prevalence in faecal than post mortem examination. This might be associated with the difference in the methods followed in the detection of lung worm larvae and the absence of larvae in the patent and post patent phases.

CONCLUSION AND RECOMMENDATIONS

Lung worms are one of the most common causes of chronic respiratory disorder that cause huge losses in small ruminants. In the present work the prevalence of lung worms was found to be higher in Jimma town. The major identified lung worm species in the study were Dictyocaulus filaria followed by Protostrongylus rufescens and Mullerius capillaries. From this study it has been concluded that goats are much prone to lung worm infection than sheep. Therefore, the following recommendations are forwarded.

- Prohibition of sheep and goats from grazing early in the morning and evening and in swampy areas to protect them from infection.
- Treating small ruminants with broad-spectrum antihelmentic at the beginning of rainy season could appear to be most effective.
- Isolation of most susceptible age groups during the season when pasture contamination occurs.
- Due to its impact on production, emphasis should be given for the control and prevention of lungworm infections.

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REFERENCES

- Hansen, J. and B. Perry,1994. The epidemiology, diagnosis and control of helminthes parasite of ruminants. ILRAD Nirobi Kenya.
- FAO (Food and Agriculture Organization of the United Nations). 1993. Ethiopia. Livestock sector development project. Preparation Report Volume 1. FAO, Rome, Italy, pp. 51.
- Torncy, P.M., 1989. Mannual of Tropical veterinary parasitology. Helminths of Livestock and poultry in Tropical Africa. The International Technical Center for agricultural and Rural co-operation, C, A.B. International, pp. 81-85.
- 4. Jovanovic, M., 1977. Report to the Ministry of Agriculture, Addis Ababa, Ethiopia.
- Tsegaye, S., 1985. Prevalence of Dictyocaulus filaria in Gaint Awraja. DVM Thesis, AAU-FVM, Debrezeit Ethiopia.
- 6. Wedewosen, T., 1992. Prevalence of lung worm in ovine in and around Assela. DVM Thesis AAU-FVM, Debrezeit Ethiopia.
- Netsanet, B., 1992. Study on the prevalence and control of lung worms Dictyocaulus and muellerius) in local Ethiopia high land sheep in and around Debre Birhan. DVM Thesis, Addis Ababa University, Debrezeit, Ethiopia.
- 8. Teffera, S., 1993. Prevalence of ovine lung worms around Dessie and Kombolcha. DVM Thesis, Addis Ababa University, Debrezeit, Ethiopia.
- 9. Mezgebu, M., 1995. Study on bovine fasciolosis and lung worm infection in Addis Ababa.
- Alemu, S., G. Esayas, A. Galegay and Z. Aschalew, 2006. Study on small ruminant lung worm in Northern Ethiopia. Veterinary Parasitol., 142: 330-335.
- Thrusfield, M., 1995. Survey in veterinary Epidemilogy, 2nd ed., Black well science limited, Cambridge, USA, pp: 178-198.

- Fraser, C.M., 1991. The Merck veterinary manual. A hand book of Diagnosis Theurapy and Disease prevention and control for the veterinarians. 7th ed; Merck and co; Inc, Rahway, NIT USA, pp: 714-717.
- 13. Urquhart, G.M., J. Armour, J.L. Duncan and F.W. Jenning, 1994. Veterinary Parasitologica, Glasgow, Scotland, pp: 35-61.
- 14. Brook, L., G. Fesseha and T. Shibru, 1986. The seasonal occurrence of Dictyocaulus filaria (Rudolphi: 1990) in four selected sites of Ethiopia: K. Sci., 9(1.2): 25-38.
- Uqubazghi, K., 1990. Preliminary study of the prevalence of ovine lung worm of small ruminants in Hamasien Awraja, DVM Thesis AAU-FVM, Debrezeit Ethiopia.
- Dawit, M., 2009. Prevalence of ovine lungworm in and around Tse-Ada-Emba. DVM Thesis, Jimma University School of Veterinary Medicine, Jimma Ethiopia.
- Frewengel, S., 1995. Prevalence of ovine Dictyocaulus in and around Mekelle (Tigray) DVM Thesis, AAU, FVM, Debrezeit, Ethiopia.
- Sissay, A., 1996. Preliminary study on the prevalence of study on the prevalence of ovine lungworm infestation in and around Bahir Dar. DVM Thesis, AAU-FVM, Debrezeit Ethiopia.
- 19. Eyob, E., 2008. Preliminary study on prevalence of ovine lung worm infection in Asela, DVM Thesis Jimma University, Jimma, Ethiopia.
- 20. Wilson, G.I., 1970. The straight and duration of immunity to Dictyocaulus filaria infection in sheep and goats. Research in Veterinary Sci., 2: 331-6.
- 21. Wilsmore, T., 2006. Disease of small ruminants in Ethiopia, the veterinary epidemiology and economics research unit of agricultures policy and development the university of read, UK, pp: 602.
- 22. Soulsby, E.J.L., 1982. Helminths, Arthropods and protozoa of Domesticated Animals 6th ed. Bailliere Tindall, Londons, pp. 492-552.
- 23. Mengestom, G., 2008. Preliminary study on prevalence of ovine lung worm infection in Atsbi, DVM Thesis Jimma University, Jimma, Ethiopia.
- 24. Abebe, M., 2008. Study on prevalence of ovine lungworm infection in and around Assella, DVM thesis, Mekelle University, Faculty of veterinary medicine, Mekelle, Ethiopia.
- Hassen, A., 2009. Prevalence of ovine lungworm in and around Assela. DVM Thesis, Jimma University School of Veterinary Medicine, Jimma Ethiopia.

- 26. Sefinew, A., 1999. Survey of small ruminant lung worm in six districts of Wollo, DVM Thesis, AAU-FVM, Debrezeit Ethiopia.
- Paulos, A., 2000. Importance and seasonal dynamics of lung worm infection of small ruminants in Chillalo area, Arsi zone, DVM Thesis, AAU-FVM, Debrezeit Ethiopia.
- Mihreteab, B. and A. Aman, 2010. Ovine Lungworms in Tiyo District, South-East Ethiopia: Prevalence, Effect of Altitude and Major Host Related Risk Factors. School of Veterinary Medicine, Jimma University.
- Addis, M., A. Fromsa and Y. Ebuy, 2011. Study on the Prevalence of Lungworm Infection in Small Ruminants in Gondar Town, Ethiopia. J. Animal and Veterinary Advances, 10: 1683-1687.
- 30. Nemat, E.A. and G.A. Moghadam, 0000. A Survey On Annual Infestation Of Sheep With Lung Worms Based On Fecal Test And Slaughter House Study In Tabriz Department Of Pathobiology, Faculty Of Veterinary Medicine, University of Tabriz, Tabriz, Iran.