

Journal of Parasitology and Vector Biology

Volume 5 Number 8 August 2013
ISSN 2141-2510



*Academic
Journals*

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

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Full Length Research Paper

Parasitological examination of Ava stream used in irrigation in Enugu State, South-Eastern Nigeria: An implication for helminth transmission

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Accepted 20 August, 2013

The parasitological examination of Ava stream in Abakpa Nike, Enugu state, Nigeria, was investigated to identify the species and load of parasites in the stream. Five different sites were sampled; these sites were chosen based on the different anthropogenic activities going on around these sites. One litre sample each was collected from these sites and were preserved by the addition of 10% formaldehyde (2 ml/L), and were transported to the laboratory. The samples were examined using the flotation technique. The species of parasites identified include *Schistosoma haematobium*, *Ascaris lumbricoides*, *Hymenolepis nana*, *Enterobius vermicularis*, *Trichuris trichuria*, *Diphybotrium latum*, and *Taenia* species. Samples of Ogburugbu, recorded the highest egg count/L, as well as the highest percentage occurrence, and were followed closely by Ugwuagor, while Agbalike site recorded the least occurrence. This research has helped to identify the possible health risk of consuming raw vegetables and fruits irrigated with wastewater and organic manure sources and the unhygienic activities of the people.

Key words: Parasites, examination, anthropogenic, stream, irrigation.

INTRODUCTION

Wastewater is any water that has been adversely affected in quality by anthropogenic influence (Cornish et al., 1999). It comprises of liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. Around 90% of wastewater produced globally remains untreated, causing widespread water pollution, especially in developing countries (Tchobanoglous et al., 2003). Increasingly, agriculture is using untreated raw and wastewater for irrigation since there is no alternative to farmers due to scarcity of water resources (IWMI, 2010).

Raw and wastewater reuse for irrigation, as practiced with Ava stream, is a common practice to overcome water scarcity especially in countries with limited water resources (Mara and Cairncross, 1989). Defecation around water bodies as well as leakage of sewage materials containing human excreta is a common practice around Ava stream due to poor standard of living of those living around this area. However, there is a potential risk of transmission of diseases through excreted organisms in the case of irrigation with untreated wastewater, as well as those natural water receiving human excreta (Feachem, 1983).

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The health hazards associated with direct and indirect wastewater use in irrigation agriculture are of two kinds: the rural health and safety problem for those working on the land or living on or near the land where the water is being used, and the risk that contaminated products from the wastewater use area may subsequently infect humans and animal through consumption or handling of the foodstuff or secondary human contamination by consuming foodstuff from animals that used the area (WHO, 1989). Therefore, in order to prevent the transmission of diseases, it has been recommended that only treated wastewater be used for crop irrigation (IWMI, 2010).

The objectives of this study were to (1) determine the level of parasite contamination of Ava stream and (2) assess the health risk of consuming vegetables and other fruits (uncooked) irrigated with the stream.

MATERIALS AND METHODS

The study area is Abakpa Nike, Eastern part of Enugu State, South-Eastern Nigeria. It covers about 8,422km², with an estimated population of about 120,835 people, which fall in the group of low income earners and majority of whom are farmers, traders and students, with few civil servants.

Ava stream forms the major source of water for people living in Abakpa metropolis. It flows East-South of Enugu State capital city. The stream is utilized for both domestic and agricultural purpose. Due to low economic status of the people living in this area, majority of their residential buildings lacks basic sanitary facilities and at such, faecal matters are deposited in and around the stream, as most people choose bush method as alternative method of defecation. Cultivation of vegetables of different kinds such as those used uncooked, in making African salad (spinach, tomato, pepper, lettuce, *Solanum* species, and *Telferia* species), and those eaten after cooking (pumpkin, bitter leaf, curry leaf, okra, etc.) form the major farming activities around the stream since the stream serves as the only source of irrigation water.

Experimental

Water samples from Ava stream, were collected from five (5) different sites: Ogwuagor, Ogburugbu, Agbalike, Silas, and Ifoo, respectively. These sites were chosen based on personal observation of the anthropogenic activities such as washing and bathing, scooping of the water into irrigation farms, promiscuous discharge of human and animal waste direct into the water, use of animal and poultry dropping for organic fertilization of the vegetable within and around the stream. Samples of 1 L were collected from each site and preserved by the addition of 10% formaldehyde (2 ml/L) in sterile bottles and are transported to Applied Biology Laboratory, Faculty of Biological Sciences, Ebonyi State University Abakaliki. In the laboratory, the samples were allowed to settle for 8 h to sediment overnight.

The supernatant was carefully removed and discarded without disturbing the sediment, using a siphon. The sediment was then transferred to centrifuge tubes. The walls of the sedimentation container was washed thoroughly using a spray bottle with detergent solution and the rinsing was added to the sediments in the centrifuge tubes. All the recovered materials were centrifuged at 1000 g for 15 min. The supernatant was removed and discarded and the sediments transferred to one tube. Flotation technique which is ideal for the recovery of helminth eggs was used by suspending the pellet in an equal volume of aceto-acetic buffer (pH

4.5) (Cheesbrough, 1999).

The mixture was re-centrifuged at 1000 g for 15 min and the pellets was transferred to microscope counting cell for final examination using objectives 10 and 40× magnification. The total number of eggs/L(N) present in the original sample of the water was determined from the equation:

$$N = AX/PV$$

Where N is the number of eggs per litre of sample, A is the number of eggs counted in the counting slide or the mean of counts from two or three slides, X is the volume of the final products (ml), P is the volume of the microscopic counting cell (0.3 ml), and V is original wastewater sample volume.

RESULTS

The analysis of this survey shows that Ava stream is contaminated with helminth eggs of different classes of Nematode, Trematode and Cestodes, respectively. Among the Trematodes encountered were the eggs of *Schistosoma haematobium*; Nematodes encountered were *Ascaris lumbricoides*, *Enterobius vermicularis*, and *Trichuris trichuria*. Among the Cestodes implicated were the eggs of *Taenia* spp., *Hymenolepsis nana*, and *Diphyllobotrium latum*. The quantification of these helminth eggs in Ava water samples from the five different sites is shown in Table 1. As shown in Table 1, the Ava stream contained different groups of helminthes, including those mentioned earlier. There was significant difference between the total egg count/L in samples of the five different sites sampled ($P < 0.05$). This difference was the most significant between Ogwuagor and Ogburugbu.

Table 2 shows the percentage of occurrence of different helminth eggs from the five different sites sampled. The effluents of Ogburugbu had the greatest percentage followed by Ogwuagor. Silas and Ifoo shared equal percentage occurrence while Agbalike has the least occurrence.

Of all the parasitic eggs isolated in the Ava stream from the five sites sampled, *A. lumbricoides* was the most frequently recovered (Table 1). The mean total egg count/L in the five different sites was much higher in Ogburugbu (mean= 2.4 egg/L) than the other four sites. This was closely followed by Ogwuagor with the mean total egg count/L of 2.2 eggs/L. However, the difference between the mean total egg count/L in Ogburugbu and that of Ogwuagor was 2.4 eggs/L.

DISCUSSION

Water is a vital need of both plants and animals and this has necessitated to a higher demand to maintain our day to day needs. The Enugu State Government has developed water production and sanitation scheme whose sole responsibility is to service water to the entire people of Enugu and environs. This notwithstanding has led to scampering of water by villagers who do not have access

Table 1. Quantification of parasite eggs in Ava water samples from five different sites of sample collection.

Parasite	Sites of collection and number of parasites identified					
	Ogwuagor (n=11)	Ogburugbu (n=12)	Agbalike (n=5)	Silas (n=6)	Ifoo (n=6)	
<i>Schistosoma haematobium</i>	1	4	1	0	0	
<i>Ascaris lumbricoides</i>	3	3	1	4	1	
<i>Hymenolepis nana</i>	3	2	0	1	1	
<i>Enterobius vermicularis</i>	2	0	2	0	2	
<i>Trichuris trichuria</i>	0	0	1	0	0	
<i>Diphylobotrium latum</i>	0	2	0	0	1	
<i>Taenia</i> spp.	2	1	0	1	1	

Table 2. Percentage occurrence of helminth eggs from the five different sites sampled.

Site	Volume of sample examined (V, L)	No. present (%)
Ogwuagor	1	11 (1.1)
Ogburugbu	1	12 (1.2)
Agbalike	1	5 (0.5)
Silas	1	6 (0.6)
Ifoo	1	6 (0.6)

to either good portable water sources to service their domestic needs. Ava people lives at the Eastern part of Enugu State. This has affected their easy access to portable water, may be because of their bed rock. Ava water is one of the most accessible water sources among their inhabitants as this is seen to assist their fast need of water. As pertaining to food and water, this stream has served a dual purpose of agricultural irrigation and drinking sources not only, but washing, bathing and even serving their domestic animals. This has transposed to the emergence of other uncalled act of defecating within and around the stream, dumping refuse and other ill transmitting activities which has yielded propagation of parasitosis among their users and domestic animals. This was evident in earlier work of El kettani et al. (2008) which showed prevalence of intestinal helminthiasis in a group exposed to raw wastewater to be 4.7%. The use of domestic animal dung as organic fertilizers are not left out in their agricultural practices, but this is seen to do more harm than good, because these waste animal dungs serve as a vehicle of parasite transmission especially their infective stages as well as other human pathogens. Of these, helminth infections pose the greatest risks and are a serious public health concern (WHO, 1989). However, the result of this study showed that Ava stream contained different helminth eggs. These parasite species identified were classified into three groups: Cestodes, Trematodes, and Nematodes. The commonest among the identified species however, was *A. lumbricoides* with mean concentration of 2.4 eggs/L. This result agrees with other studies which showed the frequent presence and high concentration of *Ascaris*

eggs in wastewater and raw sewage worldwide as reported by Stott (1997), Valbuena (2002) and Coelho et al. (2001).

In addition, the resistance of *Ascaris* to external conditions (Crompton, 1989), allows the eggs to remain viable longer than other helminth eggs (Arfaa, 1987; Erdogru and Sener, 2005).

The stream used in irrigation in this study does not respond to the WHO standard of <1 helminth egg/L (WHO, 1989), since this study implicated 1 egg/L of water. Consequently, it is not recommended for irrigation of vegetables especially those which are eaten raw. This finding is in accordance with the earlier work of Hajjani et al. (2013) which recorded mean concentration of 8.98 eggs/L. There was a great variation in parasite eggs identified in the five different sites sampled, with Ogburugbu, recording the highest number of parasite eggs/L. This was probably due to the rate of human activities going on around these sites as earlier discussed in this work. The consumption of raw vegetables and fruits irrigated with this stream also plays an important role in the transmission of parasitic contaminations (Arther et al., 1981; Daryani et al., 2008). More reason could be attributed to the fact that population around this area were economically poor, they have a very low standard of living and as such, they lack basic sanitary facilities and therefore, all faecal and other human waste are discharged directly into the stream. The occurrence of the egg of *S. haematobium* indicates the presence of infected individual who possibly could have passed their infected urine into the stream few hours before the time of sample collection.

An earlier survey conducted by Emenyonu et al. (2010) on the effect of waste water use on vegetable crop production in Imo State, Nigeria, shows that wastewater use has negative effect on vegetable crop production some of which they mentioned to include bad odour as well as parasitic infection of the irrigated crops and vegetables. Similarly, studies in different countries by World Health Organization (WHO, 1989), have served to highlight the impact of these practices on the environment and health, especially when these wastewaters, are used in irrigation agriculture.

CONCLUSION/RECOMMENDATION

Ava stream is highly loaded with eggs of helminth parasites and this could mean a serious danger to people, animals and the environment in general. Poor sanitary hygiene as observed within Ava stream illustrates the sanitary and environmental risks associated with the use of this stream as shown by the result of this study. These risks would even be higher if crops and vegetables irrigated with this stream are consumed raw. Both people working around Ava stream and the consumers of the vegetables and other agricultural products produced and irrigated with the stream have the greatest risk of helminthic infections.

However, it is recommended that government should issue warning to owners of residential houses around this stream, forcing them to build good toilets or have their houses sealed by her sanitation agency, this will discourage defecation around the stream. Also, inhabitants of these areas should be educated on the dangers of urinating inside the stream as well as direct discharge of sanitary waste into the stream. A survey should be carried out to identify those individuals passing blood in urine. Lastly, agricultural produce around this stream should be washed with clean water and properly cooked before consumption.

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Full Length Research Paper

The prevalence and risk factors associated with ovine lungworm infestation in the Asella province, Central Ethiopia

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Accepted 27 August, 2013

A cross sectional study design was conducted in Asella province, Central Ethiopia from November 2009 to May 2010. The study was carried out for the purpose of determining the prevalence of lungworm infection in ovine through coproscopic and postmortem examination in the Asella province. Moreover, the study assesses the association of some bionomic factors on the different risk factors and the correlation between adult worm burden and fecal larval output during the study time. Faecal samples (381) were collected from sheep of different age groups, husbandry system and health care, of both sexes; and additionally from 71 sheep slaughtered at Asella town restaurant and hotels, with both intact lung and fecal samples were randomly selected. The investigation also consists of questionnaire survey. Out of 381 fecal samples coproscopically examined, 276 (72.44%) were found to be infected with one or more species of lungworm. Sheep showing respiratory clinical signs, non-dewormed with anthelmintics and raised in extensive husbandry system when compared with those sheep that apparently appeared healthy, dewormed ones and of semi-intensive husbandry system shows statistically significant variation ($P < 0.05$). The difference in the prevalence of infection, both among age groups and between sexes did not show statistically significant variation ($P > 0.05$). The postmortem examination carried out on 71 sheep revealed that coproscopic examination had limited value in terms of estimating the prevalence of lungworm infection in live sheep and implies as there was no direct correlation between the adult worm burden and faecal larval output. The prevalence of lungworm infection observed through postmortem coproscopic examination and postmortem intact lung incision was 39.4 and 26.8%, respectively.

Key words: Asella province, Central Ethiopia, lungworm, ovine, prevalence, risk factors.

INTRODUCTION

Ethiopia has estimated 12 million sheep together with its great variation in agro climatic zones represents a good reservoir of sheep genotypes. Despite the low input extensive management system an estimated, 132,000 tons of sheep meat is produced annually, providing more than 30% of all domestic meat consumption. Sheep also generate cash income to the farming community and significantly contribute to the foreign exchange earnings of about 43 and 5% economy from exports and meat,

respectively. In spite of the large population and importance of sheep however, morbidity and mortality are high in the traditional agro-pastoral production system. In highland area including Arsi Asella, respiratory lungworm parasites are the most common cause of high mortality and morbidity rates of sheep (FAO, 2006). Lungworms of small ruminants are limited to two super families such as Dictyocaulidae and Metastrongylidae. Although these parasites are widely prevalent, the clinical signs they

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showed in infected sheep can be less obvious than signs of other ovine diseases (Hansen and Perry, 1996). Infestation to lungworms in sheep is characteristic by dyspnea, sneezing and cough due to pneumonia. Lungworm infection produces nodules and brownish spots in lung. These signs are diagnosed at postmortem examination during slaughter (Etminani, 1980).

Primary finding of lungworm infection in Ethiopia (Netsanet, 1992; Teffera, 1993; Ayalew et al., 2011; Bekele and Aman, 2011; Hasen et al., 2013) indicated high prevalence and importance of the infection in certain areas of the country. In order to investigate a sound lungworm control strategy at local and regional level, further and detailed investigation on epidemiology and importance of lungworm infections with respect to its temporal distribution is necessary. Therefore, the objective of this research is to determine the prevalence of lungworm infection in ovine through coproscopic and postmortem examination, to assess the association of some biotic factors on the different risk factors during the study period and to determine the correlation between adult worm burden and fecal larval output during the study in the Asella province.

MATERIALS AND METHODS

Description of study area

Asella is situated at 6°59' to 8°49'N latitudes and 38°41' to 40°44' E longitude in Central Ethiopia, 175 km South East of Addis Ababa. The area covers 23674.72 km² representing only 2% of the total land surface of the country. Topographically, Asella province has high land escarpment and low land areas. Vegetation of the area changes with altitude and rainfall ranging from scattered trees and bushes to dense shrubs and bushes. Livestock are the major agricultural resources in the area and has livestock population of 82190 cattle, 51292 sheep, 11479 goats, 162015 poultry, 11,716 camels and 22055 equines (APEDO, 1999).

Study animal

The study population includes indigenous sheep raised in the Asella province and includes sheep grazed in extensive system, reared in in-door and those brought to Asella Veterinary Clinic. Sheep of 1 to 7 years of age and of both sexes, with extensive and semi-intensive husbandry system, dewormed (oral administration of Albendazole at 7.5 g/kg) and non-dewormed sheep; and additionally sheep with no clinical respiratory signs and those that appeared apparently healthy are included. For convenience, the age of every sampled sheep was categorized into young and adult (ESGPIP, 2000) using dentition and information from the owners.

Sample size and sampling methods

The desired sample size for the study was calculated using the formula given by Thrusfield (1995) with 95% confidence interval and at 5% precision. As pulmonary helminthosis prevalence of 54.6% (previous average prevalence of sheep lungworm infection in the areas recorded by the Asella Veterinary Regional Laboratory) was taken into consideration, a sample size of 381 sheep was considered. A stratified sampling method was used to sample

sheep. For postmortem examination, from sheep which was presented for slaughter at Asella hotels and/or restaurants, 71 sheep intact lungs and fecal samples were randomly selected. The desired sample size for the study was calculated using the same formula as for the coproscopic examination (except that the estimated previous average prevalence of pulmonary helminthosis in the area by postmortem examination is 4.86%).

Experimental procedures

For coproscopic examination purpose, about 10 to 15 g of fresh fecal pellets taken from the rectum of sheep were transported to Asella Regional Veterinary Laboratory within 4 h. Individual samples were processed by the modified Baermann technique described by Glovirina (1984) and Hanse et al. (1994). All larvae were identified morphologically as described by Soulsby (1982) and Urquhart et al. (1992). For postmortem intact lung incision, the lungs were removed with trachea immediately after slaughter, then put on clean metallic frailty and examination of adult lungworm was done as per the method described by William (1998). Hence, it was described as: after intact lung removed incision was made with fine blunt-pointed scissors and scalpel blade along trachea, bronchi and then bronchioles. Description was given by Soulsby (1982) used for identification of each adult lungworm morphology.

Closed type questionnaire survey was also carried out to interview individual owners to obtain general information about sheep age, husbandry practices, usage of previous anthelmintics administration and appearance of respiratory clinical signs.

Statistical analysis

A computer based statistical software package interceded (STATA-7) was used to carry out different statistical analysis. The prevalence variation of species within parameters as well as statistical significance of the association between risk factors and the infection was assessed using descriptive statistics and Chi-square (χ^2). Additionally, to make comparison between post-mortem (PM)-intact lung incision and PM-coproscopic examination, sensitivity and negative predictive value were used. For the purpose of this study, a 95% significant level was considered.

RESULTS

Overall prevalence

Of the total 381 sheep examined, 72.44% (confidence interval (CI)=95%; $\chi^2=3.1484$) were found to be infected with either one or more of the lungworm species. The lungworm species encountered during the study period were *Dictyocaulus filaria*, *Protostrongylus rufescens* and *Muellerius capillaries* which accounted for a prevalence of 12.3, 33.7 and 5.4%, respectively.

Prevalence of lungworm infestation by age and sex

The overall lungworm infection prevalence in young and adult sheep was accounted to be 70.7 and 73.2%, respectively; revealing no statistically significant variation ($\chi^2=0.2562$; $P>0.05$) between the two age groups. Lungworm infection in relation to the sex of sheep

Table 1. Prevalence of lungworm infection in ovine hosts in relation to hypothesized risk factors (variables).

Variable	No. of sheep examined	No. of sheep infected				Overall infection (%)
		<i>D. filaria</i> (%)	<i>P. rufescens</i> (%)	<i>M. capillaries</i> (%)	Mixed infection* (%)	
Age						
Young	116	3 (2.6)	34 (29.3)	7 (6.0)	38 (32.8)	82 (70.7)
Adult	265	31 (11.7)	59 (22.3)	8 (3.0)	96 (36.2)	194 (73.2)
						$\chi^2=0.26$
Sex						
Male	168	16 (9.5)	42 (25.2)	4 (2.4)	59 (35.1)	121 (72.2)
Female	213	18 (8.5)	51 (23.9)	11 (5.2)	75 (35.2)	155 (72.8)
						$\chi^2=0.03$
Husbandry system						
Extensive	244	25 (10.2)	69 (28.3)	12 (4.9)	82 (33.6)	188 (77.0)
Semi-intensive	137	9 (26.6)	24 (17.5)	3 (2.2)	52 (37.9)	88 (64.2)
						$\chi^2=7.23$
Anthelmintics usage						
Dewormed	130	8 (6.2)	18 (13.8)	5 (3.8)	39 (30.0)	70 (53.8)
Non-dewormed	251	26 (10.0)	75 (29.0)	10 (3.9)	95 (37.8)	206 (82.1)
						$\chi^2=34.18$
Resp. clinical sign						
Showing respiratory clinical sign	197	23 (11.7)	59 (29.9)	11 (5.6)	78 (39.6)	171 (86.8)
Apparently healthy	184	11 (5.9)	34 (18.5)	4 (2.2)	56 (30.4)	105 (57.1)
						$\chi^2=42.14$

*Infestation of sheep with more than one lungworm species.

accounted for 72.8 and 72.2% prevalence in female and male, respectively. Statistically, significant variation ($\chi^2=0.0262$; $P>0.05$) was not found between the two sex groups (Table 1).

Prevalence of lungworm infection by husbandry system and anthelmintics usage

The result of questionnaire survey revealed the effect of husbandry system on the prevalence rate of lungworm infection and it has statistically significant association ($\chi^2=7.2180$; $P<0.05$). The analysis of questionnaire survey from sheep owner also revealed the effect of anthelmintics usage on the prevalence rate of lungworm infection and the variation was found to be statistically significant ($\chi^2=34.1766$; $P<0.05$) (Table 2).

Prevalence variation with clinical respiratory sign

Of the total sheep sampled for the study; 184 of them were apparently found clinically healthy while the remain-

ing 197 sheep presented with clinical respiratory signs (like coughing and nasal discharge). Thus, there was a statically significant ($\chi^2=42.1404$; $P<0.05$) difference in lungworm infection prevalence between sheep those showing respiratory clinical signs and apparently healthy (Table 1).

Postmortem examination

With postmortem intact lung incision, 39.4% (28) sheep were found to contain lungworm infection. However, only 26.8% (19) sheep were detected to have the infection with the coproscopic examination and presented in lung incision to the coproscopic examination was 3:2.04. The estimated probability of missing sheep with lungworm infection using coproscopic examination was 47.4%.

DISCUSSION

In the present study, coproscopic results disclosed an overall lungworm infection prevalence of 72.44%

Table 2. Prevalence of lungworm infection by postmortem examination of slaughtered sheep.

Lung worm species	Larval encounter during coproscopic examination		Adult worm encountered during PM intact lung incision	
	Male	Female	Male	Female
<i>D. filarial</i>	1	3	1	4
<i>P. rufescens</i>	1	4	2	6
<i>M. capillaries</i>	1	3	2	3
Mixed infection	2	4	3	7
Total (%)	5 (20)	14 (30.43)	8 (32)	20 (43.48)

(276/381) in sheep of Asella province. Of the total infected, 12.3, 33.7, 5.4 and 48.6% prevalence was due to *D. filaria*, *P. rufescens*, *M. capillaries* and carried mixed infection with two or all the three species of lungworm, respectively. The overall prevalence result almost coincides with the previous Ethiopians researchers report, 70.7% prevalence in DebreTabor Awraja (Yohannes, 1989), 73.75% in Debre Birhan (Netsanet, 1992) and 71.3% in six district of Wollo at high altitude (Sefinew, 1999). But, it was lower than the observation made from the same area by Bekele et al. (1981) 59.4%, Wondwossen (1992) 58.8%, Paulos (2000) 52.54%, Mireteab and Aman (2011) 57.1% and Hasen et al. (2013) 55.10%. The studies analysis observed in other area of the country also disagrees very highly than with this study conducted in different parts of Ethiopia; 50% in Dessie and Kombolcha (Teffer, 1993), 13.24% in and around Mekele (Frewengel, 1995), 44.7% in BahirDar (Sisay, 1996), 53.6% in Northwestern Ethiopia (Amenu et al., 2006), 40.4% in Dessie and Kombolcha districts, Northern Ethiopia (Regassa et al., 2010), 32.7% at Gonder (Mekonnen et al., 2011) and 13.4% in Mekele town (Ibrahim and Degefa, 2012), while 83 to 99.5% in highland of Shoa province (Jovanovic et al., 1962). The possible explanation for such infection rate variation could be attributed variation in agro-ecology, altitude, rainfall, humidity and temperature difference and season of examination on the respective study areas, which favor or disfavor the survival of parasite larvae (Blood, 1976; Soulsby, 1982; Bradford, 2002).

An attempt was furthermore made to know the influence of age and sex on the overall prevalence of lungworm infection and there is no statistically significant difference ($P > 0.05$) in the infestation rate of all lungworm species. However, a high infection prevalence of overall lungworm was recorded in youngest than adults which is in agreement with the observations of Geotray (1962), Blood (1979), Soulsby (1982) and Mireteab and Aman (2011). This has been partly explained by the analysis given as though, infection with *M. capillaries* and *P. rufescens* did not show development of acquired immunity. The acquired immunity developed older sheep due to previous exposure of *D. filaria* and that recovered from the infection have better immunity against re-infection (Soulsby, 1982; Craig, 1998). In the present

study, sex dependent variation was not encountered, hence both sexes showed almost equal susceptibility to infection with lungworms. This was coinciding with research study reported by Addis et al. (2011), Nibret et al. (2011), Dawit and Abdu (2012) and Hasen et al. (2013), but disagree with report of Alemu et al. (2006) and Mireteab and Aman (2011). These may be due to the fact that improper distribution of sample selection between the two sexes (Paulos, 2000); or else most of the sampled females are not in preparturient period during the study time (Urquhart et al., 1996).

In addition, husbandry system revealed statistical significant variation on the infection prevalence of sheep. Higher prevalence of infection was noted out where the husbandry of sheep was extensive type than in the semi-intensive. This result coincides with that of the observation recorded by Sisay (1996) and Dhar (1982). The reason why sheep which have extensive management type has the highest infection prevalence can be explained due to the sheep in these managerial system that have higher chance to ingest the intermediate host (Snail and slugs) for *P. rufescens* and *M. capillaries* in large number; or are they possibly infested with larvae as well as easily obtained *D. filaria* from the herbage (Radostitis, 1994; Souls, 1982). Another possible explanation for the massive infection of sheep in extensive managerial type is that they were not supplied with appropriate nourishment which provides high computation, getting wide of lungworm infection (Kimberling, 1988). The variation with anthelmintic usage is clearly indicating as the non-dewormed sheep have high infection prevalence than dewormed counter parts. When the infection prevalence on anthelmintic usage base was subjected to analysis, the difference is statistically significant ($P < 0.05$). The observation noted on the dewormed sheep in this study was in agreement with the work of Yohannes (1989), Netsanet (1992) and Sefinew (1999). Even though the dewormed sheep revealed low infection prevalence compared to non-dewormed groups, about 53.8% of them are infected with lungworm. The reason behind this result probably, is that sheep which have only cough and/or tachypnoeic are usually in the prepatent stage of the disease or have small adult worm burden and the anthelmintic used for the treatment of these sheep may be only temporarily

suppress egg production of the adult worms (Blood et al., 1997; Urquhart et al., 1996).

The higher infection prevalence was recorded in sheep showing clinical respiratory signs than those apparently healthy and significantly higher ($P < 0.05$) prevalence infection was revealed on comparison of coproscopic larvae burden. The result coincides with the observation of Paulos (2000) and Hasen et al. (2013). *D. filaria* appeared with the highest prevalence and coproscopic larvae output in sheep with respiratory sign. This may indicate that *D. filaria* is responsible for clinical respiratory sign of sheep in Asella. However, *P. rufescens* infection may weaken the lung and assists in reducing the appearance of respiratory clinical signs (Geotray, 1956; Radostitis, 1994).

The observation of intact lung from slaughtered sheep revealed 39.4% infection, whereas 26.8% infection prevalence was discovered in the examination of intestinal pellet. Wondwossen (1992) and Teffera (1993) reported the prevalence of 60.5% in Asella area and 67.83% in Dessie and Kombolcha, respectively. Infection prevalence of 40.09% (Paulos, 2000) and 53.6% (Sefinew, 1999) were observed by coproscopic examination of intestinal pellets from sheep slaughtered in Chilalo area and six district of Wollo, respectively. The prevalence of coproscopic examination reported by the aforementioned two investigator have significant variation with their respective prevalence revealed by intact lung incision which accounted 72.8% in Chilalo area and 62% in the six district of Wollo. Thus, the statistical variation of infection prevalence between the two techniques observed by Paulos (2000) and Sefinew (1999) almost coincides with the present study.

In an attempt done to assess the value of coproscopic examination in estimating the worm larvae in infected sheep, it was found that, of the confirmed adult worm infection, only 67.86% infection prevalence was detected. It was also shown that there was no statistically significant correlation ($P > 0.05$) between adult worm recovery and faecal larvae outputs for any of the three lungworm species as well as mixed lungworm species. One of the probable reasons attributed for such difference could be that those larvae which reach the lung of sheep remain in the parenchyma and became encysted in fibrous nodules. Because, such nodules may not contain adult worm of both sexes; fertile egg may not be deposited in air passage (Radostitis, 1994; Blood et al., 1997). Therefore, the finding of the present study strongly supports that coproscopic examination had limited value in estimating the burden of lungworm infection in sheep and interpretation of coproscopic value. Hence, coproscopic examination requires serious cautions. In addition, it was more appropriate to use coproscopic examination for clinical purpose than in scientific investigations. The other reason may be that the larvae are found (50 to 1000 g) only in the faces/pellet of patent cases, otherwise, in the rest phase it may not be dispersed throughout the pellet (Urquhart et al., 1996; Rose, 1965). Likewise, through

PM-intact lung incision, the present study revealed that about 60.6% of sheep were found to be apparently healthy, but it may not mean that only 28 sheep were infected with lungworm. This is because of the fact that, although sheep are infected with lungworm at the penetration, prepatent and post-patent phases, the adult worm may not be observed with the aid of necked eye or else there be the case of completion of sufficient duration and self-cure, at these time no adult worm was visible and careful microscopic examination of bronchial mucus is to find out the larvae (Urquhart et al., 1996; Soulsby, 1982; Blood et al., 1997).

In conclusion, the infection prevalence of lungworm has no statistical significant association with the age and sex of sheep. Husbandry system and anthelmintics usage has great contribution on the prevalence of lungworm infection. Although most sheep infected with lungworm are clinically characterized by respiratory signs; some sheep appeared apparently healthy. Both coproscopic and postmortem examination results justify high prevalence of lung worm infection during the study period. The postmortem examination also implies as the coproscopic examination has limited value in terms of estimating the prevalence of lungworm infection in live sheep.

ACKNOWLEDGEMENTS

The authors are indebted to the Asella Regional Veterinary Laboratory for material and logistic supports, and cooperation of the staff members to bring this research to completion.

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