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PREVALENCE OF OVINE FASCIOLOSIS AND ITS ASSOCIATED RISK FACTORS ON SLAUGHTERED SHEEP AT HOTELS OF MERAWI TOWN NORTH WEST OF ETHIOPIA

Desalew Salew[1]* and Dr. Abaineh Munshea[2]

[1]Amhara Public Health Institute, Bahir Dar, Po.box 477, Ethiopia.
[2]Bahir Dar University Faculty of Science, Department of Biomedical Science, Bahir Dar, Po. Box 79, Ethiopia.

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***Corresponding Author**
Desalew Salew
Amhara Public Health Institute, Bahir Dar, Po.box 477, Ethiopia.
desalew131@gmail.com,

ABSTRACT

A cross-sectional study was conducted in Merawi town from January to May with the objective of determining the prevalence of ovine fasciolosis and identify its risk factors in the study area. The fecal samples of (384) sheep were collected and tested to identify eggs of *Fasciola* spp. Moreover, the liver of this sheep were examined for the presence of adult *Fasciola* worm. The overall prevalence of *Fasciola* species infection of slaughtered sheep was 18.75%. During sample collection, information concerning history of sheep was collected using semi-structure interview. The results of the study showed that there is a significant ($p<0.05$) difference in the prevalence of ovine fasciolosis in; age groups, FAMACHA eye color, body condition score, agro-ecology, nature of grazing area, practice of mixed grazing and deworming history. The higher prevalence being observed in old aged, white eye color, poor body condition, high risk "Dega" areas swampy grazing areas and with an often practice of mixed grazing respectively. Sex, breed type, feeding management, Awareness of sheep owners have not showed significant ($p>0.05$) difference in the prevalence of fasciolosis. Animals should be prevented either by keeping them away from these area or by fencing of dangerous areas and swampy areas should be well drained and also Strategic anthelmintic treatment with appropriate flukicide drug should be administered.

KEYWORDS: ovine; fasciolosis; prevalence; Merawi.

1. INTRODUCTION

1.1 Back ground and justification

Ethiopia has the largest livestock inventories in Africa, with livestock ownership currently contributing to the livelihoods of an estimated 80% of the rural population. Ethiopia's economy depends heavily on the agricultural sector. Agriculture accounts for 83.4% of the labor force, about 43.2% of the Gross Domestic Product (GDP) and 80% of exports.[1] The regular droughts combined with poor cultivation practices, make Ethiopia's economy very vulnerable to climatic changes. However, the full exploitation of these huge resources is hindered by a combination of factors such as drought, poor genetic potential of animals, traditional system of husbandry and management as well as the presence of numerous diseases.[2] Production of sheep for meat, milk, wool, hair, skin, and manure is an attractive agricultural enterprise for Ethiopian farmers because of the relatively low cost of breeding stock and the high productive rate of sheep. Sheep require minimal inputs and maintenance costs to live in various conditions, from desert to humid rainforest.[3] 99.9% of the total sheep population is indigenous breeds that are owned by resource poor smallholder farmers and pastoralists under traditional systems.[4] Ethiopia is the second most populous country in sub-Saharan Africa. Sheep serve as a major means of livelihoods of poor livestock keepers, and thereby contribute to poverty reduction and means of attaining sustainable agriculture and food security. Very often, there are no banking facilities in rural areas and an easy way to store cash for future needs is through the purchase of sheep and goats. In fact, in some areas, small ruminants are described as the 'village bank'. It has to be noted that this is beyond the cash value of the animal.

Parasitism is one of the major bottlenecks to livestock development in tropics.[5] Among many parasitic problems of farm animals, fasciolosis caused by *Fasciola hepatica* and F. *gigantica*, is one of the most prevalent helminthes infections of ruminants in different parts of the world including Ethiopia.[6] Infestation is highest in livestock particularly sheep.[7]

F. hepatica has cosmopolitan distribution, mainly in temperate zones, while *F. gigantic* is common in tropical regions of Africa and Asia. Thus, the two Fasciolid species overlap in many Africa and Asian countries.[5] *F. hepatica* and *F. gigantica* are responsible for wide spread morbidity and mortality in sheep, Deaths account for only a part of this loss. Other significant losses in sheep include reduced production and quality of wool, poor growth rate of lambs and increased costs for replacement stock.[8] Recently worldwide losses in animal productivity due to fasciolosis is conservatively estimated at over US$3.2 billion per annum with prevalence ranging from 11.5% to 87.0%. In Ethiopia, the annual loss due to ovine fasciolosis was estimated to be 48.4 million Ethiopian Birr of which 46.5, 48.8, and 4.7% were due to mortality, loss of productivity and liver condemnation respectively.[9] The prevalence and economic significance of fasciolosis has long history and was reported by several researchers like.[10,11] Likewise; different researchers reported the prevalence of fasciolosis in sheep to be 13.2%.[9] 26 49%.[12,13] in different parts of Ethiopia.

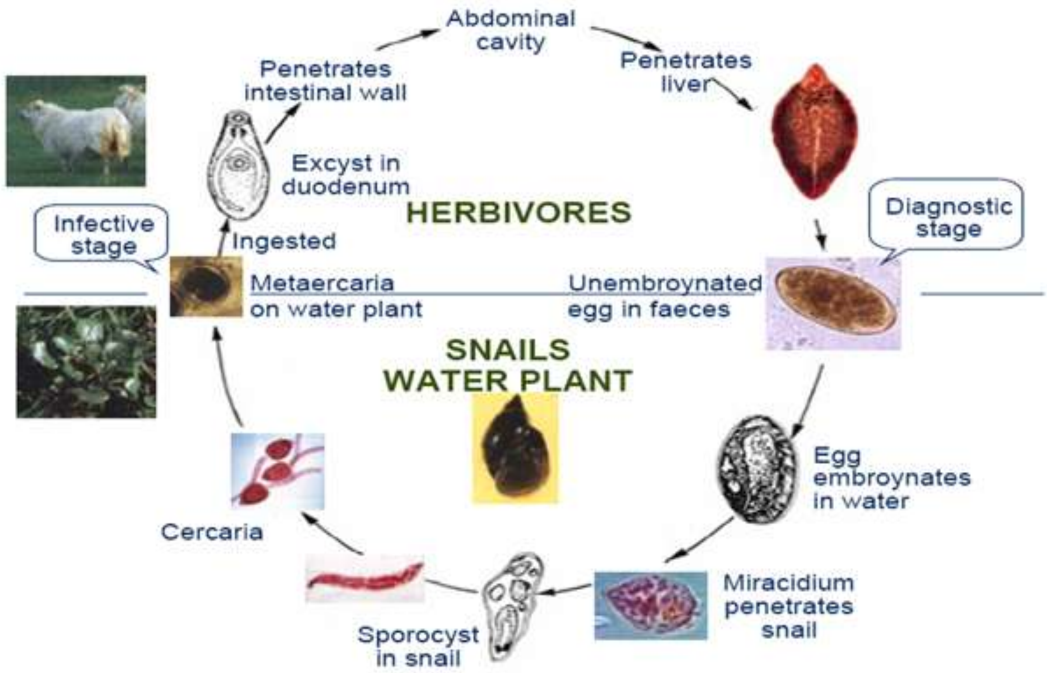

Figure 1: Life cycle of *Fasciola* species (Adapted and modified from:www.soton.ac.uk/…/fasciola/CEB_ posterscan.jpg).

It is evident that water resources can play a significant role in improving food security and household income.[14] Agricultural production that depends on rain is mostly aimed at self-provision and this kind of production system is severely affected by climatic irregularities. An effective method to reduce vulnerability of climatic irregularities and coping with periods of inadequate rainfall is by the use of irrigation for the agricultural production.[15] Wrongly, planned irrigation, however, hinders production and results in wasted effort by favoring the incidence and spread of common waterborne animal diseases such as fasciolosis.[14] Current trend towards food self-sufficiency is by irrigation and implementations of irrigation projects are expected to bring about changes in land use patterns, and intensification of labor.[15] The increasing number of dams and irrigation canals built to boost energy and food production will increases the number of potential snail habitats and with them the risk and incidence of fasciolosis. Several studies have been conducted on the distribution and prevalence of ovine fasciolosis in Ethiopia, although there are still several localities in which epidemiological information is not available including (Mecha) Merawi the area of my study.

In the study area, small scale traditional practices using small streams and large scale irrigational practices using Koga dam are the common life supporting activities by the community of Mecha district. These activities may create suitable condition for survival of snail intermediate hosts and are expected to increase the risk of water born trematode parasites. In addition to this, the sheep in the study area usually graze at natural fields and drink at ponds that are suitable for the snail intermediate hosts of *Fasciola*.[16] Mecha district (Merawi) is one of the areas where environmental conditions and altitude of the area is conducive for the occurrence of fasciolosis However little information is available about its prevalence and its economic significance in the study area. Any intervention that improves the productivity of sheep is important in creating wealth and improving the standard of living of resource-poor farmers of Ethiopia. Therefore, there is a need for the development of good preventive and control measures against ovine facioliasis in the study area through improved programs.

Thus, the purpose of this study is to determine the current prevalence of ovine fasciolosis and its associated risk factors in the study area at the current time. Specifically, the study is initiated to provide information about the prevalence of ovine fasciolosis in sheep slaughtered by hotels of Merawi town. More over to help the stakeholders to take actions, prevention and control measures, and the study may also help as a source of information for future further research on the field in the study area.

1.2 Objectives

1.2.1 General objectives

- To determine the prevalence and associated risk factors of ovine fasciolosis among slaughtered sheep in hotels of Merawi town.

1.2.2 Specific objectives

- To identify the major *Fasciola* species in sheep slaughtered at different hotels in Merawi town.
- To evaluate the association between the prevalence of ovine fasciolosis with age, sex, body condition, FAMACHA eye color, and to relate practices of sheep owners and environmental conditions with ovine fasciolosis.
- To assess the association between mean worm burden, FAMACHA eye color and body condition score of the studied animals with the prevalence of fasciolosis.

2. MATERIALS AND METHODS

2.1 The study area

The study was conducted in Merawi town the main town of Mecha district of west Gojjam zone in Amhara regional state, North west of Ethiopia located about 525 km North West of Addis Ababa and 34 km South East of Bahir Dar the capital city of Amhara region. In Mecha, district the climatic condition, alternate between along summer rainfall and winter dry season with mean annual rainfall of 1500-2200 mm. The mean temperature is between 24-27 °C and altitude ranges from 1800 to 2500 m.a.s.l. The study area is located at latitude 10°30' N and longitude 37°29' E. The land is covered by Savanna grassland and bush lands vegetation. Agriculture is the main economic sector in the study area. The main agricultural activities currently practiced include irrigation (Modern and traditional) and mixed farming. The major agricultural products seasonally harvested include sorghum, maize, teff, wheat and other legume groups. In this district there are 192, 556 cattle, 148 971 ovine, 23, 106 equine and 204, 181 poultry.[17]

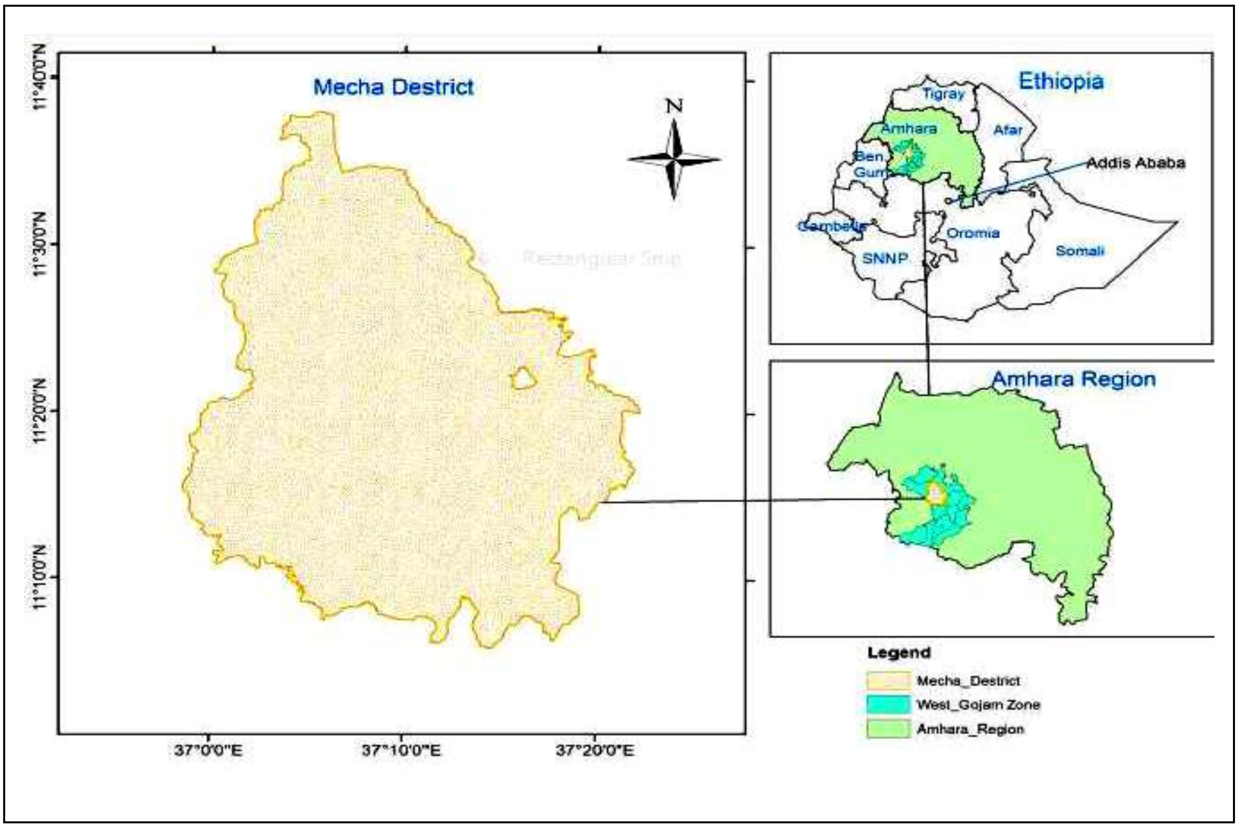

Figure 2: Map of the study area.

2.2 Study animals

The study was conducted on indigenous sheep of both sex groups that have different body condition, developed under different management system and come from different agro ecological areas of the district that are bought and slaughtered at selected hotels and restaurants of Merawi town.

2.3 Sampling procedure

The study houses were selected purposely because of their more customers (clients) and more mutton consumption. Excluding the fasting days, Wednesday and Friday, because of minimal slaughter rate at these days, four slaughter days are selected randomly by lottery system from the remaining five days.

2.4 Study design and period

A cross-sectional study was conducted from December (2015 to April 2016) involving indigenous sheep to determine the prevalence of ovine fasciolosis in the study area and to assess the association between ovine fasciolosis and associated risk factors.

2.5 Sample size determination

To determine the sample size, prevalence of 50% for sheep at Merawi was taken into consideration since there was no previous research made on the prevalence of ovine fasciolosis in the study area. The desired sample size for the study was calculated using the formula given by.[18] with 95% confidence interval and at 5% absolute precision.

N=1.962xPexp (1-Pexp)/d2

Where N= required sample size

1.96=the value of Z at 95% confidence level

Pexp= expected prevalence

d= desired absolute precision (5%)

Therefore, at 50% expected average prevalence, the sample size is 384.

2.6 Data collection

2.6.1 Ante-mortem Inspection

Sheep owners who brought and sold their sheep to hotel owners for demand of hotel consumption were interviewed. In the interview, factors of epidemiological relevance such as age of the sheep, grazing management system, the agro-ecological area where the sheep had grown and came from, practice of mixed grazing, experience of canal cleaning, knowledge of the sheep owners on the disease and deworming history on the sheep were included. Information on the agro-ecology of the area has been obtained by classifiying the areas "kebeles" in to nearly three categories such as, high risk, medium risk, and less risk. "Kebeles" with high surface water and, or 'Dega' kebeles were classified as high risk; "kebeles" with some surface water and, or 'Woine Dega' were classified as medium risk and "kebeles" with no surface water and dry areas or 'Kola' were classified as low risk.

Pre-slaughter examinations of sheep were conducted in order to determine the breed type, age, and body condition of the sheep. The age grouping was performed based on arbitrary classification according to.[3] It was based on the information obtained from the sellers and dentition of the sheep. Those that have not erupted permanent incisor teeth, were classified as young, while those with one pair or more permanent incisor teeth will be classified as adults and sheep with more than one pair of permanent incisor teeth and fallen out teeth were classified as old.[3,18] The study animals were selected by simple random sampling among sheep slaughtered at the same day on the same sampling house. During the study, body condition score, was recorded. The body conditions were grouped in to three and animals that score 1- 2, 3 and 4- 5 are classified as poor, medium, and good body conditions respectively. The animal's origin based on its agro-ecological condition, sex, age, and breed of animals were recorded according to.[18] The age of sheep were classified in to three; sheep with the age of up to 1 years as young and sheep between one and two years as adult and more than two years old.[18] The FAMACHA eye-color score estimation was used during data collection.[19] The color of the mucous membrane of the lower eyelid of each animal was examined in good natural light and compared to the colors of the FAMACHA chart.[19] Each sheep was scored on a scale of 1–5 (1. red, non-anemic; 2. red-pink, non-anemic; 3. pink, mildly anemic; 4. pink-white, anemic; 5. White, severely anemic). FAMACHA eye-color score values of 3, 4 and5 or 4and 5 were considered anemic.

2.6.3 Fecal Laboratory Technique

The equipments and tools that were used for the study were beaker, strainer, measuring cylinder, mortar and pestle, test tube, test tube rack, microscope slide, cover slip, microscope, centrifuge and refrigerator. According to.[20] the investigation procedure for the examination of *Fasciola* egg will be as follows: About 3gram of feces were collected from the rectum of each selected sheep using sterile surgical gloves or a top uncontaminated part of fresh feces was taken. Moreover, the sample was collected in a bottle. The fecal sample was crushed with mortar, pestle and 40-50ml of tap water was added, and mixed with fork and filtered the fecal suspension through a tea strainer into a beaker, the filtered material was poured into a centrifuge tube. The centrifuge tubes centrifuged the sample at about 1500 rpm for three minutes the supernatant fluid was discarded carefully, transferred a small amount of the top of the layer of the sediment to a microscope slide and covered with cover slip, then examined under 40xs magnification power. Coproscopy was used to detect the presence of *Fasciola* eggs using the standard sedimentation techniques recommended by.[20] Fecal samples were examined by sedimentation techniques for the presence of fluke eggs. Identification of fluke egg from *Paramphistome* egg was done based on morphology and by using methylene blue solution.[21]

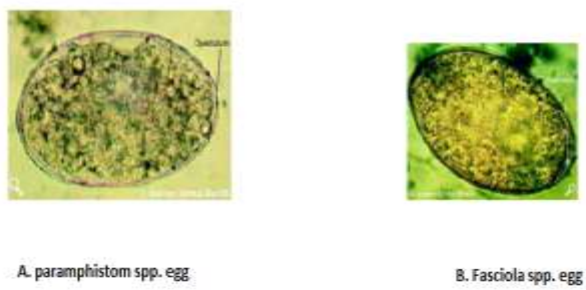

Figure 3: The morphology of paramphistomum species and fasciola species egg (Adopted from Michael *et al.*, 2005).

2.6.4 Postmortem examination of liver

The prevalence of fasciolosis was investigated by postmortem examination technique from liver parenchyma and major bile ducts to recover the young flukes and adult parasites, respectively. The previously identified animals and their livers were carefully supervised and examined, Postmortem examination of liver and associated bile duct was carefully performed by visualization and palpation of the entire organ followed by transverse incision of the organ across the thin left lobe in order to confirm the presence of the parasites.[7] Data were obtained by macroscopic examination of most livers according to.[23] in which the liver size, consistency, dilatation of the bile ducts, presence of sinus tract due to migration of the immature flukes, fibrous tissue formation, presence of immature flukes in the liver parenchyma and mature. Flukes within the bile ducts were examined during the routine post-mortem meat inspection. The liver was palpated and the biliary tracts were milked for consistency and presence or absence of flukes respectively. Incisions were also made on the parietal surface of the liver and along the length of the bile ducts to observe fibrosis and presence or absence of flukes. Each mature flukes were identified to species level according to its shape and size morphologically identified as *Fasciola hepatica* and *Fasciola gigantica* according to.[21] All intact immature and mature flukes was found.[21] Liver fluke burden was determined by counting the recovered *Fasciola* parasite. The liver was cut in to slices of about 1cm thick and put in a metal trough (plate) and then the heads of the flukes were counted. The presence of more than 50 flukes per liver indicates high pathogenicity.[21]

2.7 Data Quality Control

To insure the quality of laboratory results, the standard operating procedures of the laboratory was followed every time and tests were performed under the supervision of experienced veterinary laboratory technologist. A structured interview was adapted from related study and according to the objective of the study and local situation. Information on the previous history of sheep, and other risk factors were obtained using the interview.

2.8 Data Analysis

All raw data generated from this study was coded and entered in Microsoft Excel spreadsheet for data analysis using SPSS software version 20. Descriptive statistics was used to determine the prevalence of the parasite. Chi-square ($\chi 2$) test was used to assess the association of the prevalence of ovine fasciolosis with in associated risk factors such as age, nature of grazing area, irrigational status, agro ecology, deworming history, breed and body condition score of the animals. Mean comparisons was used to compare means and determine the association of the mean worm burden of infected sheep with FAMACHA eye color score and body condition score. Chi-square ($\chi 2$) was used to determine the risk of infection prevalence with feeding management, sex and breed type of sheep. Statistical significance will be set at $P < 0.05$ to determine whether there are significant associations between the parameters measured and groups.

3. RESULTS

3.1 prevalence of fasciolosis in agro-ecological conditions

Ante-mortem and postmortem examinations were carried out on 384 sheep to determine *Fasciola* infection, of which 72(18.75) were found positive for fasciolosis in the study area. The prevalence of ovine fasciolosis in the three agro-ecological conditions of the the study area was 40.29%, 27.41% and 5.69% in 'Dega', 'Woina Dega' and 'Kola' agro-ecologies of the study area. The prevalence of fasciolosis varied significantly among the three agro-ecologies (χ^2=48.116; P=0.000).

Figure 4: The adult stage morphology of *Fasciola hepatica* and *Fasciola Gigantica.* Source: a photo of adult *Fasciola* parasite found in liver of infected sheep slaughtered at Hotels of Merawi town by Desalew Salew.

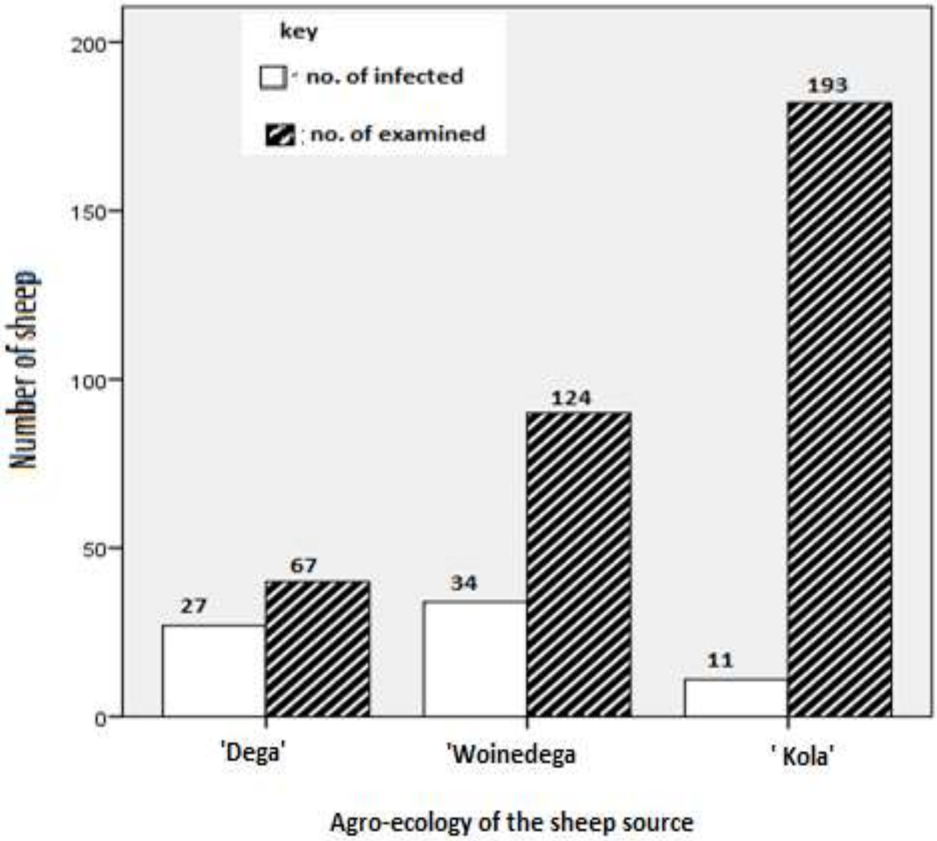

Figure 5: Prevalence of ovine fasciolosis at areas of different risk condition.

3.2 Prevalence of ovine fasciolosis based on feeding management

From a total of 206 sheep having an exposure of many hours of free grazing and little hours of home feed, 32(8.3%) were found positive. From 178 sheep that had an experience of completely free grazing 40(10.4%) were found positive for fasciolosis. However no statistically significant (χ^2=3.017; p=.089) association was observed between prevalence of ovine fasciolosis and feeding management of sheep

Table 1: Prevalence of ovine fasciolosis based on different back history and environmental Conditions of sheep.

Factor		No. of Examined	No. of positive	% prevalence	95%.CI.	DF.	χ^2	p. value
Agro-Ecology	Dega	67	27	40.29	(0.00-0.000)	2	48.12	0.000
	Woinedega	124	34	27.41				
	kola	193	11	5.69				
	total	384	72	18.75				
Nature of grazing area	Very swampy	143	35	24.47	(0.000-0.008)	2	11.65	0.000
	Partial-swampy	160	32	20				
	Non swampy	81	5	6.2				
	Total	384	72	18.75				
Irrigational status of grazing area	Highly irrigated	186	43	23.12	(0.005-0.032)	2	8.546	0.018
	semi- irrigated	144	26	18.05				
	non-irrigated	54	3	5.55				
	total	384	72	18.75				
Practice of Mixed grazing	Very often	218	51	23.4	(0.016-0.052)	2	8.024	0.034
	Some times	158	21	13.3				
	Not at all	8	0	0				
	Total	384	72	18.75				
Deworming history of sheep	Two or more	31	1	3.22	(0.003-0.028)	2	9.343	0.016
	Once	44	4	9.1				
	Not at all	309	67	21.68				
	Total	384	72	18.75				

Age in year.	<one year. B/n 1 & 2 > two year Total	65 151 168 384	4 32 36 72	6.15 21.2 21.4 18.75	(0.002-.024)	2	8.152	0.017
Famacha	White Pink Red Total	39 281 64 384	10 59 3 72	25.64 20.99 4.68 18.75	0.000-0.008	2	10.454	0.003
Canal cleaning experience	Often Seldom Not at all Total	16 65 303 384	1 8 63 72	6.25 12.3 20.8 18.75	(0.108-0.178)	2	4.241	0.143
Body condition score	Poor Medium good total	32 294 58 384	9 59 4 72	28.12 20.06 6.89 18.75	0.008-0.039	2	7.531	0.023
Awareness of sheep owners	Enough Limited None Total	1 20 363 384	0 1 71 72	0 5 19.55 18.75	(0.266-0.359)	2	2.869	0.313

3.3 Prevalence of fasciolosis based on awareness of sheep owners

A total of 384 sheep owners were interviewed about their awareness in the biology and epidemiology of fasciolosis and 5% (1/20) and 19.6% (71/363) of the sheep owned by owners who had limited awareness and those who do not have any idea about fasciolosis tested positive respectively. Though a fourfold of *Fasciola* infection was detected among sheep owned by those who lack knowledge of the disease, no significance association was found between prevalence of ovine fasciolosis and awareness this disease ($\chi 2$ =2.869; p=0.313).

3.4 Prevalence of ovine fasciolosis based on grazing area of sheep

Prevalence of ovine fasciolosis in sheep grazing on very swampy, partially swampy and non-swampy sites of the study area was 24.5% (35/143), 20% (32/160) and 6.2% (5/81) respectively. Analysis showed that there was a statistically significant ($\chi 2$=11.652; p=0.000) difference on the prevalence of ovine fasciolosis among sheep grazing at the three sites of the study areas under consideration.

3.5 Prevalence of fasciolosis based on the irrigational status of grazing areas

The prevalence of the infection in sheep grazing on highly irrigated areas was the highest 23.11% (43/186). It was 18.1% (26/144) and 5.5% (3/54) in sheep grazing on areas which are moderately irrigated and with little or no irrigation respectively. Statistically significant ($\chi 2$=8.546; p=0.018) variation in the prevalence of *Fasciola* infection was observed among sheep grazing on places with different irrigational status.

3.6 Prevalence of fasciolosis based on the experience of mixed grazing

From the data on sheep management system 218 sheep had an often experience of mixed grazing of which 70.8% (51/218) were found positive for *Fasciola*. A prevalence of 29.2% (21/158) was observed from 158 sheep with a seldom experience of mixed grazing and none of them were positive from eight sheep with no experience of mixed grazing. Statistical analysis showed that there is a significant ($\chi 2$=8.024; p=0.034) variation on the prevalence of ovine fasciolosis among sheep with different experience of mixed grazing. (Table 2).

3.7 Prevalence of fasciolosis on the basis of canal cleaning experience

Prevalence of ovine fasciolosis on sheep that came from an area with an often practice of canal cleaning was 6.2% (1/16). Moreover, a prevalence of 12.3% (8/65) and 20.8% (63/303) was observed on sheep that came from an area of a seldom and no experience of canal cleaning respectively. Statistical analysis showed that there is no significant ($\chi 2$=4.241; p=0.143) variation in prevalence of ovine fasciolosis among sheep that came from areas of different canal cleaning experiences.

3.8 Prevalence of fasciolosis on the basis of deworming history of sheep

Of the total 384 sheep included in the present study 8.07% (31/384), 11.45(44/384) 80.46% (309/384) had a two or more time, one time and no deworming history respectively. The lowest 3.22% (1/31) prevalence of ovine fasciolosis was detected among the sheep which were dewormed at least two times followed by 9.1% (4/44), 20.2%(67/309) among sheep having one time and no history of deworming respectively. There was statistically significant ($\chi 2$=9.343; p= 0.016) association between prevalence of the disease and deworming history of the sheep as shown below (Table 1).

Table 2: Prevalence of fasciolosis on different natural conditions of sheep.

Factor		No. of Examined	No. of positive	% preval.	OR.	95%.CI.	DF	χ^2	p. value
Sex	Male	336	62	18.45	0.86	0.407-1.819	1	0.156	0.694
	Female	48	10	20.8					
	Total	384	72	18.75					
Breed	Washera	348	66	18.96	1.17	0.468-2.926	1	0.113	0.827
	Farta	36	6	16.66					
	Total	384	72	18.75					
Feeding management	home and field.	206	32	15.53	0.634	0.379-1.063	1	3.017	0.089
	field grazer	178	40	22.47					
	total	384	72	18.75					

3.9 Prevalence of ovine fasciolosis based on sex

Difference in *Fasciola* species infection was observed in male and female sheep. Gender wise almost similar rates of infection 18.45% (62/336) in males versus 9 /48 (20.8%) was found and not statistically significant (χ^2 =0.156; p=0.694).

3.10 Prevalence of ovine fasciolosis based on breed type

The prevalence of ovine fasciolosis in Washera breed is 19.0 % (66/348) and it was 16.7% (6/36) among Farta breed type. Statistically no significant (χ 2=0.113; p=0.827) variation of ovine fasciolosis between breeds was obtained.

3.11 Prevalence of ovine fasciolosis on the basis of age of sheep

The prevalence of *Fasciola* species in different age groups was 1% (4/65) in sheep aged less than one year 8.3 % (32/151) among sheep aged between one and two years old. In addition, it was 9.4 % (36/168) in sheep older than two years. Statistical analysis showed that there is a significant (χ2=8.152; p=0.017) variation in the prevalence of ovine fasciolosis between age groups of sheep as shown below in (Table 1).

3.12 Prevalence of ovine fasciolosis on the basis of body condition of sheep

The prevalence of infection was 28.1% (9/32), 20.1% (59/294) and 6.9% (4/58) among sheep with poor, medium and good body condition scores respectively. Statistically significant (χ2=7.531; p=0.023) variations on the prevalence of *Fasciola* species in different body condition score of animals was observed.

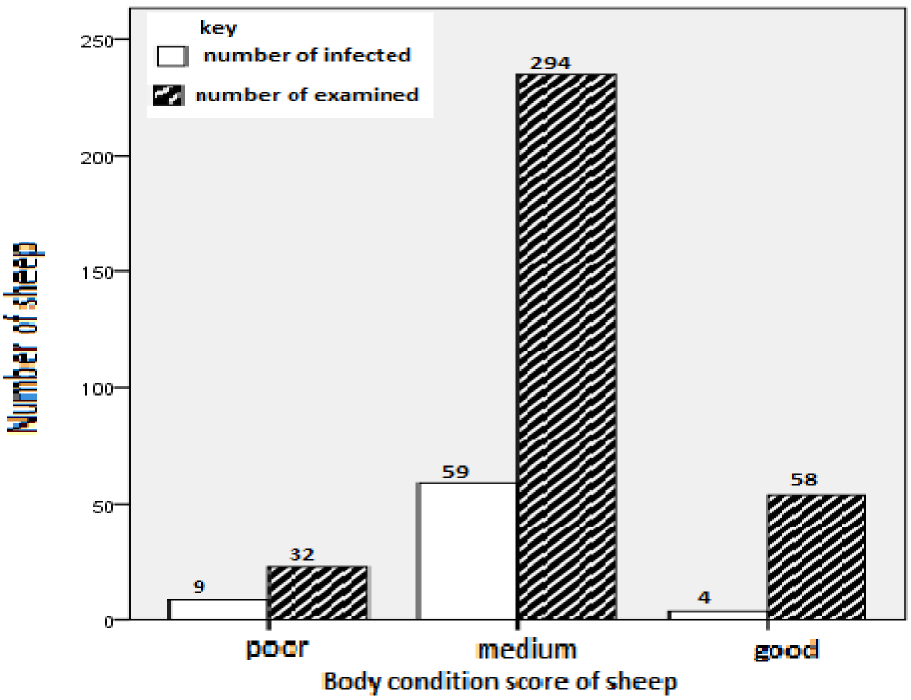

Figure 6: Prevalence of ovine fasciolosis based on body condition of sheep.

3.13 Prevalence of ovine fasciolosis on the basis of FAMACHA eye color score

Prevalence of *Fasciola* species based on FAMACHA eye color score showed that 25.6% of infection was detected among sheep with in white FAMACHA eye color group, 21% from pink FAMACHA eye color, and 4.7%from red FAMACHA eye color. The association between prevalence of ovine fasciolosis and FAMACHA eye color scores was statistically significant ($\chi2$=10.454; p=0.003).

3.14 The worm burden of different Fasciola species in infected sheep

As it is shown in the (Table below) from the total 72 *Fasciola* infected ovine livers, the prevalence of *Fasciola hepatica* was 65.28 % (47/72) *Fasciola gigantic* with a prevalence of 20.83%(15/72), mixed infection with a prevalence of 8.33% (6/72) and that of unidentified immature worms with a prevalence of 5.55% (4/72). *Fasciola hepatica* has the highest total worm burden 69.9% (535/765). The total mean worm burden was 10.63 per infected liver.

Table 3: The worm burden of different Fasciola species in slaughtered sheep.

Species of fasciola	Number of cases	% of Total N	Total worm burden	% of Total Sum	Mean worm burden	Standard deviation
Fasciola epatica	47	65.3%	535	69.9%	11.38	5.080
Fasciola gigantic	15	20.8%	120	15.7%	8.00	5.292
Mixed infection	6	8.3%	69	9.0%	11.50	4.135
Unidentified	4	5.6%	41	5.4%	10.25	8.180
Toatal	72	100.0%	765	100.0%	10.63	5.309

3.15 Mean worm burden and FAMACHA eye color score of infected sheep

There was a statistically significant (p=0.002) difference in worm burden among sheep with different anemic status, the mean worm burden was 15.00, 10.2, and 4.33 for (white-eye color), anemic, (pink eye color) mildly anemic and non -anemic (red eye color) FAMACHA eye color score of sheep respectively. The mean worm burden of anemic sheep is higher than the mean worm burden of mildly anemic and non-anemic sheep.

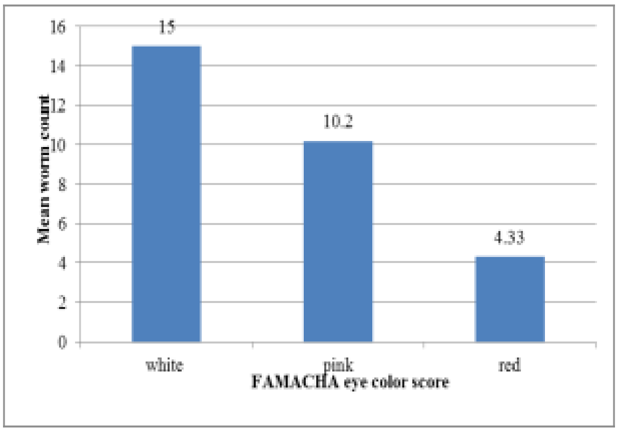

Figure 7: association between FAMMACHA eye color and mean worm burden of infected sheep.

3.16 Association of the mean worm burden and body condition score of sheep

Statistical analysis showed a mean worm burden of 17.33, 9.93 and 5.75 in sheep with poor, medium, and good body condition score respectively. As indicated from the analysis there was a statistically significant (p=0.000) variation on mean worm burden between body condition scores of sheep (figure 8).

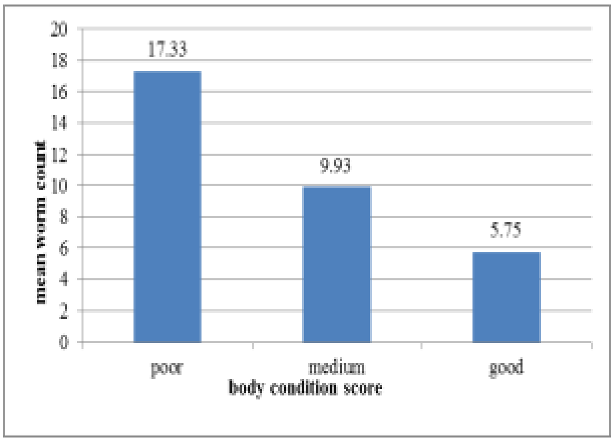

Figure 8: association between body condition score and mean worm burden of infected sheep.

3.17 Test of agreement of fecal examination with liver examination

The sensitivity and specificity of direct sedimentation technique is calculated from the number of positive and negative fecal tests in sheep with or without the fluke in their liver. In the present study, 59 cases from 72 liver positive cases (livers with flukes) were found to have eggs in their feces. Moreover, 13 liver positive cases (liver with flukes) were found to have no eggs in their feces. The agreement of the two tests were done calculating kappa that showed (k=0.881 at p=0.000) Statistical analysis showed that there is a statistically significant agreement that is 81.94% agreement between fecal and liver examinations as shown in (Table 4).

Table 4: Test of the agreement of fecal examination with liver examination.

Fecal examination	Presence of fasciola species in ovine liver		
	Fluke present	Fluke absent	Total
Egg present	59	0	59
Egg absent	13	312	318
Total	72	312	384

4. DISCUSSION

Ethiopia has the largest livestock population in Africa and this sector has been contributing considerable portion to the economy.[24] Fasciolosis is an important zoonotic disease of domestic livestock, especially cattle, sheep, and goat, as well as occasionally man. Several studies showed that ovine fasciolosis in particular poses the major constraints to small ruminant production in the country.[24]

The overall prevalence of ovine fasciolosis in the present study was 18.75 %. The prevalence of fasciolosis in the current study is in line with the previous report by.[25] who reported prevalence of 18.2 % in Dembecha District, northwest Ethiopia. The present finding is also nearly similar to the findings of.[26] who found the prevalence of 15.8% in and around Bahir Dar. The similarity in the prevalence rate of ovine fascioliasis in different parts of the country and year may be due to the similarity of the climatic condition such as altitude, rainfall, temperature, humidity, and management system.

The prevalence of *Fasciola* species infections in Merawi is higher than previous reports 4.38% prevalence in Pakistan,[27] 10% prevalence in Egypt by,[28] and 5.8% prevalence reported at Amol city abattoir in northeastern Iran,[29] and 0.35% prevalence reported by in northeastern Iran.[30]

In contrast to the present finding, significantly higher prevalence rates of ovine fasciolosis were reported from different parts of Ethiopia, including 70.20% in Lalo Mider district,[31] 54.17% in Debreberhan,[32] 48.21 % from Debreberhan Sheep Breeding and Forage Multiplication Center,[33] and 49% prevalence in and around Dawachefa.[12] This disparity may be due to different agro-ecological conditions, traditional pasture management practices, the pattern of movement of the animals from grazing near water logged areas, and agricultural irrigation practices during rainy season. The variation in prevalence between the different locations was also likely due to the differences in sampling time, variation in accessibility of sheep to swampy communal grazing areas, and agricultural irrigation practices.

Analysis of the prevalence of fasciolosis among sheep raised in three different agro-ecologies of the study area showed significantly higher rate of infection among sheep raised in “Dega” agro-ecology (p=0.000). This may be because of the presence of the existence of permanent surface water that may favor high infection prevalence. In this regard,[34] and,[35] have made similar observations. In this study, no statistically significant difference in the prevalence of fasciolosis was seen in sheep with a completely free field grazing experience and in those with partial field grazing experience (p=.089). Nevertheless, a higher prevalence of fasciolosis is expected among sheep having in a complete field grazing habit than those having partial field grazing habit. This may be because of the longer exposure of completely free grazer sheep to the metacercaria of the parasite than sheep that are partial field grazers.

Although, there was no statistically significant difference, almost four times higher *Fasciola* infection was detected in sheep owned by those who have no idea about fasciolosis than those who have limited knowledge about fasciolosis. The observation of,[36] is in support of the present finding in which they noted that knowledge of sheep owners about the nature of disease problems, symptoms of diseases and their prevention and control, and the life cycle of parasites is necessary in taking appropriate disease prevention and control measures.

In the present study, sheep which were allowed to graze in very swampy area were the most infected with *Fasciola* species than those grazing in partially and non-swampy places of the study area (p=0.000). This agrees with the observation of,[37] who noted that the presence of swampy or waterlogged areas is suitable for *Fasciola* to perpetuate and increase the chance of infection of sheep grazing in such areas. Sheep that graze and drink water around marshy and stagnant water areas, which were favorable environment for the growth and development of snails as well as *Fasciola* species, are more at risk than those that are managed at drier areas.[37,38] Nowadays, there is an expansion in small-scale irrigation schemes by making use of local rivers, streams, and ponds in the study area for the purpose of food security and income generation. This schemes have created conducive environment for the development and survival of the intermediate host, snail. Because of shortage of animal feeds during the dry season, the farmers have the tendency to graze their sheep in these marshy and damp areas. These factors may have also contributed significantly to the prevalence of infection in this area.

Statistically significant difference was observed between prevalence of ovine fasciolosis and irrigational status of the sheep’s grazing sites. Sheep that were grazed on highly irrigated grazing areas were the most infected than those sheep that were grazed on areas with moderate and little or no irrigation. This result is agreement with previous studies by,[39] who stated grazing on highly irrigated area increases the rate of *Fasciola* infection prevalence.[22] and,[40] also stated that irrigation canals provide a suitable condition for snail intermediate host, life cycle progression. As irrigation, increases the animals that tend to graze along the riverbanks and sides of irrigation canals. And this could easily dispose to fasciolosis in dry season. Mixed grazing of sheep and cattle is one of the causes of cross contamination, so continues follow up and treatment of cattle are necessary. Rotational grazing management is also a recommended means of control of fasciolosis.[38] Alternating crops and livestock would reduce the intensity of infection, as would the use of different livestock species. In the current study, sheep that were fed very frequently with mixed grazing type were the most affected with fascioliasis. The result of the study is in agreement with previous study by.[39]

The higher prevalence of fasciolosis in sheep from areas of high stocking and with the experience of communal mixed grazing of cattle with sheep increases the risk since the parasite *Fasciola* is non- host specific trematode infection thus cross infection can occur between cattle and sheep so that frequent mixing of this species have increased the rate of infection. Risk factors such as drinking water on the same stream or river with cattle, goat, and donkey, other domestic animals, temperature and altitude of the study area were favorable for the developmental life cycle of Fasciola species, especially for *Fasciola hepatica.*[38]

In irrigated areas, the prevalence of ovine fasciolosis has been shown to be high in sheep that frequently graze near the water canals than those that graze on areas far from water canals.[22] This might be due to frequent exposure of these sheep to metacercariae of *Fasciola* species since edges of canals which are filled by water plants serve as a suitable place for survival of snail intermediate host and consequently for the metacercaria of the parasite. The current study demonstrated non-significantly elevated liver fluke infection in sheep coming from areas where there is no canal cleaning experience. It is generally recognized that, during the dry season, when biomass for animal feed are generally scarce, animals tend to graze along the banks of rivers and sides of irrigation channels , which provides an ideal environment for fasciolosis infection to occur.

Evaluation of infection prevalence in dewormed and non-dewormed sheep in this study revealed statistically significant variation in prevalence of fasciolosis and deworming histories of the sheep under investigation. Sheep having no previous history of deworming were found to be the most infected as compared to those sheep which were dewormed once or two and more times. This might be due to lack of proper worm control strategies. However, re-infection had observed among dewormed sheep groups due to repeated re-exposure to cercarie of *Fasciola* in study area, that is why a small percent of dewormed sheep remain positive to *Fasciola* infection.[12] also reported similar observations in and around Dawa-Cheffa, Kemissie and,[41] In Menz Gera Midr Woreda of North Shoa Zone, Ethiopia

Slightly higher prevalence rate of ovine fasciolosis was observed in female than in male animals but this difference was not statistically significant. The reports in this regard are contradictory. However, consistent with the current study,[34] also reported the absence of sex related difference. This might be because all the animals were grazing on similar pastureland.

No statistically significant ($p>0.05$) variation in prevalence of ovine fasciolosis was seen between Washera and Farta breeds.[40] in Selected Sub-Districts of Alamata District, Ethiopia made similar observation. This may be the similar husbandry and the management system common for all breeds.

Analysis of age related prevalence of *Fasciola* species in the study animals revealed a statistically significant difference in distribution of the disease across age categories of the sheep. There was also an increase in prevalence of fasciolosis with increase age. The highest rate of infection was recorded among sheep belonging to the older age category (> 2 years old) while the lowest prevalence in younger sheep aged less than 1 year. This could be because young animals may not be allowed to go far with adult animals for grazing reducing the chance of exposure to infective metacercaria as compared to adults and old sheep.[42] It may also be because the adults may be at risk to the infection due to their physiological differences, such as stress, pregnancy, lambing, inadequate nutrition, and infectious diseases. The result of this study is in accordance with the findings of the studies conducted in the middle Awash River Basin by,[9] Dawa-Cheffa, Kemissie Zone, Ethiopian by,[12] and Adamawa state, Nigeria by.[43]

In this study, the association between ovine fasciolosis and body condition scores was statistically significant ($p<0.05$) and sheep with poor body condition scores were the most infected. The highest fasciolosis prevalence in these sheep might be because the poor body conditioned sheep possess the less resistant and are therefore more susceptible they are to infectious diseases or else fasciolosis causes progressive weight loss, lack of appetite and the sheep became weak and more susceptible to the parasite.[27,44] The result of this study agrees with previous reports by,[45] in Jimma Area of South Western Ethiopia,[46] in Adigrat, North East Ethiopia,[47] in Debre Zeit town, and,[25] Dembecha District, Northwest Ethiopia.

FAMACHA eye-color scoring is one of the methods used by veterinarian for clinical diagnosis of anemia in *Fasciola* infected sheep.[48] In the current study, prevalence of fasciolosis based on FAMACHA eye-color scores was highest in sheep with white-eye color (anemic), and the least being in sheep with red eye color (non-anemic). There was statistically significant variation in the prevalence of fasciolosis and FAMACHA eye color scores of sheep ($p<0.05$). This is consistent with the findings of,[49] in Basona Worana district central Ethiopia.

In the present study, the most common species of *Fasciola* reported everywhere, *F. hepatica* and *F. gigantica were* identified and ovine fasciolosis due to *F. hepatica* was the most predominant, while *F. gigantica* was the second most abundant followed by mixed infections of the two species. The predominance of *F. hepatica* in the current abattoir survey can be explained by the presence of ecological and climatic conditions favorable for development of intermediate host, *Lymnea truncatula*, the main intermediate host of *F. hepatica*. It is well documented that *L. truncatula* is the most amphibious snail having a wide distribution throughout the world including Ethiopia.[50] This is in agreement with the work of,[13] who stated that in Ethiopia, *F. hepatica* is wide spread in areas above 1800-2000 m.a.s.l. while *F. gigantica* is predominant in those areas having altitude bellow 1200 meters. The co-existence of both species' was reported in areas with altitude ranging from 1200 to 1800 meters above sea level. The present study areas and its surroundings have altitudes ranging from (1800-2500) meter above sea level that may favor the co-existence of both species of *Fasciola*.

The mean worm burden of infected livers in relation to FAMACHA eye color scores of the studied animals showed the highest worm burden in anemic (white eye color) sheep, followed by mildly anemic (pink eye color), and the least worm burden in non-anemic (red eye color) sheep. There was statistically significant (p=.002) variation between mean worm burden and FAMACHA eye color score of sheep. According to Jean-Richard and his coworkers report, liver infection with a worm burden of between10 and 100 is considered as moderately affected liver.[51] and thus the infected sheep in the present study can be considered as they have moderately affected livers. However, the mean worm burden of the present study is considerably less than the result of the previous studies by,[52,53] who reported the mean burden of,[55,56.79,78] flukes per affected liver, respectively. The possible explanation of the variation in the average mean worm burden of the present study and that of the previous authors might be due to climatic and ecological factors such as edaphic and topographic factors, which contributes major role for survival and distribution of *Fasciola* species. More over the intermediate host, the sheep management system, and the feeding condition (nourishment) of the sheep might be the causes of this difference.

Body condition scoring (BCS) is a subjective way of measuring the level of muscle and body fat carried on your sheep. Body condition scores can give a good indication of the health, nutritional state and potential reproductive success of sheep in a single easy measurement.[20] In the current research, significantly highest mean worm burden was observed among sheep with poor body condition score as compared to sheep with medium and good body condition score (p=0.00). This might be because sheep that are not supported by additional food in dry season may develop poor body conditions, and so are less resistant and very susceptible to infectious diseases with a higher rate of developing infection with strong parasitic burden.[27] It may also be due to the high worm burden has minimized the food conversion rate of the sheep causing weight loss and emaciation resulting poor body condition score.[54]

Degree of agreement between the two tests

The agreement of direct sedimentation technique with post mortem examination is calculated from the number of positive and negative fecal tests in sheep with the presence or absence of the fluke in their liver. In this study, 59 of 72 liver positive cases were found to have eggs in their feces. Moreover, 13 liver positive cases were found to have no eggs in their feces. The agreement of fecal examination can be calculated taking postmortem examination as a gold standard technique for diagnosing *Fasciola* species infection. The agreement of the two tests were estimated calculating kappa that showed (k=0.881 at p=0.000) Statistical analysis showed that there is a statistically significant agreement that is 81.94% agreement between fecal and liver examinations. The agreement of sedimentation technique with post mortem examination in the present study was higher than the reports of,[55] at Bahir Dar Municipal Abattoir, who reported 64.61 %. This strong agreement may be obtained due to the repeated sampling and testing made in the current study.[56] stated that traditional coproscopy can be very efficient if there is repeated sampling, resulting in sensitivity of approximately 92%. Nevertheless, fecal examination alone is not enough for the diagnosis of fasciolosis. Additionally, postmortem liver inspection should be employed for more accurate assessment of the prevalence of fasciolosis.[57]

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Previously many researches/studies were done on fasciolosis in different regions of Ethiopia. This study also witnessed that fasciolosis is one of economically important helminth infection in sheep in and around Merawi. The result of the present study indicated that Fasciolosis is a highly prevalent disease of sheep in the study area. However, it is increasingly evident that a proper evaluation of the epidemiology of Fasciolosis is lacking. The relatively high prevalence reported in this study has clearly indicated lack of strategic control measures against the disease as well as poor livestock health management. This high prevalence found in the study area could be also due to the water lodgment from koga dam that increased irrigated landmasses and ponds at grazing areas of animals and the tendency of farmers to graze their animals in these areas because of feed scarcity. As it is evidenced by the present study, agro ecology of the district, the nature of the grazing area and the practice of mixed grazing were found to be significant factors for the prevalence of fasciolosis in the study area. In addition to this body condition score and FAMACHA eye color score of sheep were found to be associated with the prevalence of fasciolosis and the high parasite burden. In the study several snails' species were found in and around merawi that act as intermediate hosts of different trematodes that affect our livestock Based on the above conclusion, the following recommendations are for warded:

5.2 RECOMMENDATION

- As this was the first survey of its kind for the district that indicated the prevalence and risk factors of the disease, further study on the epidemiology(covering a wider area, capturing all seasons and involving a large population of animals) of the disease is required for establishment of an effective control program.
- Provision of animal health extension services, which includes regular monitoring of faecal egg output of selected animals, assessment of anaemia using the FAMACHA chart, and treatment of animals based on the outcome of these analyses.
- Community awareness should be created regarding correct ways to improve animal management systems, the importance of parasites and major signs of worm infections in their animals will facilitate the control measure.
- Detailed study on ecology and epidemiology of the intermediate host and seasonal dynamics of parasites in the study area should be conducted to implement integrated control strategies.
- Strategic antihelmintic treatment with appropriate flukicide drug should be practiced twice a year; before and after rainy seasons to eliminate fluke burden of the host animals and minimize pasture contamination by fecal egg shedding thus interrupting the life cycle.
- Animals should be prevented either by keeping them away from these area or by fencing of dangerous areas and swampy areas should be well drained.

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Appendix

Annex 1:- Age estimation.

Age	Age categories
Less than one year	Young
Between one to two year.	Adult
Greater or equal to two year.	old

Adapted from (Mbaya *et al.* (2009).

Annex 2:- Methods of simple sedimentation technique

- Measure 3 gm of faeces in to a beaker and add 42ml of water. mix (stir) thoroughly with stirrer.
- The mixture poured through a wire mesh screen (tea strainer) with aperture of 0.15mm and strained fluid cought in a funnel like flask. The debris left on the screen was discarded.
- The strained fluid was stirred and a sample of it poured in to centrifuge tube.
- The tube containing strained fluid was centrifuged for three minutes at 1500 rpm and the supernatant was poured off and discarded carefully.
- The sediment was allowed to settle for 5 minutes.
- The sediment was transferred to a microscope slide and covered with a cover slip.
- 1% single drop of methylene blue solution was added to stain the sediment.
- The sediment was examined under a magnification power of 40x.

Adapted from (Hanson and Perry, 1994).

Annex 3:- method of counting fluke burden in infected livers

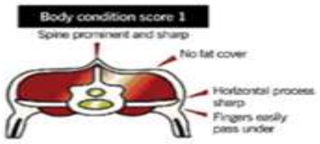
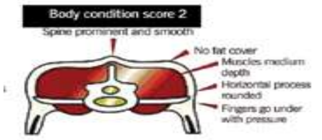
- Place the liver in a metal tray; visualize, palpate and cut it in to slices about 1cm thick with a sharp knife across the thin left lobe.
- Apply pressure to the liver to squeeze out the flukes and wipe these of gently before making the next cut.
- Remove the gall bladder and wash to screen out mature flukes.
- When the whole liver has been sliced, place all of the slices on a tray and cover with warm water to allow mature flukes lodged in small bile ducts to escape.
- Remove the pieces of liver, again squeezing each piece as it is removed from water.
- Pour water and flukes into a sieve and wash parasites until clean.
- Pour in to petri dishes and count the flukes.

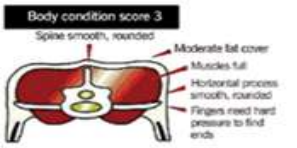
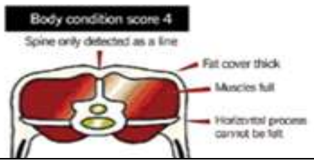
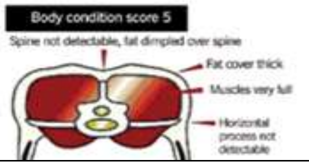
Adapted from (Hanson and Perry, 1994).

Annex 4:- Criteria for identification of sheep breeds.

Basic characteristics of Sheep		Washera	farta
1	Hair length	Short smooth	Coarse fleece, hairy (wooly)
2	horn	Both males and females are Hornless or polled	Adult males are horny
3	Body size	Large body size and good conformation	Smaller body size
4	Growth performance	Fast and better growth performance, good fattening potential	Limited growth performance, low fattening potential
5	Tail	Large and wide fat tail	Short and narrow fat tail
6	Coat colour	Predominantly brown, sometimes light and dark red with white legged and or white faced.,	Commonly white, brown or black with brown belly, white/ brown with brown/ white patches.
7	Face	Long and oval face	Short flat face

Annex 5:- Showing the body condition categories of sheep.

Cc Body Condition	Pictural view	Demonstrational view	Description	Group
1			The Spinal processes are prominent and sharp.	poor
2			The spinal processes still feel prominent.	medium

3			The spinal processes can only be felt as smooth and rounded elevations. over the ends.	
4			The spinal processes can just be detected, with pressure, as a hard line between the fat-covered muscle areas	
5			The spinal processes cannot be detected even with fifirm pressure,	good

Adapted and modified from (Solomon Gizaw, *et al.*, 2013)
Table appendix showing the body condition categories of sheep.
Adapted and modified from DR. David Fernandez (1977) and body condition scoring hand book 2013).

Annex 6:- Determination of famacha eye colour score of sheep.

Famacha eye score	Pictural view	Colour	Description	Grouping
1		Red	Optimal no dose	Non-anemic
2		Red pink	Acceptable no dose	
3		pink	Border line dose	Mild
4		Pink white	Dangerous dose	
5		white	Fatal dose	Severly anemic

Adapted from, Sheep and Goat Production Handbook for Ethiopia (**Kaufmann, J.** 1996).Information at famacha@vet.uga.edu