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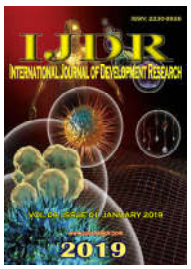

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

MAJOR TREMATODE INFECTIONS OF SHEEP IN LEMO WOREDA AND ASSOCIATED ECONOMIC LOSS DUE TO LIVER CONDEMNATION AT HOSSANA TOWN, SOUTHERN ETHIOPIA

[1]Bekele Chakiso Gugero and [2]Tarekegn Gebreyesus Abisso

[1]Department of Biology, College of Natural and Computational Sciences, Wachemo University, Hossana, Ethiopia
[2]Department of Life science and Bio-Engineering, Beijing University of Technology, Beijing, China

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ABSTRACT

Trematode infection is a prevalent parasitic disease of livestock that causes one of the major problems leading to a huge economic loss in livestock production. The cross sectional study was conducted from October 2015 to June 2016 at Lemo Woreda and Hossana Town to determine the infection rate, predominant trematode species, fluke burden and severity of liver pathological lesions of sheep slaughtered at Hossana Town, and to assess the direct economic loss due to liver condemnation. The sample population of 384 sheep from the study area were randomly selected and examined for the presence of eggs by sedimentation techniques and 384 sheep were also selected by systematic sampling techniques for the trematode species identification from slaughter houses at Hossana Town. The overall infection rate of trematode species of sheep from PAs was 32.6%.The infection rate of trematode species of sheep on this study was significantly associated ($p<0.05$) with body condition, age, breed and location of study animal. However, there was no statistically significant association of trematode infection with sex of study animal ($p>0.05$).It was noticed that, a higher infection rate was identified in young (85.7%) than adult (29.5%) sheep, and cross breed (66.7%) were most affected as compared to local (28.8%) sheep breed. Sheep having thin (53.5%) body condition were higher in infection rate of trematode species than average (31.8%) and fat (16.3%). The major trematode species recognized was *Fasciola hepatica* (60.8%) followed by mixed trematode species (28%).The overall prevalence of trematode infection of sheep due to liver condemnation in this study was 33.9%. The average mean flukes were 34.90 per affected liver. The average mean worm burden that was responsible for the liver pathology types such as moderately, severely and lightly affected livers were (54.56±4.469), (34.75±1.620) and (18.67±0.311), respectively. A liver associated economic loss identified in sheep by trematode species at Hossana Town was estimated to 116,860.08 Ethiopian birr or 5564.80USD Per annum. Trematode infection was highly spreaded parasitic disease and causes a considerable economic loss in the study area. Therefore, environment for the development of snails should be clearly identified by all stakeholders in order to understand their effects on the disease occurrence as well as in control and prevention of trematode infection.

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INTRODUCTION

Trematode infection is a predominant parasitic disease of sheep, goats, cattle, camels and equines that causes one of the major problems associated to a huge financial loss in livestocks by decreasing direct and indirect productivity (Ahmed , 2007). The two species, that are commonly known as *Fasciola hepatica* and *Fascioa gigantica*, as usual they referred to as liver flukes.

***Corresponding author:* Bekele Chakiso Gugero**
Department of Biology, College of Natural and Computational Sciences, Wachemo University, Hossana, Ethiopia

It is extremely serious disease of herbivorous animals, while human infection has long been appeared as unintentional (Mufti and Saira, 2011). Trematode infection, caused by *Fasciola hepatica* and *Fasciola gigantica*, is one of the most helminthic infections of ruminants in different parts of the world. It causes significant morbidity and mortality. However, the distribution of *Fasciola hepatica* is limited to temperate areas and high land of tropical and sub-tropical regions while *Fasciola gigantica*is highly spreaded in most parts of tropical Africa. Thus, the two fasciolide species overlap in many African and Asian countries and sometimes in the same

country, although in such cases the environmental requirements of the flukes and their snail intermediate host is different (Walker, 2008). *Fasciola hepatica* and *Fasciola gigantic* are commonly reported to cause disease in ruminant and their life cycle that cause fasciolosis involve snail as an intermediate host. The trematodes infection is responsible for considerable cause of enormous economic loss in the livestock production mostly via liver condemnation (Brown, 2005). The presence of fasciolosis in sheep due to trematode species in Ethiopia has long been known and its occurrences and economic loss has been reported by several authors (Yilma, 2000 and Mohammed, 2010). The estimated annual loss of 48.4 million ETB per year was reported due to liver trematode species in sheep from the middle Awash River basin (Ahmed, 2007). Most of published reports in Ethiopia were from the central, western and northern parts of the country. However, the liver trematode infection and associated economic loss as the result of liver condemnation in sheep was also reported by some scholars of Southern Ethiopia.

Even if, the study area has an optimum environmental settings such as, favorable climatic conditions, mixed farming system of livestock, altitude, and water logged grazing areas for the presence of intermediate snail host.

However, the major trematode infections and its associated economic loss due to liver condemnation was not yet studied. Hence, this study was focused on determining the major trematode infections from the sheep owned by smallholder farmers in Lemo Woreda and its economic loss due to liver condemnation at Hossana Town.

Therefore, the objectives of this study were:

General objective

To assess the major trematode infections of sheep at Lemo Woreda and associated economic loss due to liver condemnation at Hossana Town.

Specific objectives

- To determine the infection rate of trematode infections in Lemo Woreda
- To assess the major trematode species of sheep from peasant associations in the study area
- To associate worm burden with severity of liver patholo gical lesions of sheep slaughtered at Hossana Town
- To assess direct economic loss associated due to liver condemnation of the study animals

MATERIALS AND METHODS

Study area: The study was conducted at selected areas of Lemo Woreda and Hossana Town of Hadiya Zone, Southern Ethiopia. The highest altitude in the Zone is 2970 m.a.s.l at the Summit of Sengiya Mountain in the Duna Woreda and the lowest is 800 m.a.s.l in Gibe River valley. The zone is divided into ten Woredas and one city administration. Lemo Woreda is one of ten administrative Woredas found in Hadiya zone and located at a distance of 230 km from Addis Ababa. The study area is located approximately at an altitude of 2400 m.a.s.l with the mean annual rainfall of 1173 mm and mean annual temperature of $18c^0$. Geographically, it is located between $7^0.42'$-7.75′N latitude and $37^0.80'$-38.07′E longitude (HZSA, 2010).

Study Population

It includes sheep of PAs at Lemo Woreda and slaughtered at H ossana Town.Those sheep have taken antihelmenthic drugs for any intestinal helminthes within three months before the study period were excluded.

Study Design and Sampling Techniques

The study was conducted from October 2015 up to June 2016. Simple random (used to collect fecal sample) and systematic sampling techniques (used to conduct abattoir survey) were used. Accordingly, the study was conducted at selected area of PAs at Lemo Woreda, and Hossana Town. There are 35 kebeles at Lemo Woreda and out of which, six selected as the representative PAs such as Haysie, Jewie,Shecha, Shurmodubancho, Ashebukuna and Belessa were selected purposively by considering sheep populations environmental conditions, distance and transport access.

Sample Size Determination

Since, there was no adequate information on the current status of trematode infections of sheep and associated economic loss due to liver condemnation at the study area, the sample size was determined by taking the infection rate of 50% (Thrusfield, 2005).

$$n = (Z\alpha/2)^2 . P_{exp} (1-p_{exp})/d^2 = \frac{1.96^2 \times P_{exp} (1-P_{exp})}{d^2}$$

Where n = required sample size; Pexp = expected prevalence (50%) =0.5; d = desired absolute precision (5%) = 0.05; Zα/2 = critical value at 95% certainty (1.96). Therefore, 384 sample population were selected from total of 28,312 sheep owned by smallholder farmers of PAs and 384 samples were collected from sheep slaughtered houses at Hossana Town.

Fecal examination

Prior to collection of fecal samples, risk factors associated with the host were identified. Age of the study animal was determined based on dentition. Those sheep which are not erupted incisor teeth were considered as young where as those sheep which are erupted one or more incisor teeth were classified as adults (FAO, 2009). Fecal sample collection and processing were performed according to the procedures described by Ministry of Agriculture, Animal Industry and Fisheries. Fecal sample was collected directly from rectum of randomly selected sheep by hands protected by rubber gloves, using two fingers. Each sample was clearly labeled with animal's identification, date and place of collection. Samples were packed and dispatched in cool box to avoid development of eggs and hatching, and then were transported to the parasitological laboratory by preserving it with 5% formalin in the universal bottles (Antonia, 2010). Most trematode eggs are relatively large and heavy compared to nematode eggs. In the laboratory, coprological examination was performed to detect trematode species eggs using the sedimentation technique. The collected samples for each case; 3g of faeces was measured and put it in to a container 1. Then, 40-50 ml of tap water was poured in to the same container and mix thoroughly

with a stirring device. The fecal suspension was filtered through a double-layer of cheesecloth and put it into container 2 and then, the filtered material was poured into a test tube and allows it to sediment for 5 minutes. The supernatant is removed and then, re-suspended in a 5ml of water very carefully and allow it to sediment for five minutes. After discarding the supernatant very carefully, the sediment was stained by adding one drop of methylene blue. Finally, examine under low power objective and the presence and the absence of trematode eggs were identified (Shimizu and Robyn, 2006). Morphologically, liver trematode species eggs were notable from the eggs of large sized *Paramphistome* species by having yellowish brown shell due to the presence of tanned protein shell with an indistinct operculum. As *Fasciola* eggs may be confused with the eggs of *Paramphistome* species, addition of methylene blue in fecal suspension can facilitate easy identification by providing a blue contrasting microscopic field (Shimizu and Robyn, 2007).

Abattoir survey

Preslaughter inspection was conducted in the lairage by grouping the sheep based on sex, age, body condition and location. The age was grouped based on dentition. Such as, those which have not erupted permanent incisor teeth, were classified as young, while those with one pair or more permanent incisor teeth were classified as adults (Pasquini, 2008). Body condition of the sheep in abattoir was classified similarly to the field study as thin, average and fat and then, followed up of their postmortem examination. The livers of the slaughtered sheep identified during antemortem examination were examined through systemic incision to recover *Fasciola* species (Rahmeto Abebe 2010). Those livers condemned as unfit for human consumption due to worm burden during postmortem examination were registered and representative samples were taken to laboratory to observe the adult flukes. The gall bladder was removed and washed to screen out mature flukes. The liver was cut into slices of about 1cm thick and put in a metal trough of warm water to allow mature flukes lodged in smaller bile ducts to escape and then all flukes were identified and collected (Shimizu and Robyn, 2007). The decisions at postmortem inspection was classified into the following categories of judgment such as approved as fit for human consumption and totally condemned as unfit for human consumption (FAO, 2004).

Identification of liver trematode species

The recovered trematode species were identified based on shape and size and classified them as *Fasciola hepatica*, *Fasciola gigantic* and immature forms (FAO, 2009).

Pathological categorization of affected livers

Pathological category of affected livers of sheep was determined as lightly, moderately and severely affected, which was described based on Ogunrinade and Adegoke (Taylor, 2007).

Fluke burden

The intensity of fluke infection (mean fluke burden) was linked with the pathological lesions. The flukes recovered from the affected livers during postmortem examination of sheep were made to determine the fluke burden in the sheep. The total fluke burden per affected liver was also counted (Taylor, 2007).

Financial loss assessment

The financial loss (direct) due to liver condemnation of sheep by trematode species was assessed from October 2015 up to June 2016.The total number of livers condemned due to trematodes species and the total number of sheep slaughtered during the study period was considered on daily/ monthly and annual basis. The average weight of sheep liver and price per kilogram (based on the information obtained from butchers at Hossana Town) was also considered for calculation.

Data Analysis

Data obtain from fecal and post-mortem examination was recorded. Then, it was entered and managed using MS Excel work sheet and analyzed by using SPSS version 16. The prevalence of trematodes infection was calculated as the number of infected individuals divided by the number of individuals sampled x 100 (Thrusfield, 2005). Chi-square ($\chi 2$) test was used to evaluate the association between the prevalence of major trematode infections and sex, age, breed, study location and body condition of sheep. In all statistical analysis, confidence level was held at 95% and P-value is <0.05, which was considered as significant. Direct economic loss was also calculated as

ALC = MCS x MCL x P, and Mean value was used to associate the total worm burden with liver pathological lesion of sheep (Ogunrinade, 1982).

RESULTS AND DISCUSSION

Infection rate of trematode species in sheep: The infection rate of trematode species in sheep from the selected peasant association of Lemo Woreda was conducted by collecting faecal samples. The variables considered during this period were sex, age, location, breeds and body condition, which were discussed in Table 1-3. Moreover, fluke burden that associated with liver pathological lesion from the affected livers at Hossana Town were indicated in Table 4. From the total of 384 sheep coprologically examined at Lemo Woreda for the occurrence of trematode species, 125 were found infected and resulting in an overall prevalence of 32.6% (Table1).The overall infection rate of trematode species (32.6%) in the present study was in agreement with the earlier findings that was 33.42% from northwestern part of Ethiopia (Yilma, 2000). However, the overall prevalence of trematode infections of sheep at the present study was lower than the earlier findings of 54.17% (Zerihun, 2006). This may probably due to the seasonal variation of the study period. As shown in Table 1, from the total of 374 males and 10 females, 120 (32.1%) and 5 (50%), respectively were positive for of trematode infection. Among 21 young and 363 adult sheep examined during study period, 18 (85.7%) and 107 (29.5%), respectively were positive for trematode infection. Out of 372 local breed and 12 Cross-breeds examined, 107 (28.8%) and 8 (66.7%), respectively were positive for trematode infection.
Though, the number of males cattle were higher in number as compared to female, the number of positive females was higher in proportion than males, hence in this case, the variation in between positive males and very few females sheep were high as compared to their total number.

Table 1. Infection rate of trematode species by sex, age and breed based on fecal examination of sheep at Lemo Woreda from October 2015 up to June 2016

Variables		№ of examined	№ of positive	95% CI	OR	χ2 (P-value)
Sex	Male	374	120 (32.1%)	15.9-24.3		
	Female	10	5 (50%)	7.6-77.8		
	Total	384	125 (32.6%)	28.2-44.3	1.751	0.726 (0.531)
Age	Young	21	18 (85.7%)	28.6-76.0		
	Adult	363	107 (29.5%)	21.6-42.4		
	Total	384	125 (32.6%)	35.7-41.2	3.438	10.491 (0.000)
Breed	local breed	372	107(28.8%)	31.5-44.2		
	Other breeds	12	8 (66.7%)	27.8-86.2		
	Total	384	125 (32.6%)	19.8-46.9	0.964	9.227 (0.017)

Table 2. Association of trematode infection by PAs (location) and body condition of sheep in the study area

Variables		№ examined	№ Positive	%	χ^2	p-value
Body condition	Thin	86	46	53.5		
	Average	255	72	31.8		
	Fat	43	7	16.3		
	Total	384	125	32.6	23.746	0.000
Location	Shecha	77	20	25.9		
	Dubancho	62	15	24.2		
	Jewie	90	50	55.6		
	Haysie	44	20	45.5		
	Ashebukuna	50	20	40.0		
	Belessa	61	13	21.0		
	Total	384	125	32.6	9.114	0.010

Table 3. The predominant trematode species of sheep identified during fecal examination during the study period

Trematode species (N=384)	№. of positive	(%)
F.hepatica	76	60.8
F.gigantica	14	11.2
Mixed species	35	28.0
Total	125	32.6

Table 4. Association of worm burden with severity of liver pathological lesions of sheep slaughtered at Hossana Town

Trematode species	Liver pathological levels			Total	F-test (P- value)
	Lightly (Mean±SEM)	Moderately (Mean±SEM)	Severely (Mean±SEM)	(Mean±SEM)	
Fasciola hepatica	22.16 ± 1.161	69.22 ± 1.864	44.27 ± 2.238	46.24 ± 6.137	
Fasciola gigantica	26.80±0.655	38.70± 8.716	24.00±3.786	28.16±4.240	
Mixed species	0.00	52.06±10.425	31.44±6.245	27.00±2.255	
Total	18.67±0.311	54.56± 4.469	34.75±1.620	34.90±2.735	167.52(0.000)

This study shown that, there was no significant difference (χ2=0.726; P=0.531) in trematode infection on sex basis (Table 1). As Table 1 shown, young sheep (85.7%) have significantly higher (χ2= 10.491; P=0.000) prevalence rate of fasciolosis when compared to adult (29.5%).This may be attributed to the fact that the protective ability against disease increases with age (Mufti, 2011). Regarding of the effects of breed as showed in Table 1, the infection was significantly associated with breed (χ2= 9.227 ; P=0.017) and the highest prevalence was observed in cross breed (72.7%) and few in local breed (33.7%). This could be explained by the fact that, the higher prevalence rate at other breeds than local breed may be due to the less resisting capacity with the environment and are acquired a high degree of immunity as a result of repeated natural exposure to trematode infections. The association between trematode infection and body condition scores were statistically significant (χ 2=23.746; P=0.000).The highest prevalence of trematode infection was identified as thin (53.5%) and lowest prevalence was at fat (16.3%) body conditioned sheep.

As shown in Table 2, the prevalence of trematode infection in thin, average and fat body conditioned sheep was 46 (53.5%), 72 (31.8%) and 7(16.3%). The prevalence of trematode species infection was higher in thin body condition 46 (53.5%), which indicated (Table 2) that the parasite causes progressive weight loss, lack of appetite and the sheep became weak. Because when the body condition was thin they have less resistant and are consequently susceptible to infectious diseases. The difference might be the period of sheep exposed to trematode species infection which was increased due to their choice of grazing around the water bodies. The current prevalence of major trematode infection varies with respect to Peasant Associations (Table 2). The highest prevalence of trematode infection was in Jewie 50 (55.6%) and the lowest was in Belessa 13(21%) peasant association. The relationship between prevalence of trematode infection and peasant associations were statistically significant (χ2=9.114, P= 0.010). Moreover, the owners of sheep lack of experience of deworming their sheep during the dry season. In addition, risk factors such as drinking water on the same stream or river with cattle, goat, and other domestic animals, optimum temperature

and altitude of the study area were favorable for the developmental life cycle of trematode species. The finding of this study was in line with the previous findings, such as the interaction of various environmental factors that increase the likelihood of trematode infection in sheep and variation of infection rate between the localities (Biffa, 2006).

Trematode species identified from study animal during fecal examination

The eggs of trematode species such as; *Fasciola hepatica*, *Fasciola gigantic* and mixed Fasciola species (*Fasciola hepatica* + Fasciola gigantica) eggs identified during fecal examination was 60.8%, 11.2% and 28%, respectively (Table 3). The result obtained from the fecal examination (Table 3) indicated that the predominant trematode species in the study area was *Fasciola hepatica*. This condition might be associated with the existence of favorable ecological and climatic condition for the development of intermediate host Lymnaea truncatula (Brown, 2005; Zerihun, 2006). The present study was supported by Yilma and Mesfin, (2000) from Gonder and reported the prevalence of *Fasciola hepatica*, *Fasciola gigantic* and mixed species was 67.14%, 14.1% and 18.77%, respectively.

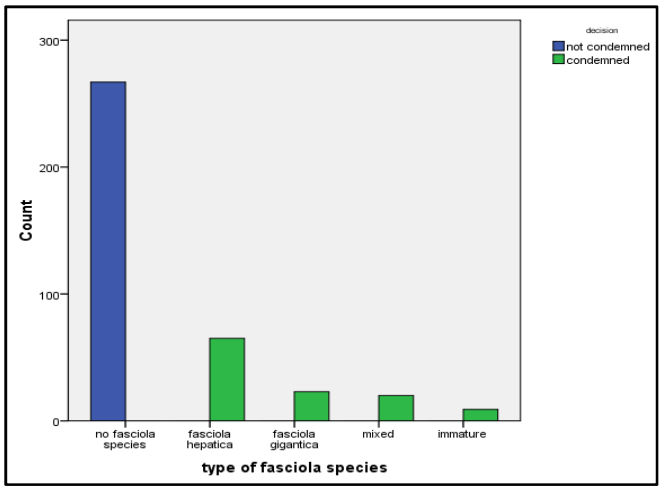

Figure 1. The major trematode species of sheep at the peasant associations in the study area

The prevalence of *Fasciola hepatica* (63.89%), *Fasciola gigantic* (24.07%) and mixed Fasciola species (12.04%) was also obse-rved from Jimma (Zerihun, 2006). Infection rate of trematode species of liver based on postmortem examination of sheep at Hossana Town. A total of 384 sheep slaughtered at Hossana Town from October 2015 to June 2016, 130 were identified as positive for the presence of major trematode infection on postmortem examination. The overall prevalence of trematode infection from postmortem study of sheep in this study was 33.9%, which was in line with 29.76% reported from Helmax Debrezeit Abattoir and 31.8% from Assela abattoir (Melkam Abate, 2008). However, the current study was much less than 94.5% prevalence of ovine this infections, which was reported from Ikom Abattoir of Cross River State of Nigeria; 84.5% was reported from West Shoa and 54.2% prevalence of ovine fasciolosis was from Sarajevo (Ozung, 2011 and Pasquini, 2003). There was a variation in overall prevalence of trematode infection among different study areas. The variation may depend on some factors such as livestock management system, suitability of the environment for survival and distribution of the parasite as well as the intermediate host might have played their own role in such differences. Overall prevalence (32.6%) recorded due to trematode infection from fecal examination of sheep from the PAs of Lemo Woreda was nearly similar with the overall prevalence of 33.9% at Hossana Town from sheep slaughtered at the present study. This might be due to the reason of similarity in study period (dry season for both studies and almost all sheep were coming from the similar ecological boundry). As the result, overall prevalence of trematode infection on farm was closely related with abattoir studies. In other words, the slight difference in overall prevalence on farm (32.6%) and abattoir (33.9%) studies may be due to the difference in the animals origins and deworming status (deworming some, but not the others) to protect trematode infection.

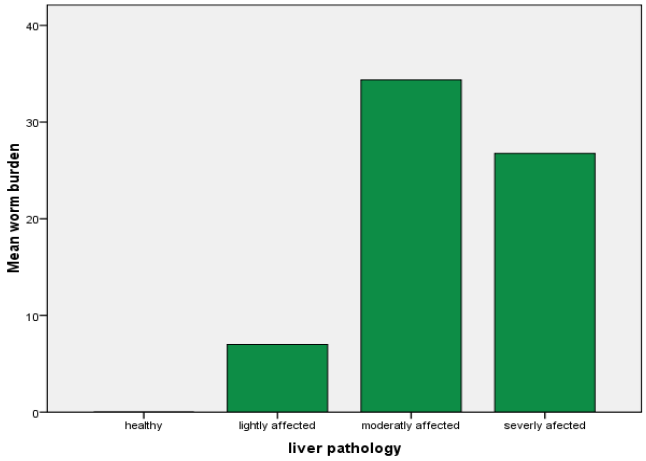

Figure 2. Association of severity of liver pathology with fluke burden in Sheep Slaughtered at Hossana Town

Fluke burden and associated liver condition

There was a statistically significant association ($P<0.05$) between the different levels of intensity of pathological lesion and mean fluke count (Table 4). The average mean fluke count identified during the study was 34.90 flukes per affected liver. It was relatively lower as compared to 50 and 55 flukes per affected livers from Jimma and Hawassa Municipal Abattoirs, respectively (Dechasa, 2012 and Rahmeto, 2010). Relative variation in the average mean worm burden of the present study as compared to the previous authors might be due to high calcification and fibrin formation of liver bile duct of sheep, lack of rainfall and moisture at a point of study period for the replication of snails as well as trematode species, anthelminthic and managing system of sheep (Brown, 2005). In the present study, the average mean worm burden of 34.90 flukes per affected liver indicates the existence of high pathogenesis of flukes at the study area. The mean worm burden in lightly, moderately and severely affected livers was found to be 18.67±0.311, 54.56± 4.469 and 34.75±1.620, respectively. It was higher in moderately affected livers (54.56± 4.469) and followed by severely affected livers (34.75±1.620). The result of this study was in agreement with the previous works done from Adwa and Jimma Municipal Abattoirs, respectively (Dechasa, 2012 and Mihreteab, 2010). The difference in the mean worm burden count might be due to the difference in species, altitude, anthelminthic and

management system of sheep. The mean worm burden in moderately affected livers was higher than severely and lightly affected livers. This may be relatively minimized calcification of bile ducts and having less acquired resistance to block the further passage of undefined immature flukes in moderately affected livers as compared to severely affected livers (Yilma, 2000).

Direct economic loss associated to liver condemnation of sheep at Hossana Town

The direct economic loss due to condemnation of affected livers of sheep in Hossana Town was considered. Direct economic loss of trematode infections of sheep was according to the average number of sheep slaughtered per year, mean selling price of the sheep livers at Hossana Town at the period of time and the recent prevalence (33.9%) due to condemnation of liver by trematode species in this study. The average market price of 1Kg of meat and one liver at Hossana Town was taken as 160 and 62 Ethiopian birr, respectively. The mean number of sheep slaughtered in this Hossana abattoir was 5560 per year depends on five years recorded data. The information obtained was subjected to analyze using the formula given [16].

ALC = MCS x MCL x P

Where ALC=Annual loss from Liver Condemnation
MCS=Mean annual sheep Slaughtered at Hossana Town
MCL= Mean Cost of one Liver in Hossana Town and
P= Prevalence due to liver condemnationtrematode infection at the present study.

ALC = MCS x MCL x P

= 5560 x 62ETB X 33.9%
= 116,860.08 ETB or (5564.80USD per annum)

The direct economic loss due to condemnation of infected livers in Hossana Town was estimated to be 116,860.08 Ethiopian Birr or 5564.80USD (1USD~20.99ETB). It was in agreement with 106,400 ETB from Hawassa and lower as compared to Melkam from Hemax Abatttoir, that is 281,707.27 ETB per annum (Melkam Abate, 2008 and RahmetoAbebe, 2010), but highest as compared to the research reported as 32,38.00 ETB from Kombolcha (Suleman Mohamed, 2010). This variation of economic loss in different study areas might be due to the variation in study area and average market price of one liver at different localities. The finding of this study confirmed that, trematode infection was identified as the major problem in the study area and causes of substantial economic loss due to the liver condemnation of sheep. *Fasciola hepatica* is the predominantamong the liver trematode species at the study area. Therefore, the conducive factors for the occurrence of trematode species should be obviously identified by sheep owners and other stakeholders in order to identify their roles on the disease occurrence as well as in control and prevention.

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