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Prevalence and Evaluation of Sedimentation and Floatation Techniques for the Diagnosis of Fascioliasis in Sheep from Selected Areas of the Country, Slaughtered at Export Abattoirs in DebreZeit

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Abstract

Fasciolosis is a serious problem in Ethiopia where sheep raising is of major importance to the local economy. A cross sectional study was conducted from October 2011 to March 2012 to determine the prevalence of ovine Fasciolosis and to evaluate sedimentation and flotation techniques for the diagnosis of ovine Fasciolosis at export abattoirs in Debrezeit, Ethiopia. Out of a total of 520 sheep examined, 114 (21.9%), 78 (15%) and 32 (6.2%) were positive for Fasciolosis using sedimentation, flotation and liver inspection techniques respectively. From the total of 114 liver infected by fasciola species, *F.hepatica* was found to be the most prevalent species in the study accounting for 12.3% whereas *F.gigantica*, mixed and immature forms of Fasciola species recovered were 1.7%, 3.2% and 5.6% respectively. The prevalence of fasciolosis has shown statistically significant ($p<0.05$) variation on origins and body condition scores of the sheep. The mean fluke burden in the affected livers was 11flukes per liver. As to the severity of infection, from a total of 520 examined liver 79.2%, 4.4 %, 6.1% and 10.6% were normal, lightly, moderately and severely affected respectively. Taking liver examination as gold standard for diagnosis of Fasciolosis, the sensitivity and specificity of the direct sedimentation technique was found to be 68.42% and 100% respectively with substantial agreement ($k = 0.76$) between the two methods and the sensitivity and specificity of flotation technique was found to be 28.07% and 100% respectively with fair agreement ($k=0.363$) between the two tests. In conclusion the study has shown high prevalence of Fasciolosis and proper control measures should be followed.

Keywords: *Ovine, Fasciolosis, coprological, postmortem, prevalence, sensitivity and specificity, Abattoirs, DebreZeit*

I. INTRODUCTION

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, the high productive rate of sheep and the source of cash income. Sheep require minimal inputs and maintenance costs to live in various conditions from desert to humid rain forest (6).In Ethiopia, sheep are the dominant livestock providing up to 63% of cash income and 23%of food subsistence value obtained from livestock production (23).

The sheep population of the country is estimated to be 25.5 million (4). Despite the large of the sheep population, the productivity per animal and the contribution of this sub sector to the national economy is relatively low. Endoparasitic infections, malnutrition and management problems are known to be the main factors that affect productivity (21). The various species of gastro-intestinal and pulmonary nematodes, trematodes and cestodes are known to be prevalent in Ethiopia (21).

Fasiolosis is one of the major parasitic diseases that cause immense economic losses in livestock productivity. Fasciolosis is caused by Fasciola; commonly referred to as liver flukes. Fasciolosis is a wide spread parasitic disease of sheep, cattle and occasionally humans. *F.hepatica* and *F.gigantica* were commonly implicated while fasciola hepatica has a worldwide distribution, but predominates in the temperate zones and cool areas of high altitude in tropics and subtropics (6).

Diagnosis of fascioliasis is based primarily on clinical signs, seasonal occurrence, previous history of fasciolosis on the farm or the identification of snail habitats; post mortem examination, haematological tests and examination of feces for fluke eggs useful and may be supplemented by two other laboratory tests. The first is the estimation of plasma levels of enzymes released by damaged liver cells and the second is the detection of antibodies against components of flukes; the ELISA and passive haemoagglutination test being the most reliable (20). Proper diagnosis of the disease is important in prescribing effective drugs and assists any control program directed to fasciolosis. However the different diagnostic techniques used today to confirm fasciolosis in ruminants has there limitation. Coprological analysis is still commonly employed to diagnose fasciolosis despite the overwhelming consensus that this method is not wholly reliable using this method eggs are not detected until the latent period of infection when much of the liver damage has already occurred (16). On the other hand that weak infection cannot be detected becoming the source of new infection. The most direct and reliable technique for the diagnosis of fasciolosis is liver examination at slaughter or

necropsy. But using this diagnostic technique is impossible to detect fasciolosis in live animals (17).

Sedimentation and flotation are the two coprological examination methods which are the major tests in the diagnosis of Fascioliasis in ruminants. There are several studies carried out in Ethiopia to determine the prevalence of the disease by using these techniques. However, the Sensitivity and Specificity of the tests is variable in different populations and evaluative assessments of the tests are not available. Therefore this study was conducted to know the prevalence of ovine fasciolosis and its risk factors in export abattoirs located in Debreziet, originated from selected areas of the country and to evaluate the sensitivity and specificity of Sedimentation and floatation techniques for the diagnosis of ovine fasciolosis**.**

II. MATERIALS AND METHODS

Description of the study area and study population

Study Area

The study was conducted at export abattoirs in Debreziet from November 2011 to April 2012 .The abattoirs are found in Debreziet town which is located about 45 K.m. South East of Addis Ababa at $8^{o}7^{o}$N latitude and 39^{o}E longitude at altitude of 1850 meters above sea level and situated in central high lands of Ethiopia. DebreZeit has bimodal annual rain fall of 866 mm of which 84% falls in the long rainy season extending from June to September. The mean annual maximum and minimum temperature ranges are 26^{o}c and 14^{o}c respectively. The relative humidity of the area is about 61.3%. The dry season extends from September to May (11; 12).

Study population

The study animals was all sheep that are from selected areas of the country presented to slaughter in export abattoirs and these animals was categorized based on their origin, age and body condition.

Study design and methodology

The study design used was a cross sectional study from October 2011 to March 2012 to determine the prevalence of faciolosis and to evaluate the two coprological diagnostic techniques (flotation and sedimentation) for the diagnosis of fasciolosis with post mortem examination.

Sampling methods and determination of sample size

The sampling method employed was a simple random sampling and using the 95% confidence interval, the sample size was determined by using the formula given by Thrustfield (1995).

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

Where; n=required sample size

P_{exp} =expected prevalence

d=desired absolute precision

As it is stated above the confidence interval chosen is 95%and the expected prevalence is 50% so that d=5% (0.05) by substituting the values in the formula the value of n was 384 but to increase the precision the sample size was increased to 520.

Study Methodologies

Coprological examination

Coprological examination was carried out on sheep that were brought to export abattoirs from selected areas of the country.A fresh fecal sample was collected from each sheep by retrieval per rectum, using disposable plastic gloves during anti mortem examination in the liarage one day before slaughter. The sample was placed in a separate disposable plastic container and it was transported in a cool box to the laboratory on the same day of collection and it was examined for the presence of fasciola eggs. Sedimentation and flotation technique was employed. At the time of sampling, the origin of the sheep, the date of sampling, consistency of the feces (diarrhea, semi-solid and dry), the body condition, the age and the sex of the sheep was recorded for each sheep on a recording format. And an identification mark was put on each sheep for post mortem examination during anti mortem examination and the age of the animal was determined by using dentations.

Post mortem Examination

The liver was examined using routine meat inspection procedure where by the liver was incised and the bile ducts was opened to find the adult parasite as described by (7). Identification of the fluke species was carried out by using size parameters described by (18) and characterization of pathological lesions observed in affected livers was based on the approach by (14) as follows; a) mildly affected if small portion of the organ is affected and only one bile duct is enlarged on the visceral surface of the liver; b) Moderately affected if half of the organ is affected and two or more bile ducts are enlarged and (c) severely affected if most portion of the organ is involved and the liver is cirrhotic.

Data analysis

The data collected and recorded on specifically designed forms and prepared for analysis was entered in the Microsoft excel spread sheet and analyzed with SPSS version 16 statistical software. Descriptive statistic was used to summarize the data generated from the study. The prevalence of fasciolosis was calculated by using percentage values and possible association of disease with risk factors was analyzed by using Chi –Square test and

predictive value (P-Value) and the results of diagnostic techniques were analyzed using Kappa statistics (agreement between the two tests) sensitivity and specificity were also analyzed.

III. RESULTS

A total of 520 sheep were examined for the presence of fasciolosis using both coprological and postmortem examination methods at export abattoirs. The prevalence under coprological and post-mortem examinations were found to be 15 % and 29.1 % respectively. The results are summarized in the following Tables.

Coprological examination results based on different factors:

From a total of 520 (133 young and 387 adult sheep) fecal sample collected during anti mortem examination, 20(15%) young and58 (15%) adult sheep were positive for fasciolosis. Therefore the prevalence of Fasciolosis in young and adult sheep was the same and found statically insignificant ($p > 0.05$) as shown at table 1.

Table1.Coprological prevalence of ovine fasciolosis based on the two age groups:

Age	Positive	Negative	Total
Young	20(15%)	113(85%)	133(100%)
Adult	58(15%)	329(85%)	387(100%)
Total	78(15%)	442(85%)	520(100)

χ^2=0, df=1, P= 0.989

From the total of examined sheep 185, 246 and 89 were good, medium and poor body conditions respectively. From this poor body condition showed higher prevalence (60.7%) as compared to medium and good body conditions during fecal examination and it was found to be statistically significant ($p<0.05$) as shown at table 2.

Table2.Coprological prevalence of fasciolosis based on body condition**:**

Body condition	positive	Negative	Total
Poor	54(60.7%)	35(39.3%)	89(100%)
Medium	19(7.7%)	227(92.3%)	246(100%)
Good	5(2.7%)	180(97.3%)	185(100%)
Total	78(15%)	442(85%)	520(100%)

χ^2=1.778, P=0.0, df=2

From the total of 520 sheep that originated from different parts of the country, the highest prevalence was found in Sodo (39.9%) and the lowest prevalence was seen in Awash (4.6%) and it was found to be statistically significant ($p<0.05$) as shown at table 3.

Table3. Coprological prevalence of ovine fasciolosis based on different sites

Origin	Positive	Negative	Total
Ambo	14(16.9%)	69(83.1%)	83(100%)
Arbaminech	10(15.6%)	54(84.4%)	64(100%)
Sodo	21(39.4%)	40(60.6%)	66(100%)
Shashemeni	11(18%)	50(82%)	61(100%)
Metahara	7(9.9%)	64(90.1%)	71(100%)
Awash	3(4.6%)	62(95.4%)	65(100%)
Borena	3(5.5%)	52(94.5%)	55(100%)
Somalia	4(7.3%)	51(92.7%)	55(100%)
Total	78(15%)	442(85%)	520(100%)

X2=44.96 P=0.00, df=7

Post mortem examination result based on different factors

From a total of 520 (133 young and 387 adult sheep) examined during post mortem examinations, 35(26.3%) young and79 (20.4%) adult sheep were positive for fasciolosis and the prevalence between the two age group is statically insignificant (p> 0.05) shown on table 4.

Table4: Abattoir prevalence ofovine fasciolosis based on age groups:

Age	Positive	Negative	Total
Young	35(26.3%)	98(73.7%)	133(100%)
Adult	79(20.4%)	308(79.6%)	387(100%)
Total	114(21.9%)	406(78.1%)	520(100%)

X2=2.015, df=1, P=0.98

From the total of 520 sheep that originated from different parts of the country, the highest prevalence was observed in Sodo (55.5%) and Arbamich (28.1%) and the lower prevalence was seen in Borena and Somalia (9.1%) and it was found to be statistically significant (p< 0.05) at table 5.

Table5: Abattoir prevalence of ovine fasciolosis based on origin:

Origin	Positive	Negative	Total
Ambo	17(20.5%)	66(79.5%)	83(100%)
Arbamench	18(28.1%)	46(71.9%)	64(100%)
Sodo	34(51.5%)	32(48.5%)	66(100%)
Shashemene	13(21.3%)	48(78.7%)	61(100%)
Methara	9(12.7%)	62(83.3%)	71(100%)
Awash	13(20%)	52(80%)	65(100%)
Borena	5(9.1%)	50(90.9%)	55(100%)
Somali	5(9.1%)	50(90.9%)	55(100%)
Total	114(21.9)	406(78.1%)	520(100%)

χ^2=49.587, df=7, p=0.00

From the total examined sheep 185, 246 and 89 were good, medium and poor body conditions respectively. From this poor body condition showed highest prevalence (78.7%) compare to medium (13.8%) and good body condition (5.4%) during post mortem examination and the prevalence was found to be statistically significant (p<0.05) (Table6).

Table6: Prevalence of ovineFasciolosis based on body condition scores

Body condition	Positive	Negative	Total
Poor	70(78.7%)	19(21.3%)	89(100%)
Medium	34(13.8%)	212(86.2%)	246(100%)
Good	10(5.4%)	175(94.6%)	185(100%)
Total	114(21.9%)	406(78.1%)	520(100%)

χ^2=2.063, df=2, p=0.00

From the total of 520 livers the prevalence of, *F.hepatica* was found to be the most prevalent species in the study (12.3%) during post mortem examination.

Table7. Shows the distribution of fasciolaspecies found in infected liver

Species fasciola	No of liver infected	Percentage %
F.hepatica	64	12.3%
F.gigantica	9	1.7%
Mixed	12	2.3%
Immature	29	5.6%
Total	114	21.9%

The results of fluke count made on 114 infected livers are given in Table7. The mean fluke burden was 11 per liver (1-80). Of the total sheep subjected to fluke count, 15.4% had between 1 and 25 flukes in the liver. Similarly, 3.8% had counts between 26 and 50 and only 2.7% had counts between51and 80 flukes in their liver shown on table 8.

Table 8: Fluke count in the representative liver

Fluke count interval	No of livers	Relative proportion (%)
3-25	80	15.4
26-50	20	3.8
51-80	14	2.7

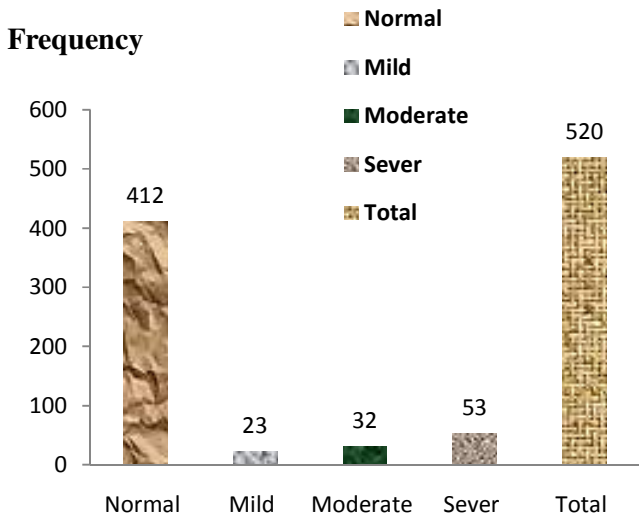

Fig. 1 Classification of liver pathology

Comparative results of different diagnostic tests

Test agreement between post mortem and sedimentation and test agreement between post mortem and flotation were compared using kappa statistics where kappa value 0.8-1.0 donates very good, 0.6-0.8 donates substantial and 0.2-0.4 fair (19).

Sensitivity and specificity test for sedimentation technique

The sensitivity and specificity of the direct sedimentation technique were calculated from the results in table 9 which sets out the numbers of positive and negative tests in animals with and without flukes in their livers (19). Out of the 520 sheep subjected to both fecal and liver examination, 114 had flukes in their livers but only 78 showed Fasciola eggs in their feces by using sedimentation technique. Accordingly, the sensitivity of a single examination by sedimentation method was found to be 68.42% and specificity was 100% with substantial agreement between the two tests (k = 0.76)

Table 9: Sensitivity and specificity test for the fecal sedimentation technique:

Fecal examination	Presence of fasciolaspp in liver		Total
	Fluke(+)	Fluke(-)	
Eggs present(+)	78	0	78
Eggs absent(-)	36	406	442
Total	114	406	520

The sensitivity and specificity test for flotation technique

The sensitivity and specificity of flotation technique were calculated from the results in table(10) which sets out the numbers of positive and negative tests in animals with and without flukes in their livers (19). Out of the 520 sheep subjected to both fecal and liver examination, 114 had flukes in their livers but only 32 showed Fasciola eggs in their feces by using flotation technique. Accordingly, the sensitivity of a single examination by flotation method was found to be 28.07% and specificity was 100% with fair agreement between the two tests (k = 0.363).

Table10: Sensitivity and specificity test for flotation technique for the diagnosis offasciola

Fecal examination	Presence of fasciolaspp in liver		Total
	Fluke(+)	Fluke(-)	
Eggs present(+)	32	0	32
Eggs absent(-)	82	406	488
Total	114	406	520

IV. DISCUSSIONS

Fasciola infections are of major concern worldwide. Fasciola species are prevalent pathogens that cause severe disease in animals and humans when ingested via contaminated feeds/ foods with the infective stages of the parasite (metacercaria). Hence, the surveillance of the prevalence is vital for the protection of the animal health, public health and consumer interests. Production of healthy animals also has important economic implications in an increasingly competitive global market.

The result of the present study has revealed that the overall prevalence of ovine fasciolosis was found to be 21.9% by using post mortem examination and the result of this study is lower than other works

done in different regions of the country with a prevalence of 51% in Debrezietand (21) with a prevalence of 49% in Holeta and greater than (5) with prevalence of 5.6% in Modjo modern export abattoir. These differences in the prevalence of ovine fasciolosis in different regions of the country may be due to the variation of the climatic conditions such as altitude, rain fall, temperature, humidity and management system of the sheep.

The prevalence of fasciolosis in the present study based on fecal examination was found to be 15% and this result was nearly the same with (8) with a prevalence of 14.6% in Hirna district of Oromia regional state and lower than (10) with reported prevalence rates of 56.3% for ovine fascioliasis in the Upper Awash River Basin.

There was no statistically significant difference ($p>0.05$) in prevalence between young and adult age groups of sheep and the prevalence is 26.3% in young and 20.4% in adult. These may be due both of the age groups have similar exposure to ingest the infective stage of the parasite (metacercaria) during grazing and the other reason may be due to the sheep that were slaughter in HELMIX export abattoir was closely related i.e there is no great gap between the young and adult age groups. Even if it is insignificant when we see the prevalence young group is more affected than adult as the age increases the level of infection decreases. This is certainly the fact that adult animal have repeatedly exposed to fluke infection than young's and develops resistance.

The high infection rates of fasciolosis were observed in Sodo (51.5%) followed by Arbaminech (28.1%) while the lowest infection rates were observed in Borena and Somalia with the same prevalence (3.5%) by using post mortem examination. This result shows that Sodo and Arbaminech are considered to be the high risk areas for fasciola infection and Somalia and Borena are considered to be low risk areas for fasciola infection due to the arid and semiarid ecology of the area. The statistical variations in the prevalence of fasciolosis between the origins of animals were may be due to the geographical location and ecology of the areas which determine the survival of the snail host of fasciola.

The prevalence of fasciolosis under post mortem examination was found to be 78.7%, 13.2% and 5.4% for poor, medium and good body condition respectively. The prevalence was highest in poor body conditioned sheep. Analysis on the prevalence of fasciolosis in relation to body condition of animal showed statically difference ($p<0.05$) indicating an inverse relation (as the body condition increases infection with fasciola decreases) this may be due to poor body condition animals were susceptible to disease causing organisms since the

animal is poor in body condition then the animal was an able to resist the infection and the other reason of the highest prevalence of fasciolosis in poor body conditioned animals may be come from fasciola infested areas.

Species identification revealed that *F. hepatica* was more prevalent (12.3%) as compared to *F.gigantica*(1.7%), Certain proportion of animals (2.3%) harbored mixed infection and others unidentified immature fluke (5.6%). The higher prevalence of *F.hepatica* might be associated with the existence of favorable ecological biotopes for the intermediate host *L.truncatula*, L. truncutula and amphibious snail with a wide distribution throughout the world. It is the most common intermediate host for *F.hepatica* (20*).*

By using sedimentation technique, lower prevalence of fascilosis was reported in this finding as compared to post mortem examination indicating the less sensitivity of the test in detecting the actual presence of the disease. In this study, the sensitivity of the sedimentation diagnostic technique was found to be 68.42% in relation to the results of post-mortem examination and a substantial agreement (kappa = 0.76) was observed between the two tests. However, this test suggests that about 31.8 % infected animals may pass undetected with single examination of feces by sedimentation technique. This may be attributed partly to the fact that Fasciola eggs only appear in feces 8-15 weeks post infection, so most of pathological lesions had already occurred (11). Furthermore, detection of Fasciola eggs can be unreliable during the patent period because the eggs are expelled intermittently depending on the evacuation of the gall bladder (3). The present result is comparable to the reports of 66.7% in Vietnam (1) and 69% in Switzerland (15). The other reason may be due to fecal sample test includes numerous steps that increase the chance of losing eggs as demonstrated by the lower number of positive result recorded in this work. Eggs may remain in the debris while filtering the faces through gauze or may get fixed on the bottom and walls if the container and within the pipette when taking the sediment for microscope observation (2).

In the present study the prevalence of fasciolosis was very low by using flotation as compared to sedimentation and postmortem examination indicating that flotation technique is the least sensitive in detecting the actual presence of the disease. In this study, the sensitivity of the flotation diagnostic technique was found to be 28.07 % in relation to the results of post-mortem examination and a fair agreement (kappa = 0.363) was observed between the two tests. However, this test suggests that about 72 % infected animals may pass undetected with single examination of feces by flotation technique. The reason for lowest sensitivity of flotation technique may be due to the

fact that commonly used flotation procedures open the operculum and sink the fluke egg rather than floating it for surface detection and fluke eggs are comparatively heavier and as a result the eggs do not float in routinely used floatation mediums such as saturated salt solution. However, flotation fluids of higher specific gravity such as saturated zinc solutions and magnesium may float the eggs of fasciola but the saturation of these solutions damage to observed eggs is very high and then the egg is distorted and may loss its morphological character and difficult to identify correctly.

V. CONCLUSIONS AND RECOMMENDATIONS

In my present study the prevalence of ovine Fasciolosis which was found to be high indicated that Fasciolosis is one of the major disease that cause immense economic losses and the major obstacle for livestock development in Ethiopia. This study confirmed that there was significant difference in the prevalence of Fasciolosis among the different origins and body condition score of examined sheep and it shows that fasciola parasites were more prevalent in poor body condition animals than medium and good body condition animals and the prevalence was high in sheep that originate from sodo and the low prevalence in the sheep that originate from Borena and Somalia. The present study indicated that sedimentation is the best diagnostic technique for the diagnosis of Fasciola eggs than flotation technique with post mortem examination as gold standard.Generally, Strategic antihelmentic treatment with appropriate flukecidal drug should be practiced, the snail population should be reduced by proper drainage, clearing of aquatic vegetations and fencing of snail breeding habitat and application of molluscide in snail habitat, awareness creation to animal owners regarding the route of infection and economic importance of the disease so that they can actively participate in the control of the disease and Coprological examination should be repeated and supported by other diagnostic methods

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