1116

ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

STUDY ON COMPARATIVE DIAGNOSTIC TECHNIQUES OF FASCIOLOSIS IN SLAUGHTERED RUMINANTS AT ELFORA EXPORT ABATTOIR DEBRE ZEIT, ETHIOPIA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in Partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Medicine

By

MESKEREM ADAMU CHERE

JUNE 2006

Debre Zeit, ETHIOPIA

1116

ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON COMPARATIVE DIAGNOSTIC TECHNIQUES OF FASCIOLOSIS IN SLAUGHTERED RUMINANTS AT ELFORA EXPORT ABATTOIR DEBRE ZEIT, ETHIOPIA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in Partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Medicine

By

MESKEREM ADAMU CHERE

JUNE 2006

Debre Zeit, ETHIOPIA

STUDY ON COMPARATIVE DIAGNOSTIC TECHNIQUES OF FASCIOLOSIS IN SLAUGHTERED RUMINANTS AT ELFORA EXPORT ABATTOIR DEBRE ZEIT, ETHIOPIA

By

MESKEREM ADAMU CHERE

Board of Examiners Signature

DEDICATION

This paper is affectionately dedicated to my beloved father, Ato Adamu Chere, who is unfortunate to see my success because he is passed away on 21 August 1978.

TABLE OF CONTENTES

TABLE OF CONTENTES I

LIST OF TABLES III

LIST OF FIGURE IV

LIST OF ANNEXES V

ABBREVIATIONS VI

ACKNOWLEDGEMENTS VII

DEDICATION VIII

ABSTRACT IX

1. INTRODUCTION 1

2. LITRATUR REVIEW 3

2.1. The parasite 3

2.1.1. Distribution 4

2.1.2. The status of fasciolosis in Ethiopia 5

2.1.3. The snail intermediate host 8

2.1.4. Life cycle of fasciola 9

2.1.5. Pathogenic significance 11

2.2. Diagnostic approaches to fasciolosis 12

2.2.1. History and Clinical Manifestation 12

2.2.2. Postmortem Examination 13

2.2.3. Laboratory Diagnostic Methods 14

3. MATERIAL AND METHODES 21

3.1. Study area 21

3.2. Study population 21

3.3. Study methodology 22

3.3.1. Coprological examination 22

3.3.2. Postmortem examination 22

3.3.3. Enzyme analysis 23

3.3.4. Serological examination 23

3.4. Data analysis 25

4. RESULTS **26**

4.1. Coprological study **26**

4.2. Post mortem inspection **27**

4.3. Serum enzyme analysis **28**

4. 4. Serological examination (ELISA) **29**

4.4.1. Comparative results of different diagnostic tests 31

5. DISCUSSION **32**

6. CONCLUSIONS AND RECOMMENDATION 36

7. REFERENCE **37**

8. ANNEXES **49**

9. CURRICULUM VITAE **56**

10. SIGHNED DECLARATION SHEET 59

LIST OF TABLES

Table 1. Prevalence of bovine fasciolosis in different regions of Ethiopia as determined on the basis of coproscopy and abattoir examination. 6

Table 2. Prevalence of fasciolosis and calculated economic losses at different abattoirs in Ethiopia 7

Table 3. Prevalence of fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on coproscopy. 26

Table 4. Prevalence of fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on abattoir survey. 27

Table 5. Comparison of the different diagnostic methods in relation to the results of coproscopy and postmortem examination. 31

LIST OF FIGURES

Figure 1.*Fasciola. hepatica* and its egg ... 4
Figure 2. Life cycle of *Fasciola gigantica* ... 10
Figure 3. Enzyme Linked Immunosorbent Assay plate lay out for sera distribution ... 24
Figure 4. Classification of liver pathology ... 28
Figure 5. Comparison of enzyme activities and liver pathology in cattle. ... 29
Figure 6. ELISA results and level of infestation within the herd. ... 30
Figure 7. Comparison of the results of ELISA and faecal sedimentation ... 30

LIST OF ANNEXES

Annex 1. The simple sedimentation method (McMaster),(Antonia *et al.*, (2002). 49
Annex 2 Parasites recorded other than fasciolosis in the study areas.. 49
Annex 3 Procedure for enzyme (LDH) analysis.. 50
Annex 4. Procedure for enzyme (GGT) analysis:.. 50
Annex 5. Materials required for serum enzyme analysis.. 51
Annex 6. Reagent composition for LDH:.. 51
Annex 7 Reagent composition for GGT :.. 51
Annex 8. Normal value of animals serum enzymes (Merck & Co, 1998) 51
Annex 9 Enzyme Linked Immunosorbent Assay (ELISA) test protocol. 51
Annex 10. Materials required for diagnosis of fasciolosis by ELISA method. 52
Annex 11. Instructions for diagnosis of fasciolosis by ELISA method.. 53

ABBREVIATIONS

ELISA	Enzyme Linked Immuno Sorbent Assay
ES	Excretory Secretory
GGT	Gamma Glutamil Transpeptidase
GLDH	Glutamate Dehydrogenase
LDH	Lactate Dehydrogenase
HA	Haemagglutination
IgG	Immunoglobulin G
PBS	Phosphate Buffered Saline
Fig	Figure
GIS	Geographical Information System
Spp	Species
Km	Killo mettres
CSA	Central Statistical Authority
Ml	Millilitter
MML	Micro moll per litter
UL	Micro litter
H_2So4	Sulphuric acid
OD	Optical Density
PMI	Post Mortem Inspection
VEIN	Veterinary Education Information Network
AST	Aspertate transaminase
AP	Alanine phosphatase
S/P	Sero positivity
Ci	Confidence interval

ACKNOWLEDGEMENTS

Above all thanks goes to GOD, the almighty creature, sustainer and lord of the universe for His innumerable favours and benevolence upon me during the last two years of my MSc studies. I do have also special spritual respect for His mother Saint Merry.

The moral support, good professional wisdom to learn from, and encouragement due to Dr. Merga Bekana, Dean of FVM, is highly appreciated.

I would like to express my heartily thanks to Dr. Getachew Tilahun for his technical advice, overall guidance and valuable comments on my draft documents to appear in the present form.

The enthusiastic moral support, diligence, meticulous and incalculable time devotion in commenting my draft in order to appear in its present form, material provision and general overall guidance based on long years professional wisdom due to Dr. Abebe Wossene have been highly indebted.

I would like to forward my special thanks to Dr. Kelay Belihu, Associate Dean for research and graduate programmes, for his motivation in all aspects of the graduate programmes.

The very vital technical support in facilitating to get the ELISA kit for serological investigation of my MSc studies due to Dr. A.K. Basu deserves my special attention and appreciation.

It is a very good opportunity to extend my special thank to the Institute of Pourquier (France) for providing me the ELISA kit on free of cost basis.

I would like to forward my special thanks to the administrative and meat inspection staffs of the ELFORA Export Abattoir, particularly Dr. Tefera Woldemariam, Dr. Hadgoligne, W/ro Kibenesh for their cooperation and support during the course of my work in the abattoir.

My deepest gratitude goes to Dr. Berhe Gebreegziabher, main manager of NVI, for allowing me to have free access to the laboratory facilities of immunology. Dr Fekadu Kebede and Dr Ahmed Issa are acknowledged for their valuable professional help and collaboration.

The technical support due to W/rt Aynalem Fentie, W/rt Aynalem Tadesse, W/rt Eskedar W/ro Berhane Wakejirra, W/ro Seble Sinkie, Ato Yosef Cherinet of the Faculty of Veterinary Medicine and Ato Kinfe Mekuria of NVI are greatly acknowledged.

The encouragement and patience due to my husband, Ato Efrem Mersiehazen, do not have any parallel and it is an excellent opportunity for me to witness his gender sensitivity.

ABSTRACT

A study was conducted at ELFORA Export Abattoir, Debre-Zeit in order to compare the different diagnostic techniques in ruminant fasciolosis. The results of standard faecal sedimentation technique for fluke egg quantification was compared to that of an enzyme linked immunosorbent assay (indirect ELISA) test and liver inspection as well as results of serum enzyme activities. Out of the 324 cattle, 350 sheep and 385 goats slaughtered at ELFORA Export Abattoir, 125 (38.5%), 4 (1.14%) and 2 (0.51%) were found to be positive for lesions of fasciolosis, respectively during post mortem inspection. During faecal examination 108(33%), 4(1.14%) and 2(0.51%) were positive for fasciola eggs in cattle, sheep and goats respectively. Further examination on 134 randomly selected samples from cattle revealed that 75%, 29% and 41% were positive using serological, faecal and postmortem examination techniques, respectively. In small ruminants, the prevalence of fasciolosis was low both in coproscopy and indirect ELISA testes: only one out of 68 sheep was positive coproscopically and 3 were positive serologically; and one out of 22 goats tested was positive both at coproscopy and serology examinations. This indicates the high sensitivity of ELISA compared to the others techniques in diagnosing fasciolosis. The overall assessment indicates the relatedness of faecal examination result with ELISA findings, though the test agreement is weak (Kappa 0.236). Similarly, there was a strong relationship between faecal examination and postmortem findings of liver lesions, Kappa value, P=0.758). Out of the 54 cattle with known liver lesions of fasciola 33 (61.1%) were having elevated levels of GGT than normal value for cattle, in support of the above statement, our findings regarding the level of GGT suggest its association with liver lesions encountered at postmortem examination. The level of test of agreement between the liver examination and GGT determination was however moderate (Kappa=0.332). On the other hand the level of LDH has shown to be generally elevated both in negative as well as positive animals for liver lesions indicating the absence of relationship between liver lesions and level of LDH. The use of ELISA is found to be a more reliable diagnostic test than coproscopy in detecting fasciolosis in ruminants.

Key Words: Coproscopy, ELISA, Fasciola, Liver enzymes, Ruminants.

1. INTRODUCTION

The trematode species (known commonly as flukes) are parasitic in livestock belong to the subclass Digenea. In general, these trematodes are dorso-ventrally flattened, some being leaf-shaped and are hermaphrodites but they have the ability to reproduce asexually and multiply in aquatic or amphibious snails, which they require as intermediate hosts in order to complete their life cycle. The geographic distribution of trematode species is dependent on the distribution of suitable species of snails.

The economic losses due to fasciolosis throughout the world are enormous and these losses are associated with mortality, morbidity, reduced growth rate, condemnation of fluky liver, increased susceptibility to secondary infections and expense due to control measures. In Ethiopia, the prevalence of bovine fasciolosis has shown to range from 11.5% to 87%. A rough estimate of the economic loss due to decreased productivity caused by bovine fasciolosis is about 350 million birr (Bahiru and Ephrem, 1979). Table 2 depicts the annual economic losses calculated from abattoir findings in different parts of the country.

Fasciola hepatica and *F. gigantica* are causing major economic losses in livestock in East Africa (Malone *et al.*, 1998). *F. hepatica* was shown to be the most important fluke species in Ethiopian livestock with distribution over three quarter of the nation except in the arid north-east and east of the county the distribution of *F. gigantica* was mainly localized in the western humid zone of the country that encompasses approximately one fourth of the nation (Yilma and Malone, 1998).

Diagnosis is based primarily on clinical signs, seasonal occurrence, previous history of fasciolosis on the farm or the identification of snail habitats; postmortem examination, haematological tests and examination of faeces for fluke eggs are 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

(Urquhart *et al.*, 1996). Proper diagnosis of the disease is important in prescribing effective drugs and assists any control programs directed to fasciolosis.

However the different diagnostic techniques used today to confirm fasciolosis in ruminants have their limitations. Coprological analysis is still commonly employed to diagnose bovine 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 (Rokni *et al.*, 2003). On the other hand that weak infections cannot be detected, becoming the source of new infection (Sanchez-Andrade *et al.*, 1995).

The most direct and reliable technique for the diagnosis of fasciolosis is liver examination at slaughter or necropsy. But using this diagnostic technique it is impossible to detect fasciolosis in live animals. Serum activities of lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) may be used as markers of the different stages of fasciola infection in sheep, indicating the presence of cell necrosis caused by juvenile migrating flukes and bile duct lesions associated with mature helminthes, respectively (Ferre *et al.*, 1997). Different immunodiagnostic tests have been used in the early immune diagnosis of fasciolosis, but they have some disadvantages, such as cross-reactions with other trematodes, leading to false positive results Kara and Kircali (2004). The present project is therefore proposed with the aim of comparing the different diagnostic approaches in the diagnosis of fasciolosis in slaughtered ruminants at Debre Zeit abattoirs.

The objectives of this study were, therefore:

To compare the results of different diagnostic techniques for fasciolosis in slaughtered ruminants at ELFORA Export Abattoir.

To assess the extent of correlation of pathological lesion caused by flukes with results of enzyme analysis.

2. LITRATUR REVIEW

2.1. The parasite

According to Urquhart, *et al*. (1996), the taxonomic classification of the organisms that cause fasciolosis is presented as follows:

Phylum:	Platyhelminths,
Class:	Trematoda
Sub-class:	Digenea
Family:	Fasciolidae
Genus:	Fasciola
Species:	*Fasciola hepatica*
	Fasciola gigantica

Fasciola are large leaf-shaped hermaphrodite flukes. The anterior end is usually prolonged into the shape of a cone and the anterior sucker is located at the end of the cone. The ventral sucker is placed at the level of the "shoulders" of the fluke. The internal organs are branched while the cuticle is covered in spines (Urquhart *et al*., 1996).

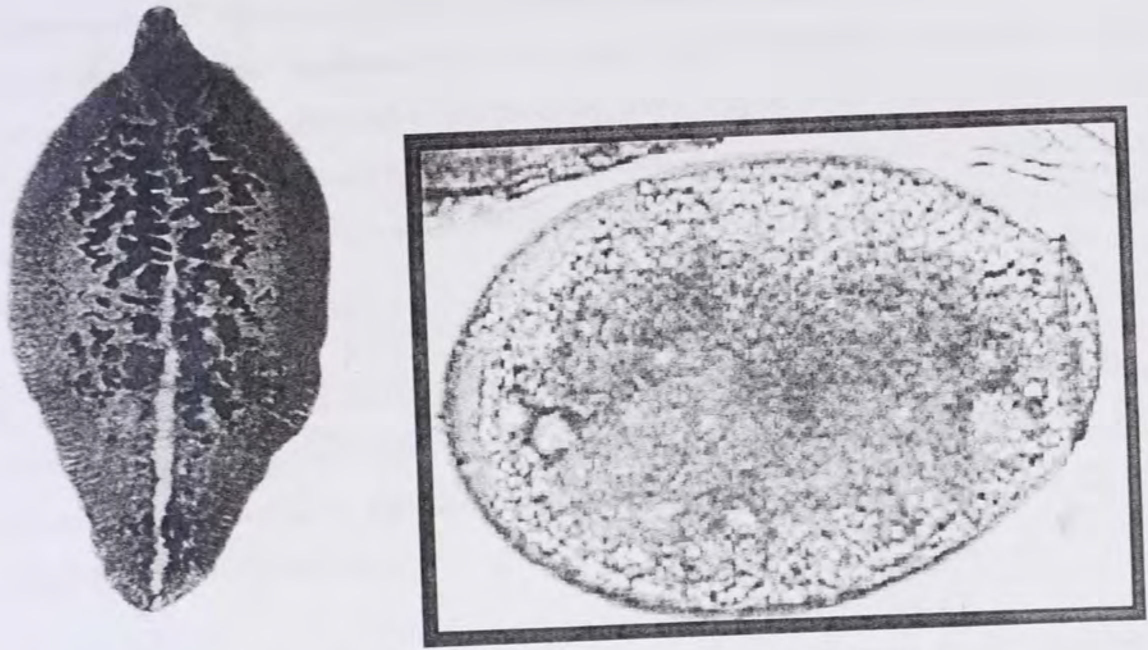

Figure 1 .*F. hepatica* and its egg by (Papas, P. W. and Wardrop, S. M.)

F. gigantica is exclusively tropical and measures 25-75mmx3.12mm. F. *hepatica* is found in temperate areas and measures approximately 20-30mmx10mm (Troncy, 1989). *F. gigantica* resembles *F. hepatica* but is readily recognized by its large size. The anterior cone is smaller than that *F. hepatica*, the shoulders are not as prominent and the body is more transparent. It is greyish-brown in colour, changing to grey when preserved (Soulsby, 1982). Fasciola eggs are small, yellow or golden in colour with an eccentric morula and indistinct cells (Reinecke, 1983).

2.1.1. Distribution

Fasciolosis is a cosmopolitan disease that occurs only in sufficiently wet areas (Troncy, 1989; Hunter, 1994). *F. gigantica* is found wherever ecological conditions are favorable to the intermediate host, such as borders of lakes, flood - prone areas, low lying marshes, and drainage ditches,(Troncy, 1989).

Those species of snails tnat transmit *F. hepatica* are found to cooler areas of high altitude while snails of *F. gigantica* are usually found in warm climates such as Africa where it

predominates over *F. hepatica* and also in southern Europe, Southern USA, the Middle East, the Indian sub-continent, Southeast Asia and certain pacific islands. In some areas, both species occur resulting in mixed infections (Soulsby, 1982; Payne, 1990; Hunter, 1994). Snails of the genus *Lymnaea truncatula* are widely distributed throughout the world particularly in Europe, Australia, New Zeland, North America, Southern USA, Caribean *etc* (Urquhart *et al.*, 1996).

Both *F. hepatica* and *F. gigantica* are found in Eastern African countries such as Ethiopia. *F. hepatica* is the most important fluke species in Ethiopian livestock, with a distribution over three-quarters of the nation. *F. hepatica* risk occur in all areas of Ethiopia except in the arid north-east and east of the county.

Using GIS forecasting model, the highest risk areas for *F. gigantica* were found to be localized in the western humid zone of the country that encompasses approximately one fourth of the nation. High risk of *F. gigantica* infection was indicated only at a small focus along the Blue Nile River. Highland areas of Ethiopia and Kenya were however identified as unsuitable for *F. gigantica* (Malone *et al.*, 1998). According to Yilma and Malone (1998), the distribution of pure *F. hepatica* and *F. gigantica* is limited to extreme high (>1800m) and low (<1200m) elevations, respectively. Mixed infections are prevalent at intermediate altitude zones (1200-1800m).

2.1.2. The status of fasciolosis in Ethiopia

In Ethiopia, both *F. hepatica* and *F. gigantica* have been reported to exist in many parts of the country. The prevalence of bovine fasciolosis based on coproscopy result varies from 11.5% in Buno Province 87% in Debre Brehan (Table 1). Abattoir studies have also reported up to 88.57% prevalence of fasciolosis in Debre Brehan.

Table 1: Prevalence of bovine fasciolosis in different regions of Ethiopia as determined on the basis of coproscopy and abattoir examination.

Administrative region	Prevalence (%) faecal	Abattoir	References
Wolaita	28	18	Getu (1987)
Nekemte	17.14; 32	22.7; 29.8	Abebe (1988); Wassie (1995)
Jimma	-	47.5; 58	Zewdu (1991); Moges (2003)
Kombolcha	51.13	53.5	Mulugeta (1993)
Kalu	15.77	-	Girmay (1988)
Bahirdar	60.2	61.97; 84.7	Fekadu(1988);Yohannes(1994)
Gondar	33.42	33.4; 50	Roman (1987); Mesfin (1999)
Debre Birhan	87	88.57	Daghe (1994); Tsegaye (1995)
Estern Gojam	50.56	-	Bayazn (1995)
Soddo	-	47	Abdul (1992)
Awassa	-	30.43	Hailu (1995)
Dembidollo	-	77.8	Abera (1990)
Arsi	53.72	41.3	Wondwosen (1990)
Asela	20.21; 32.9	-	Yosef (1993); Dinka (1996)
Wolliso	34	-	Rahmeto (1992)
Chillalo	26.2	-	Zerfu (1991)
Bale	34.6	49	Abduljebar (1994)
Zeway	32.7	-	Adem (1994)
Western Shoa	82.5	-	Yadeta (1994)
Eastern Hararge	42.9	12.1	Hymanot (1990)
Tigray	-	26	Takele (1995)
Buno province	11.5	8.1	Seyoum (1987)
Addis ababa	63.8	-	Mezgebu (1995)

Fasciolosis in sheep and cattle results in animals that show low productivity (low weight gain, low milk production, etc.) also, in many countries, including Ethiopia livers from animals infected with *F. hepatica* and *F. gigantica* are condemned as unsuitable for human consumption. A rough estimate of the economic loss due to decreased productivity caused by bovine fasciolosis is about 350 million birr (Bahiru and Ephrem, 1979). Table 2 depicts the annual economic losses calculated from abattoir findings in different parts of the country.

Table 2: Prevalence of fasciolosis and calculated economic losses at different abattoirs in Ethiopia

Place	Prevalence (%)		Annual Economic losses in the studied abattoirs (Eth. Birr)	**References**
	Bovine	Ovine/ Caprine		
Kombolcha	53.5		266,741.37 *	Mulugeta (1993)
Soddo	47		142,128 *	Abdul (1992)
Wolliso	34	133.5/12.4	78,311.60 ***	Rahmeto (1992)
Jimma	47.5		480,789.00 *	Zewdu (1991)
Gondar	75.1		497,752.36 *	Roman (1987)
Dire Dawa	12.7	14.8	309,695.00 **	Daniel (1995)
Awassa	30.43		122,775.54 *	Hailu (1995)
Bahir Dar	61.97		180,942.48 *	Yohannes (1994)
Robe	49		109,601.24 *	Abduljebar (1994)

* bovine; **bovine & ovine; ***bovine, ovine, caprine

2.1.3. The snail intermediate host

Lymnae natalensis and *Lymnae truncatula* are the intermediate hosts of *F. gigantica* and *F. hepatica*, respectively. *L. natalenisis* is truely aquatic, whereas *L. truncatula* is amphibious (Troncy, 1989). *F. hepatica* uses a mud snail as an intermediate host so that infection with this species is usually associated with herds and flocks grazing wet marshy land. On the other hand *F. gigantica* uses a water snail as its intermediate host therefore infection with this species is associated with livestock drinking from snail infected watering places as well as with grazing wet land which may be seasonally inundated (Payne, 1990).

According to Urquhart, *et al.* (1996), *L. truncatula* is the most common widely distributed snail in the world. Important Lymnae vectors of *F. hepatica* outside Europe are:

L. tomentosa: Australia, NewZealand,
L. columella: North America,
L. bulimodides: Southern USA an Caribbean,
L. humilis: North America and
L. viator: South America
L. diaphena: South America.

Lymnaea viator and *L. columella* are distributed in the regions of Brazil and Uruguay (Ueno *et al.*, 1982).

Morphologically the body – whorl of *Lymnaea truncatula* does not form the major part of the shell as in *L. natalensis* and there are at least five whorls. In the other species, there are never more than four *whorls*. Field specimens are dark brown or black in colour. The adult snails are rarely more than 4-5mm long (Reinecke, 1983).

L. truncatula are capable of withstanding summer drought or winter freezing for several months by aestivating or hibernating deep in the mud. Optimal conditions include a slightly acid pH environment and a slowly moving water medium and they feed mostly on algae, the optimum temperature range for development is 15-22^{0}C, below 5^{0}C development ceases. *L.*

natalensis is aquatic and associated with permanent water chanels or dams. Development within the snail is completed at the end of the rainy season (Urquhart, *et al*., 1996).

2.1.4. Life cycle of fasciola

The eggs of fasciola laid by the mature fluke residing in the bile duct enter the duodenum with the bile and leave the host in the faeces. The rate of development and the hatching of *F. hepatica* eggs depend on temperature but at 26^0C eggs hatch in about 10-12 days, and in 17 days for those of *F. gigantica*.

In the egg the embryo develops into a pyriform (pear shaped), ciliated larva called a miracidium. Under the stimulus of light, the miracidium release an enzyme, which attacks the proteinaceous cement holding the operculum in place. And the miracidium emerges within a few minutes and propelled through the water by its cilia, does not feed and must, for its further development, find a suitable snail within a few hours. It is believed to use chemo tactic responses to "home" on the snail and on contact it adheres by suction to the snail and penetrates its soft tissues aided by a cytolytic enzyme. The entire process of penetration takes about 30 minutes after which the cilia are lost and the mircacidium develops in to an elongated sac, the sporocyst containing a number of germinal cells, these cells develop in to rediae which migrate to the hepatopancreas of the snail.

Rediae are larval forms possessing an oral sucker, some flame cells and a simple gut. From the germinal cells of the rediae arise the final stages, the cercariae although if environmental conditions for the snail are unsuitable, a second or daughter generation of readiae is often produced instead. The cercariae are in essence a young flukes with long tails and emerge actively from the snail usually in considerable numbers. Once a snail is infected cercariae continue to be produced indefinitely although the majority of infected snails die prematurely from gross destruction of the hepatopancreas (Soulsby, 1982; Troncy, 1989; Urquhart *et al*., 1996).

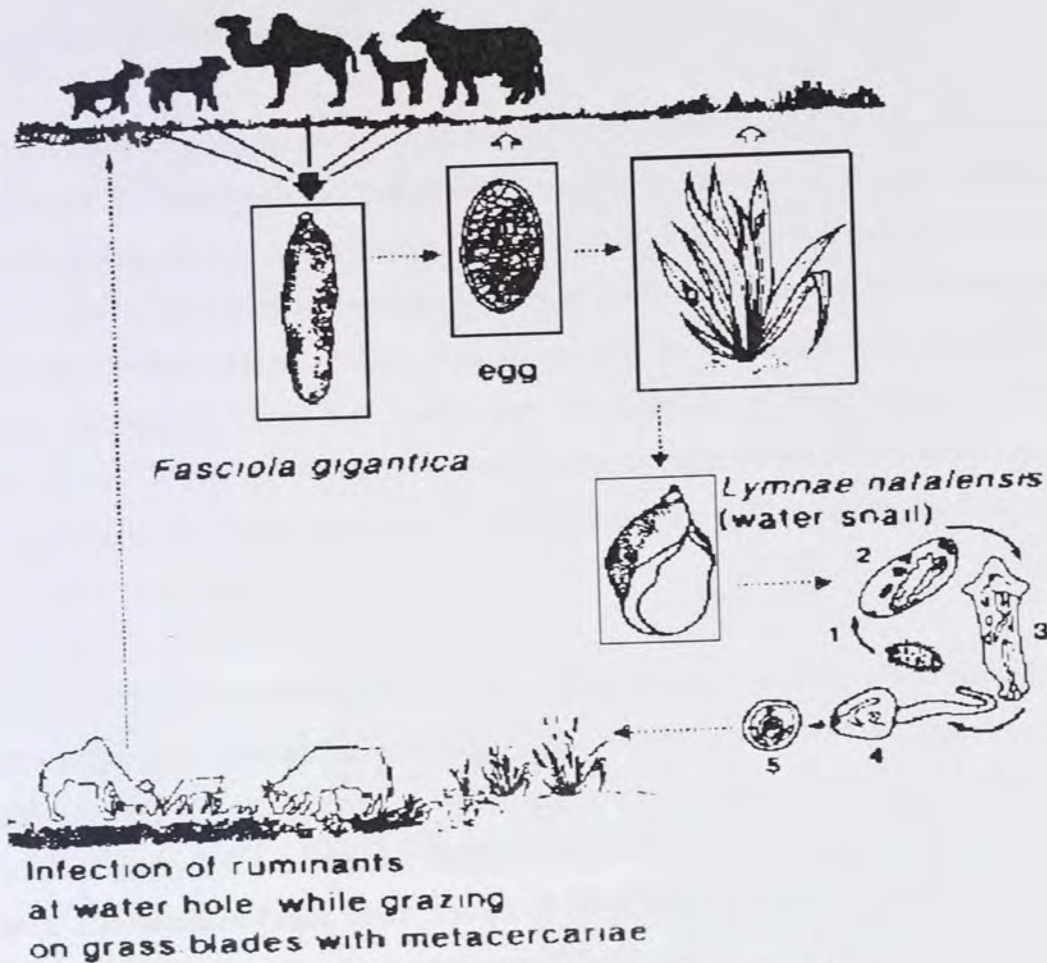

Figure 2. Life cycle of *Fasciola gigantica* (Jorgen & Brian, 1994).

Further development in the snail is complex but leads to eventual release of many motile forms, which attach to firm surfaces such as blades of grass where they encysted to infectious forms called metacercariae. If ingested by a susceptible host, the metacercariae release immature flukes in the small intestine, which migrate through the liver to the bile ducts and sometimes the gall bladder. When mature, the flukes shed eggs, which are passed out in the faeces and thus continuing the life cycle (Hunter, 1994).

One trematode egg may eventually develop in to hundreds of adults; this is due to the phenomenon of paedogenesis in the molluscan intermediate host, the production of new individuals by single larval forms (Urquhart *et al*., 1996).

2.1.5. Pathogenic significance

These vary according to the phase of parasitic development in the liver and the species of host involved. Essentially the pathogenesis is two-fold the first phase occurs during migration in the liver damage and haemorrhage. The second occurs when the parasite is in the bile ducts and results from the haematophagic activity of the adult flukes and from damage to the biliary mucosa by their cuticular spines. Fasciolosis may be acute, sub-acute or chronic. The acute ovine fasciolosis occurs 2-6 weeks after the infection of large number of metacereariae, usually over 2000, and is due to the severe haemorrhage, which results when the young flukes migrating in the liver parenchyma, rupture blood vessels. Damage to the liver parenchyma is also severe (Urquhart *et al.*, 1996).

Lesions of the acute form are those of simple traumatic hepatitis, or traumatic hepatitis linked to an infectious hepatitis is seen in the most severe infections. The liver is hypertophic and haemeorrhagic, with numerous haemorrhagic tracts. On careful examination, young flukes (*adolescariae*, 2-4 mm long) are found throughout the organ, the peritoneal cavity is infiltrated with a serohaemorrhagic fluid. Traces of local peritonitis can also be seen in the form of haemorrhagic patches on the peritoneum (Troncy, 1989).

The clinical entity of the acute and sub acute forms are seen in animals of all age and states of nutrition. Death may occur rapidly, or after several days. Animals are disinclined to move, anorexic and show a distended abdomen, which is painful to the touch. A complication of the acute condition is "Black Disease" caused by *Clostridium novyi*. These are anaerobic bacteria which proliferates in the necrotic lesions produced by the immature trematodes and the liver become putrefied as it decomposes rapidly and turns greenish, there is abundant production of gas and the term "liver rot" is used which is also applied to simple traumatic hepatitis (Soulsby, 1982 and Troncy, 1989).

Chronic fasciolosis. This is the most common form of the infection in sheep, cattle and other animals including man. The essential lesion is a progressive biliary cirrhosis, which ultimately produces a hard fibrotic liver in which the bile ducts are prominent, thickened, fibrous and in

cattle often calcified and cholangitis with dilated bile ducts containing flukes and their eggs etc (Soulsby, 1982).

The chronic fasciolosis occurs 4-5 months after the ingestion of moderate numbers, 200-500 of metacercariae. The principal pathogenic effects are anaemia, hypoalbuminaemia, calcification of the bile ducts and enlargement of the gallbladder. Aberrant migration of the flukes is more common in cattle and encapsulated parasites are often seen in the lungs. In heavy infections where anemia and hypoalbuminaemia are severe, sub mandibular oedema frequently occurs (Urquhart *et al.*, 1996).

2.2. Diagnostic approaches to fasciolosis

Diagnosis of fasciolosis both in animals and man may involve consideration of various aspects such as history, clinical findings and general epidemiology of the disease. Confirmation in all cases can be made either by faecal examination or recovery of the worms at postmortem examination. Currently serological and molecular techniques are developed by various researchers. Analysis of the enzyme and hematological profiles are also known to give important clue as to the presence or absence of fasciolosis in animals.

2.2.1. History and Clinical Manifestation

Infection with *F. hepatica* is usually associated with herds and flocks grazing wet, marshy land. On the other hand, *F. gigantica* uses a water snail as its intermediate host. Therefore, infection with this species is associated with livestock drinking from snail infected watering places as well as with grazing wet land which may be seasonally in undated (Payne, 1990).

In acute cases of fasciolosis, sudden death and severe anaemia occurs due to the migrating young flukes through the liver, however no fluke eggs are passed in the faeces. In subacute cases, signs of rapid loss of condition, severe anaemia, high fluke egg counts, death occurs 12-20 weeks after infection, and in chronic fasciolosis gradual wasting, severe anaemia with

ascites, oedema, bottle jaw and very high fluke egg counts may lead to death more than 20 weeks after infestation (Reinecke, 1983; Troncy, 1989; Soulsby, 1982).

2.2.2. Postmortem Examination

The most direct and reliable technique for the diagnosis of fasciolosis is liver examination at slaughter or necropsy. In the diagnosis of this disease, the liver of each animal will be removed and examined for gross pathological lesions and then the organ will be cut, sliced and soaked in physiological saline at 37^0C for 3-4 hr. The bile duct and the gall bladder will also be incised to recover adult and immature flukes and finally, the total number of flukes collected from each animal will be counted and recorded (Manyazewal, 1995). In acute fascioliasis, there may be peritonitis, particularly on the visceral surface of the hepatic capsule. The migrations of the flukes in the liver leave dark haemorrhagic streaks and foci. The liver is swollen, friable and has capsular perforations marked by haemorrhagic tags. Older tunnels appear as slight yellow streaks (VEIN, 2004).

Calcification of the bile ducts and enlargement of the gallbladder are characteristic lesions observed in chronic cases of fasciolosis. Progressive biliary cirrhosis which ultimately produces a hard fibrotic liver in which the bile ducts are prominent, thickened, fibrous and, in cattle, often calcified. Histologically, the fibrosis is produced by repair to the migratory tracts and a cholengitis, the bile duct walls are markedly thickened and the bile ducts are dilated containing fluke and numerous eggs (Soulsby, 1982).

While this method can provide information about the status of liver flukes on a particular property, it does not evaluate annual variations of infection level or itself to use with arriving feedlot cattle from unknown origins (Briskey, 1998).

2.2.3. Laboratory Diagnostic Methods

2.2.3.1. Faecal examination

Two points need to be kept in mind while interpreting faecal examination results for *F.hepatica*: a) the prepatent period for *F. hepatica* is 2-3 months. As a result, fluke eggs cannot be demonstrated early in the infection. A group of cattle could be carrying a high burden of young flukes, but no fluke eggs would show up in their manure. b) The quantitative value of fluke egg counts is questionable. Fluke eggs pool in the gallbladder and intermittently pass into the feces. The fluke egg count on any given day often has little relationship to the number of flukes in the liver; an animal with a negative faecal could be parasitized, whereas a high faecal fluke egg count could jest be a high number of eggs leaving the gallbladder that day, rather than a large fluke burden (Briskey, 1998).

Sedimentation procedures concentrate both feces and eggs at the bottom of a liquid medium usually water, and detects most parasite eggs or cysts that have too high a specific gravity mainly trematode (fluke) eggs (Hendrix, 1998). Faecal examination for fluke eggs requires use of faecal sedimentation, formalin-ether, or floatation techniques (Kaufman, 1996 William, 1997 and Antonia, 2002).

Commonly used flotation procedures open the operculum and sink the fluke egg rather than floating it for surface detection. Fluke eggs are comparatively heavier than strongyle eggs and as a result the eggs do not float in routinely used floatation mediums such as saturated salt solution. However, floatation fluids of higher specific gravity such as saturated zinc solutions, magnesium sulphate and potassium iodomercurate has been used to float eggs of fasciola. Due to the high specific gravity and the saturation of these solutions damage to observed eggs is very high. Several investigators have tried various types of flotation techniques but with inconsistent results because of the collapsibility of fluke eggs in solutions of high specific gravity. However a modification of sheathers sugar flotation technique with a higher specific gravity has been used to demonstrate fluke with little distortion to the eggs (Sloss *et al.*, 1978).

Advantages and limitations of faecal examination:

Faecal examination is the usual diagnostic method, but it is only useful in cases of chronic fasciolosis. It is less expensive and it doesn't require expensive equipments. As a result, it is the most widely used technique in the diagnosis of fasciolosis. (Perez *et al.*, 2005) reported that eggs in stools indicate the presence of mature adult flukes in the bile ducts and gall bladder. However, faecal examination has several limitations in that eggs only appear in the faeces 15 weeks after infection in the case of *F. gigantica*, and after 10 weeks for *F. hepatica* (Troncy, 1989). A longer period from 8-15 weeks after infection is needed for the appearance of fasciola eggs in the faeces, so most of pathological lesions had already occurred (Hillyer, 1999; Oneill *et al* 2000; Cornelisson 2001; Sanchez- Andrad 2002).

In addition, those does not detect recent infection, because eggs are not found during the prepatent period on the other hand in some cases difficult during the patent period because eggs are expelled intermittently, depending on the evacuation of the gall bladder and the biology of the fasciola. Faecal examination should be repeated for this type of diagnosis (Troncy, 1989 and Briskey, 1998).

Fasciola eggs and those of paramphistomes are very similar in appearance, except for the colour (fasciola eggs are small, yellow or golden in colour with an accentric morula and indistinct cells while those of the paramphistomes are grayish for this reason it is necessary to use a drop of methylene blue to differentiate between the two eggs during microscopic examination (Troncy, 1989 and Reinecke, 1983).

Cornelissen *et al.,* 2001 detected after natural infection of *F. hepatica* positive animals by egg count only from week 9 in sheep and even later in cattle from week 15. And Arias *et al.,* (2005) in their experimental work detected faecal egg-output in sheep 11 weeks after primary infection.

2.2.3.2. Serological Tests

Detection of circulatory antigens in ruminants using antibodies, which were reactive with defined components of the antigen repertoire of fasciola spp, has been the subject of study by various researchers through out the world.

The enzyme linked immunosorbent assay (ELISA) has been used for the detection of serum antibodies to fasciola antigens and for the detection of circulating fasciola antigens using monoclonal antibodies (Cervi *et al.*, 1996 and Rickard 1995). Circulating *F. hepatica* antigens were detected in murine fasciolosis by ELISA excretory secretory antigens in sera from patients infected with *F. hepatica* by a sandwich –type ELISA technique using monoclonal antibody Cervi *et al*., (1996). Martinez *et al*., (1996) indicated that the application of the ELISA with fasciola antigens allows the early detection of specific antibodies in goats, sheep and cattle fasciolosis and that could be interesting diagnostic method in these animals

A serological screen test by ELISA was developed using an antigen the excretory-secretary products of adult liver flukes. Using this technique it was possible to detect 39 of 40 cattle harboring liver fluke in Mauritania Bent *et al*., (2003). Anderson *et al*., (1999) indicated that counts of fasciol*a* species eggs in faeces and measurements of antibody concentration to the excretory, secretary antigens of fasciola species by ELISA were related to the numbers of flukes in the livers of 92 cattle killed in the abattoirs of Hanoi city Vietnam. No eggs of fasciola species were detected in the faeces of one third of infected cattle and 60% of the counts were less than 100 eggs per gram. The sensitivity of the egg counting method was 66.7% and specificity 100% overall accuracy was 73.9% corresponding values for the ELISA methods were 86.1, 70 and 82.6%, respectively. The positive and negative predictive values for the egg counting method were 100 and 45.5% and for the ELISA method were 91.2% and 58.3%, respectively.

A study was also conducted, in Mexico, with the aim of comparing the sensitivity, specificity and usefulness of the DIG-ELISA, DOT-ELISA and indirect ELISA tests for determining the seroprevalence of fasciolosis in cattle under tropical conditions. Indirect ELISA showed a

sensitivity of 96.5% and specificity of 98.8%, Dig ELISA 97.5% and 80.0% and Dot ELISA 93.1% and 95.4%, respectively. Indirect ELISA yielded the highest level of IgG and *F. hepatica* antibodies (Ibarra *et al.,* 1998).

An improved hemagglutination (HA) test using the purified specific F2 antigen of *F. hepatica* has also been evaluated with regard to its potential use for the prediction of chemotherapeutic success in natural bovine infections with *F. hepatica*. The result shows that the F2-HA test is useful for the prediction of chemotherapeutic success in bovine fascioliosis (Levieux *et al.*, 1992).

Different types of antigens such as whole worm antigen, excretory- secretory antigens can be prepared for the purpose of serological test. The parasites can be collected from infected animals either at postmortem examination or from local abattoir. According to Bradford (1976) cited in Fagbemi et al., 1995 whole worm antigens of the parasites can be prepared by homogenizing them in 0.01 M phosphate buffered saline (PBS), ph 7.2 in a ten-broeck tissue blender followed by centrifugation at 4°C for 1h at 100,000 g. The PBS was supplemented with 10 mM lewpeptin to inhibit proteases. Excretory – secretory (ES) antigens can also be prepared from F. gigantica by incubating the worms (one worm per 5 ml) in leupeptin-supplemented.

Advantages and limitations of serological tests

Some of the desirable qualities of a good diagnosis test are early detection, specificity and the ability to detect current infection. In sheep, serum antibodies to *F. hepatica* antigens were detected as early as 6-8 weeks post-infection by the ELISA and 2-4 weeks post infection by the dot-ELISA. Detection of serum antibodies to *F. hepatica* antigens in llamas is possible by 2 weeks post-infection with the dot ELISA Rickard (1995). Hillyer, 1999 and Martinez *et al.*, 1996 indicated that these techniques are an important adjunct for the diagnosis of fasciolosis because an earlier detection than coproscopic is allowed.

Hillyer *et al*., (1996), reported that out of 147 cattle tested, 38 were positive parasitologically while 84 were positive serologically. Of the 38 positives for fasciola eggs, 31 were positive by serology (sensitivity 82%) and, out of 184 sheep examined, 22 were positive for fasciola eggs, while 163 were positive by serology. All of the 22 sheep, which were positive parasitologically, were also positive serologically for a sensitivity of 100%.

Techniques of indirect immunoenzymatic diagnosis represent an important contribution to the early detection of infection with *F. hepatica*. By means of the indirect ELISA based on excretory, secretory (ES) antigen, it has been possible to detect experimental infections in cattle from the third to the fifth week after infection during the liver migratory phase of immature worms (Marin, 1992).

Early detection of circulating antigens, sometimes as early as the second and third weeks after infection through serological techniques enables prompt intervention in treating infected animals. On the contrary, diagnosis by faecal observation was not possible until 7 to 8 weeks after, by which time the flukes would have caused severe liver pathology (Fagbemi *et al*., 1995).

The immunoenzymatic techniques such as indirect ELISA have been found to be very suitable for the diagnosis of fasciolosis due to their high sensitivity and the possibility of processing many sera samples (Arriaga de Morilla *et al*., 1989).

An ELISA test is highly specific for trematodes and sensitive but remains positive for 5 months after treatment with or without the presence of liver flukes. The test can be particularly useful for the early diagnosis of fasciola infection (VEIN, 2004).

Nevertheless the presence of antibodies does not always correlate to active fasciolosis because antibody levels diminish slowly after cure (Hillyer, 1999; Sachez-Andrade *et al*., 2002).

The immunodiagnostic detection of Fasciolosis can be done during the prepatent stages before eggs appear in faeces. The usefulness of immunodiagnostic testes for the detection of helminth

infections, in general, is however diminished by a lack of specificity of the assays, which is due to the possession of common antigens, by several unrelated helminths. Therefore, the first step to enhance specificity in serodiagnosis of fasciolosis is the production of specific antigens. On the other hand antigen detection assays have several advantages over other diagnostic methods. They can identify animals with prepatent or occult infections, they give a more accurate indication of active infection than many serodiagnostic tests, and they provide an indirect measurement of helminthes burden (Fagbemi *et al.*, 1995).

In a comparative study involving three serological tests, indirect haemagglutination and counter- immunoelectrophorosis were found to be superior to double immunodiffsion and positive reactions in these techniques preceded the presence of parasite eggs in the faeces by at least 50 days Van-tiggele and Over (1976).

2.2.3.3. Enzyme analysis

Changes in serum enzymes are indicators of hepatic metabolism impairment (Galtier *et al.*, 1994 and Ferre *et al.*, 1996). Certain tissue cells contain characteristic enzymes, which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. GGT levels rise dramatically with obstructive diseases of the biliary tract and liver cancers. GGT is especially useful in assessing liver function associated with liver disease (Ramnik Sood, 1999; Charles, 2003; Merck, & Co, 2006; Coles, 1986).

LDH is present in nearly all types of metabolizing cells, but different cells have different forms of the enzyme, which can be distinguished. The enzyme is especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain, and lungs. The total LDH can be further separated into five components or fractions labeled by number: LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5. Each of these fractions, called isoenzymes (existence of enzymes in multiple forms), is used mainly by a different set of cells or tissues in the body. LDH-5 is found mainly in the liver, caused by hepatocellular disease and are useful in assessing acute ongoing liver disease (Coles, 1986 and Charles, 2003).

Assessing the profile of liver enzymes has also been used to monitor the extent of hepatic damage following antehelmintic treatments or artificial infection. A rise in the level of serum hepatic enzymes GGT and LDH were shown to be indicatives of liver damage (Martinez-Moreno *et al.,* 1999). In addition to the rise in serum enzymes, decreased level hemoglobin concentration and packed – cell volume were noted after infection of goats with *F. gigantica* (Haroun *et al.,* 1989).

Estimation of serum levels of enzymes released by damaged liver cells is used as supplement to faecal examination to justify the presence or absence of infection with fasciolasis. The two enzymes that are usually measured and indicative of liver damage are the glutamate dehydrogenase (GLDH) and the gamma qlutamyl transpeptidase (GGT) (Urquhart *et al.*, 1996). Serumactivities of glutamate dehydrogenase (GLDH) and gamma glutamyltransferase (GGT) may be used as markers of the different stages of fasciola infection in sheep, indicating the presence of cell necrosis caused by juvenile migrating flukes and bile duct lesions associated with mature helminthes, respectively (Ferre *et al.*, 1997).

LDH modification indicates mostly a cell necrosis during migration of young flukes through the liver parenchyma. LDH provide information on the passage of young flukes through the liver parenchyma (Coles, 1986 and Charles, 2003) whereas GGT would be more associated with bile duct damages GGT increase indicates penetration of the bile ducts by flukes, causing a hyperplastic cholangitis (Galtier *et al.,* 1986; Ferre *et al.*, 1994, 1995a, 1995b, 1996, 1997).

Other enzymes studied in connection with liver fluke infection are serum Sorbitol dehydrogenase. In artificially infected with a range of fluke burdens, serum sorbitol dehydrogenase activity exhibited several peaks, before and after week 12-post infection, when flukes should have been present in the bile ducts (Hawkins, 1984).

3. MATERIAL AND METHODES

3.1. Study area

This study was carried out in ELFORA Export Abattoir found in Debre Zeit. Debre Zeit is located some 45km south east of Addis Ababa and has total human population of 95000 (CSA, 2001). The area has an altitude of 1850 meter above sea level and a bimodal rainfall pattern with a long rainy season from June to October and a short rainy season from March to May. The average annual rainfall and average maximum and minimum temperature for the area are 800mm, and 27.7 ^{0}C and 12.3 ^{0}C respectively. (CSA, 2001).

The ELFORA Export Abattoir was established in 1967 and the main purpose of the abattoir is processing of several classes of livestock in to fresh meat for human consumption. In the abattoir, cattle, sheep and goats purchased from different parts of the country and slaughtered. Approximately 4680 cattle, 52000 sheep and 124800 goats are slaughtered annually. The abattoir exports fresh meat of small ruminants for different countries such as United Arab Emirates, Saudi Arabia, and Yemen. It also provides fresh meat for different institutions such as hotels, universities and others within the country.

3.2. Study population

The sampling units for the study were local breeds of ruminants (cattle, sheep and goats) purchased from different parts of the country for slaughtering purpose at ELFORA Export Abattoir. All the animals slaughtered at the abattoir were males usually under 1-4 years of age in (sheep and goats) and (1-10) years of age for cattle.

The study was conducted on 1059 ruminants (324 cattle, 350 sheep and 385 goats). Liver and coprological examination was conducted on all animals, whereas serological tests (indirect ELISA) and biochemical analysis (serum enzyme GGT and LDH measurements) were conducted on (134 cattle, 68 sheep and 22 goats).

3.2.1. Type of study and sample size determination

To determine the prevalence of the disease a cross-sectional study type of investigation was conducted. The desired sample size for the study was calculated using Win-Episcope 2,0 computer software program (Thrusfield, 1995) with 95% confidence interval and at 5% absolute Precision. Assuming 42.9% average prevalence of fasciolosis for cattle, 50% for sheep and goats in ELFORA Export Abattoir, 324, 350 and 385 samples were examined respectively. Therefore a total of 1059 ruminants were sampled for the study at ELFORA Export Abattoir.

3.3. Study methodology

3.3.1. Coprological examination

Faecal samples were collected from the study animals directly from the rectum during antemortem examination. The collected samples was taken to the laboratory with tightly closed universal bottles and examined for fasciola eggs according to the method described by Antonia *et al.*, (2002).

3.3.2. Postmortem examination

The liver of each animal was removed and examined for gross pathological lesions the bile duct and the gall bladder were also incised and then the organ was cut

Identification of the fluke species involved was carried out by using size parameters described by Soulsby (1982) and categorization of the pathological lesions observed in affected livers was based on the approach by Ogunrinade (1980) as follows; (a) Lightly 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.

3.3.3. Enzyme analysis

Blood samples were collected using sterile vacutainers from the jugular vein of the study animals and the serum separated and kept at-20 °C until tested. Serum activities of LDH and GGT were measured using the photometer to 0 absorbance and 340 nm commercial kits.

3.3.4. Serological examination

Blood samples were collected using sterile vacuntainers from the jugular vein of the study animals and the serum separated and kept at-20 °C until tested. The serum collected was tested for the presence of fasciola antibodies using ELISA kits as described by Cervi *et al.*, 1996 and Rickard 1995. This fasciola antigen coated ELISA diagnostic kit was used to detect fasciola antibodies against the specific "f2" antigens.

The test is made reliable by using the "f2" antigen purified from fasciola extracts. The "f2" antigen is highly specific for *F. hepatic*a. This kit has been standardized according to the hemagglutination method (HA). This kit allows determining the rate of antibodies directed to *F. hepatica*. It has been validated for bovine and ovine sera and on bovine milk. (Institut Pourquier).

3.3. 4. 1. Enzyme Linked Immunosorbent Assay plate lay out

The ELISA plate lay out for the distribution of the sample and control sera was used according to the instruction of the manufacturer. The negative controls were dispensed in A1, A2, and the positive controls were dispensed in B1, B2 and C1, C2. Serum samples were dispensed also in duplicate in all even and odd numbers in order to establish an average OD (Optical Density) value.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	N										
B	P	P										
C	P	P										
D	1	1										
E	2	2										
F	3	3										
G	4	4										
H	..	..										

Key: N = Negative control, P = Positive control, 1 = Sample no. 1, 2 = Sample no. 1, 2,

Figure 3 Enzyme Linked Immunosorbent Assay plate lay out for sera distribution

3. 3. 4. 2. Enzyme Linked Immunosorbent Assay test validation and interpretation

The sample was tested in duplicate on antigen pre-coated polystyrene microplates with a total of 45 samples per plate. Serum samples and control sera were diluted 1:20 in the ELISA buffer. Negative and positive sera were tested in duplicate (Institut Pourquier).

The optical density for each sample was read at 450nm after the photometer was blanked on air. The corrected OD 450 was calculated for each serum by subtracting the OD 450 value obtained from the uncoated well from the OD 450 value of the coated well. The test result was considered reliable when the positive control serum has a minimum uncorrected OD 450 value of 0.350 and a ratio between the corrected OD 450 value of the positive control and uncorrected OD 450 value of the negative control is greater than or equal to 3.5.

Interpretation

Calculation of, the ratio s/p:

$$S/P = \frac{\text{Corrected OD450 value of the sample}}{\text{Corrected OD450 value of the positive control}} \times 100$$

3.4. Data analysis

Ms Excel spreadsheet was used for data storage and descriptive statistics analysis.

Results of diagnostic techniques were analyzed using Win-Episcope 2.0 software program (2000), testes such as confidence interval and the kappa statistic (agreement among different tests) Sensitivity and specificity were also analyzed.

4. RESULTS

4.1. Coprological study

Out of the 324 fecal samples collected from cattle, 108 (33%) were found to be positive for eggs of fasciola. Similarly, out of 350 sheep and 385 goats examined for fasciolosis, 4 (1.14%) and 2 (0.51%), respectively, were positive for fasciola eggs (table 3).

Table 3. Prevalence of fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on coproscopy.

Spp	Sample size	Prevalence	95% ci
Cattle	324	108(33%)	28.6 - 38
Sheep	350	4(1.14%)	0.4 - 3
Goat	385	2(0.5%)	0.1 - 2

Detailed studies were done on 134 samples and out of 134 cattle examined for fasciola eggs 41(29.9%) positive and 93(70.1%) negative results were recorded. And Comparisons were made with results of direct and indirect diagnostic methods for fasciolsis. The sensitivity and specificity of coproscopy were 74% and 98.7% respectively.

4.2. Post mortem inspection

Out of the 324 cattle, 350 sheep and 385 goats slaughtered at ELFORA Export Abattoir, 125 (38.5%), 4 (1.14%) and 2 (0.51%) were found to be positive for lesions of fasciolosis, respectively during post mortem inspection (Table 4).

Table 4. Prevalence of fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on abattoir survey.

Spp	Sample size	Prevalence	95% ci
Cattle	324	125 (38.5%)	33.6 -44.3
Sheep	350	4(1.14%)	0.4 - 3
Goat	385	2(0.5%)	0.1 - 2

Out of the 324 examined cattle, detailed studies were made on 134 randomly selected livers (54 positive and 80 negative for fasciola lesions) and comparisons were made with results of direct and indirect diagnostic methods for fasciolsis. Based on the classification of liver pathology (Ogunrinade, 1980) 31(23%) of livers were lightly infected, 20(15%) moderately infected and 3 (2.2%) were severely damaged (Figure 4).

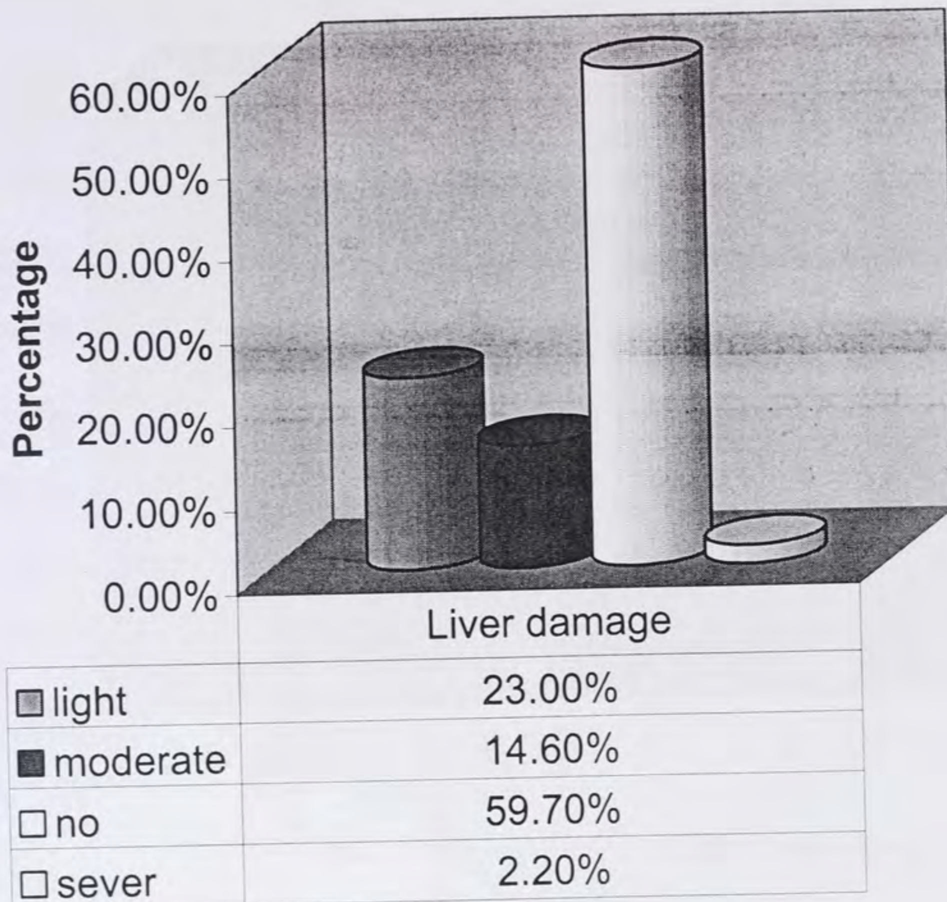

Figure 4. Classification of liver pathology

4.3. Serum enzyme analysis

Out of the 134 cattle serum samples (59% negative and 41% positive for fasciola liver lesions) examined for the level of GGT (gamma-glutamyl transferes), 56(41.8%) animals had an increase in the level of concentration of GGT suggesting the possible presence of liver lesions or increased activity of liver. On the other hand, an increased percentage in the response of LDH (Lactate dehydrogenase) enzyme activity was found in the same number of serum samples analyzed. Out of the 134 cattle serum samples, 100(74.6%) of the animals had an increase above the normal value of serum LDH activity (figure 5).

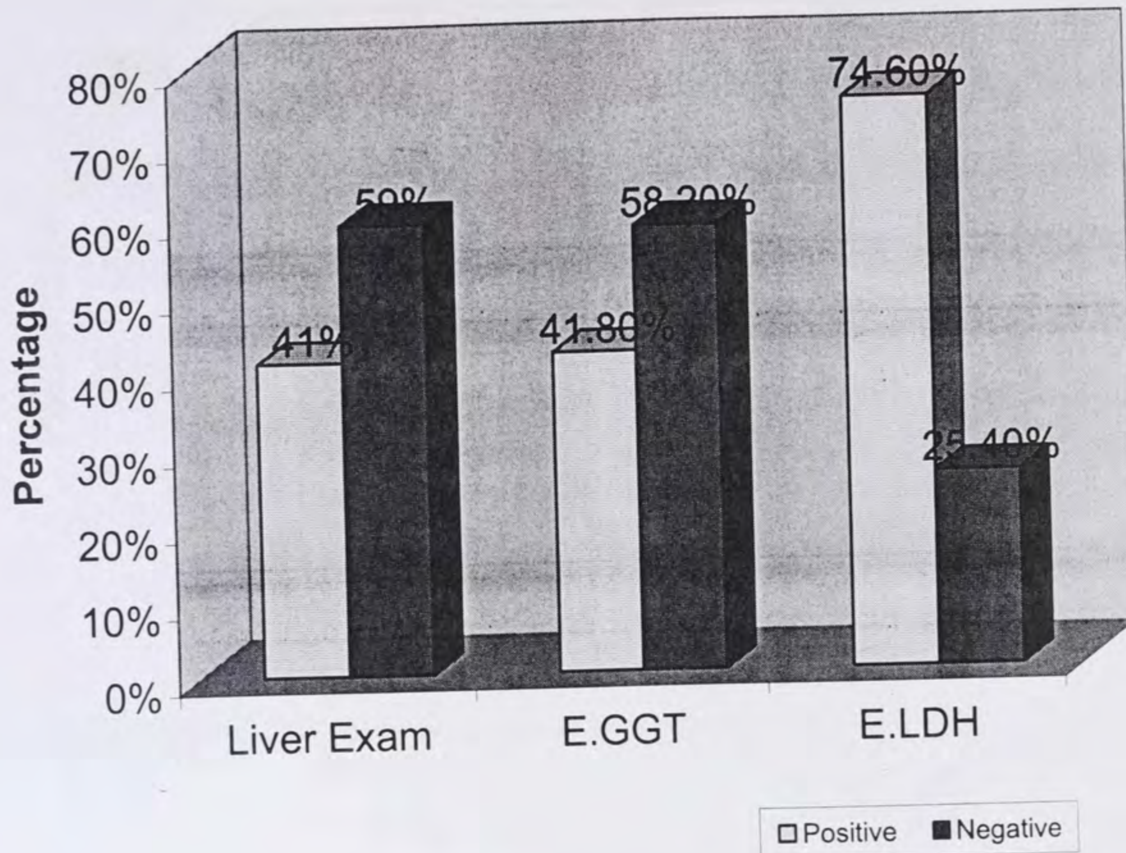

Figure 5. Comparison of enzyme activities and liver pathology in cattle.

4. 4. Serological examination (ELISA)

Out of the 134 cattle serum analyzed using indirect ELISA method, 100(75%) of the samples were found to be positive for antibodies against fasciola specific f2 antigens at various levels of infestation. 23%, 48% and 1.5% of the samples were suggestive of having low, medium and severe level of fasciola infestation, respectively (Figure 6). The sensitivity and specificity of ELISA were 100% and 42.5% respectively.

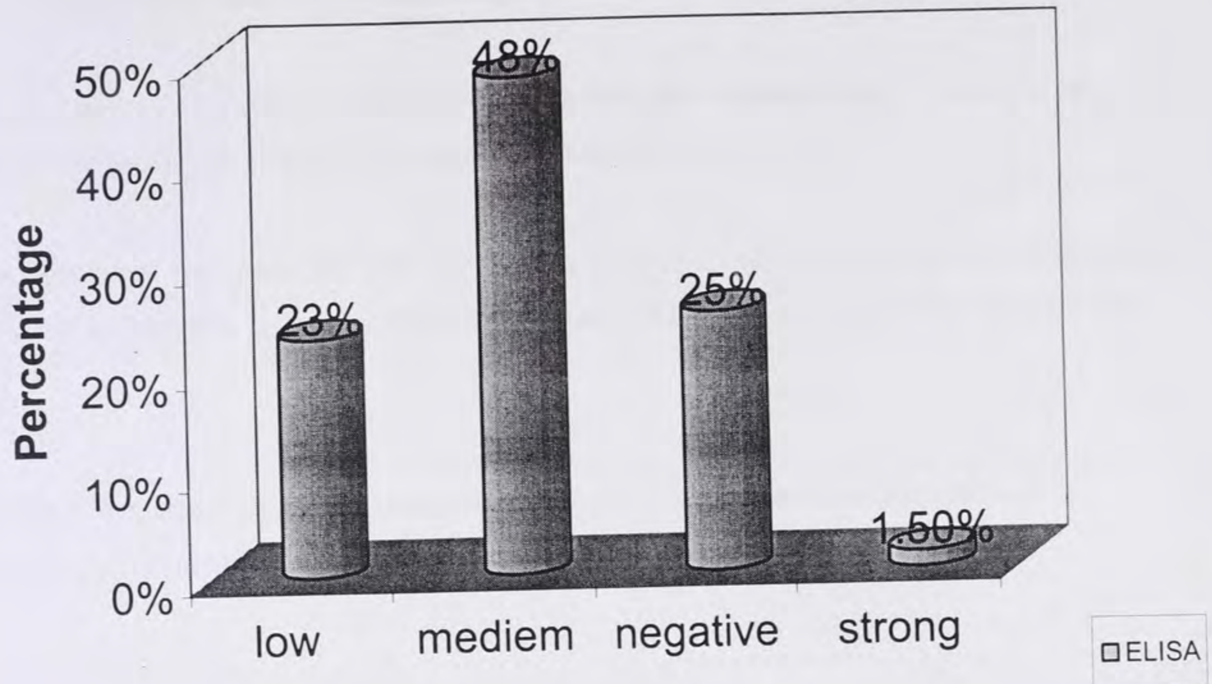

Figure 6. ELISA results and level of infestation within the herd.

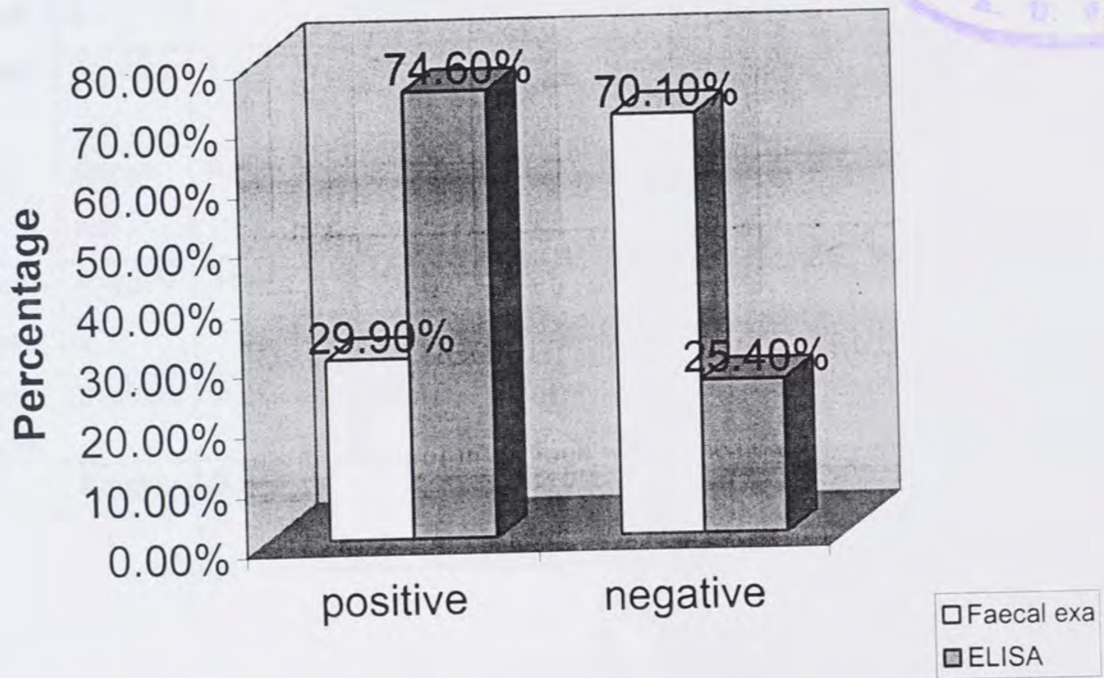

Figure 7. Comparison of results of ELISA and faecal sedimentation

4.4.1. Comparative results of different diagnostic tests

The summary of the results of the different diagnostic test employed on 134 selected cattle and the statistical analysis results are presented in (Table5) below.

Test agreement between PMI and ELISA was compared using kappa statistic where kappa value 0.8-1.0 denotes very good, 0.6-0.8 sustained and 0.4--0.6 moderate (Thrusfield, 1995).

Table 5. Comparison of the different diagnostic methods in relation to the results of coproscopy and postmortem examination.

Factor		ELISA		GGT		LDH		PMI	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Faecal examination	Negative	33	60	63	29	21	71	79	14
	Positive	1	40	15	26	10	31	1	40
	Kappa test	0.236		0.291		-0.011		0.758	
Liver Examination (PMI)	Negative	34	46	57	22	16	63		
	Positive	0	54	21	33	15	39		
	Kappa test	0.373		0.332		-0.066			

5. DISCUSSION

In the present study, the prevalence of fasciolosis based on postmortem examination at ELFORA abbttoir was 38.5%, 1.14% and 0.51% in cattle sheep and goats, respectively. Almost similar coproscopic prevalence was found in sheep and goats. A coproscopic prevalence of 33% fasciola was found in cattle. The overall prevalence in sheep and goats was very low compared to the result found in slaughtered cattle. One of the probable reasons is the difference in origin of animals slaughtered at ELFORA abattoir. Almost all small ruminants slaughtered originate from the mid and lowland parts of the eastern Ethiopia, areas most of the time considered to be low risk areas for *Fasciola hepatica* infection due to the arid or semiarid ecology of the area. *F. hepatica* is the most important fluke species in Ethiopian livestock, with a distribution over three-quarters of the nation. *F. hepatica* risk occur in all areas of Ethiopia except in the arid north-east and east of the county. According to Yilma & Malone (1998), the distribution of pure *F. hepatica* and *F. gigantica* is limited to extreme high (>1800m) and low (<1200m) elevations, respectively. Mixed infections are prevalent at intermediate altitude zones (1200-1800m). The present finding is by far lower than the report of Daniel (1995), who reported based on abattoir survey 14.8% of ovine fasciolosis in Dire Dawa, an area with a similar ecology to the origin of small ruminants of the present study. Most of the slaughtered cattle at ELFORA originate mainly from the neighboring areas of Debre Zeit. These variations in origin might have contributed to such difference in the prevalence of fasciolosis between large and small ruminants. The present finding in cattle is comparable with the results of many authors in various parts of Ethiopia (Getu, 1987; Abebe, 1988; Mulugeta, 1993; Daghe, 1994; Wondwosen, 1990; Yosef, 1993; Adem, 1994; Mezgebu, 1995).

The lower prevalence of fasciolsis reported using coproscopy in bovine compared to post mortem finding indicates the less sensitivity of the test in detecting the actual presence of the disease. According to Abdul Jebar the prevalence of fasciolosis by coproscopy were 34% while 49% by post mortem examination; the sensitivity of sedimentation technique in detecting fasciola infection is about 33%. A longer period from 8-15 weeks after infection is

needed for the appearance of fasciola eggs in the faeces, so most of pathological lesions had already occurred (Hillyer, 1999; Oneill *et al* 2000; Cornelissen, 2001; Sanchez-Andrade *et al.,* 2002). Furthermore, detection of fasciola eggs and diagnosis of the disease in some cases difficult during the patent period because eggs are expelled intermittently depending on the evacuation of the gall bladder and the biology of the fasciola (Troncy, 1989 and Briskey, 1998).

In the present study, 75% of the randomly selected 134 cattle were serologically positive for the presence of antibodies against specific antigens of fasciola. Out of the 41 cattle, which were positive in coproscopy, 98% of them were also serologicaly positive while 60/93 cattle, which were negative at coproscopy, were serologically positive. This indicates the high sensitivity of ELISA compared to the standard fecal sedimentation technique in diagnosing fasciolosis. The overall assessment indicates the relatedness of fecal examination result with ELISA findings though the test agreement is weak (kappa 0.236). Similarly, there was a strong relationship between faecal examination and postmortem findings of liver lesions, Kappa value, P=0.758).

Our finding in cattle is in agreement with Hillyer *et al*., (1996), who reported that out of 147 cattle tested, 38 were positive parasitologically while 84 were positive serologically. Of the 38 positives for fasciola eggs, 31 were positive by serology (sensitivity 82%). The higher seroprevalence of bovine fasciolosis obtained in our study might reflect the migration of immature flukes from the gut to the bile ducts which are not normally detected through faecal examination. The immunoenzymatic techniques such as indirect ELISA have been found to be very suitable for the diagnosis of fasciolosis due to their high sensitivity and the possibility of processing many sera samples (Arriaga de Morilla *et al*., 1989). Hillyer, 1999 and Martinez *et al.,* 1996 indicated that these techniques are an important adjunct for the diagnosis of fasciolosis because an earlier detection than coproscopic is allowed.

In the present study, all cattle which were positive at postmortem examination (54 out of the 134 cattle) were also serologically positive indicating the high sensitivity of the ELISA test, while 46/80 cattle, which were negative for lesions of fasciola, were having antibodies against

F2 antigens of *Fasciola hepatica*. Animals with no detectable liver lesions but positive for serology might have been at their early stage of invasion with immature flukes. Detection of antibody is a widely applied technique for the early diagnosis of different parasitic infections, such as fasciolosis. Techniques of indirect immunoenzymatic diagnosis represent an important contribution to the early detection of infection with *Fasciola hepatica*. By means of the indirect ELISA, based on excretory, secretory (ES) antigen, it has been possible to detect experimental infections in cattle from the third to the fifth week after infection during the liver migratory phase of immature worms (Marin, 1992). Cornelissen *et al.* (2001), after natural infection of animals with *Fasciola hepatica,* were able to detect positive animals by egg count only from week 9 in sheep and even later in cattle from week 15 onwards, and Arias *et al.* (2005) in their experimental work have detected positive animals through faecal examination 11 weeks after primary infection. Nevertheless, the presence of antibodies does not always correlate to active fasciolosis because antibody levels diminish slowly after cure (Hillyer, 1999, Sachez-Andrade *et al.,* 2002). (Perez *et al.*, 2005) reported that eggs in stools indicates the presence of mature adult flukes in the bile ducts and gall-bladder, but a period longer than 10 weeks after infection is required so most of the pathological damage has already occurred.

Contrary to the findings obtained in cattle, the overall finding regarding fasciolosis in small ruminants was low both through the standard faecal sedimentation techniques and ELISA. Out of the randomly selected 68 sheep only 1 was positive parasitologically and 3 were positive serologically and of 22 goats tested, 1 was positive parasitologically and serologically. These findings generally suggest the very low prevalence of fasciolosis in slaughtered small ruminants at ELFORA Abattoir, perhaps related to the origin of the animals, arid and semi arid regions of eastern Ethiopia. According to Hillyer *et al.*, 1996, out of 184 sheep examined, 22 were positive for fasciola eggs, while 163 were positive by serology. All of the 22 sheep, which were positive parasitologically, were also positive serologically for a sensitivity of 100%. An ELISA test is highly specific for trematodes and sensitive but remains positive for 5 months after treatment with or without the presence of liver flukes. The test can be particularly useful for the early diagnosis of fasciola infection (VEIN 2004).

Changes in serum enzymes are indicators of hepatic metabolism impairment (Galtier *et al.*, 1994 and Ferre *et al.*, 1996). Certain tissue cells contain characteristic enzymes, which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. GGT levels rise dramatically with obstructive diseases of the biliary tract and liver cancers. GGT is especially useful in assessing liver function associated with liver disease (Ramnik Sood, 1999; Charles, 2003; Merck & Co, 2006). In support of the above statement, our findings regarding the level of GGT suggest its association with liver lesions encountered at postmortem examination. The test of agreement between the liver examination and GGT determination was however moderate (kappa=0.332). Out of the 54 cattle with known liver lesions of fasciola 33 (61%)were having elevated levels of GGT than normal value for cattle. Where as the level of LDH has shown to be generally elevated both in negative as well as positive animals for liver lesions indicating the absence of agreement between liver lesions and level of LDH. 75% of the examined cattle had elevated levels of LDH in their serum.

LDH modification indicates mostly a cell necrosis during migration of young flukes through the liver parenchyma. LDH provide information on the passage of young flukes through the liver parenchyma (Charles, 2003) while GGT would be more associated with bile duct damages, GGT increase indicates penetration of the bile ducts by flukes, causing a hyperplastic cholangitis (Galtier *et al.*, 1986; Ferre *et al*, 1994, 1995a, 1995b, 1996, 1997).

Lactic Dehydrogenase (LDH -5) is useful in assessing acute, ongoing liver disease and neither is usually increased in chronic liver disease. LDH-5 are caused by hepatocellular disease, none of the common tests (bilirubin, GGT, GLDH, AP, LDH, AST, or alanine transaminase) for liver damage or function are clinically useful for detection of hepatic disease when used alone (Ramnik Sood, 1999 and Merck & Co, 2006).

Serum concentrations of liver-specific enzymes are generally higher in acute liver disease than in chronic liver disease. They may be within normal limits in the later stages of sub acute or chronic hepatic disease (Merck & Co, 2006).

6. CONCLUSIONS AND RECOMMENDATION

The present finding indicated that coprological examination for parasite eggs has significant limitations in detecting exactly the presence or absence of fasciolosis in animals. Although clinical disease can occur as early as 3 weeks post infection, faecal examination by coproscopy can only confirm the diagnosis after several weeks. Cattle, which were negative for liver lesions of fasciola, were having antibodies against fasciola specific antigens and cattle which were negative at coproscopy were also serologically positive. Thus, Enzyme-linked immunosorbent assay (ELISA) method that measure antibody levels against specific f2 antigen is a good diagnostic tool and may allow early detection of disease. Though the measurement of hepatic enzymes such as GGT and LDH merely indicates liver damage, the rise in theses enzymes and its association with liver lesions of fasciola in our study suggest their role in the diagnosis of fasciolosis and early treatment of animals.

Based on the above consideration the following recommendations are made:

- ✓ Coprological examination should be repeated and supported by other diagnostic methods for better diagnostic techniques due to intermittent expulsion of fasciola eggs and difficulty of detecting early infection.
- ✓ Researchers should be encouraged to develop easy and reliable diagnostic techniques like indirect ELISA that can be applied for routine diagnosis at field level to reduce the great losses due to fasciola infection in ruminants.
- ✓ Whenever possible, enzyme analysis needs to be done to know the degree of hepatic damage and recommend appropriate drugs and timely intervention.

7. REFERENCES

Abdul, J. R. (1992): Economic Significance of Bovine Fasciolosis and Hydatidiosis. In soddo. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University Debre Zeit, Ethiopia.

Abduljebar, M. (1994): Prevalence and Economic Significance of Bovine Fasciolosis in Sinana District, Bale-Region. DVM Thesis, Faculty of Veterinary Medicine Addis Ababa University Debre Zeit, Ethiopia.

Abebe, M. (1988): Prevalence of Bovine Fasciolosis and its Economic Significance at Nekempte. DVM thesis, Faculty of Veterinary Medicine Addis Ababa University Debre Zeit, Ethiopia.

Abera, B. (1990): Prevalence and Economic Significance of Fasciolosis in "Neur cattle" Slaughtered at Dembi–Dollo Slaughter House. DVM Thesis, Faculty of Veterinary Medicine Addis Ababa University Debre Zeit, Ethiopia.

Adem, A. (1994): Prevalence of Bovine and Ovine Fasciolosis: A Preliminary Survey Around Ziway Region (Shewa). DVM Thesis, Faculty of Veterinary Medicine Addis Ababa University Debre Zeit, Ethiopia.

Anderson, N., Luong, T T., Vo, N. G., Bui, K. L., Smooker, P.M. and Spithill, T. W. (1999): The Sensitivity and Specificity of two Methods for Detecting fasciola infection in cattle. Hanoi, Viet Nam, *Elev. Vet Parasitol,* **83**(1): 15-24.

Antonia, M., Conceiçao, P., Rute, M., Durao, Isabel, H., Costa, Jose., Correia de Costa. (2002): Evaluation of a Simple Sedimentation Method (modified MacMaster) for diagnosis of bovine fasciolosis. *Vet Parasitol* **(105):** 337-343.

Arias, M., Hillyer, G. V., Sanchez-Andrade, R., Suarez, J. L., Pedreira, J., Lomba, G., Diaz, P., Morrondo, P., Diez-Banos, P., Paz-Silva, A. (2005): A 2.9KDA *Fasciola hepatica* recombinant protein based ELISA test for the detection of current Ovine fasciolosis trickle infected. . Spain ***Elsevier***, *Vet Parasitol*, **(2005)**12-15 .

Arriaga de Morilla, C., Paniagua, R. Ruiz., Navarrete, A., Bautista, C. R., Morilla, A. (1989): Comparison of dot Enzyme Linked Immunosorbent Assay (dot-elisa), passive haemaglutination test (pht) and thin layer immunoassay (tia) in the diagnosis of natural or experimental *F. hepatica* infections in sheep. *Vet. parasitol.* **30**, 197–203.

Bahiru, G and Ephrem, M. (1979): Preliminary survey of bovine fasciolosis. *Ethiopian. Control rh. Agil. Science*. **1**(1): 50-127.

Bent, M. A., Cheikh, D., Thiam E and Jacquiet, P. (2003): ELISA for Serological Diagnosis of Cattel Fasciolosis due to *Fasciola gigantica* in Mauritania. *Revue Elev. Med. Pays Trop.* **56** (3-4): 135-140.

Beyazn, C. (1995): Preliminary Study on Epidemiology of Bovine and Ovine Fasciolosis in Eastern Gojjam Rigion, Ethiopia. DVM Thesis, Faculty of Veterinary Medicine Addis Ababa University Debre Zeit, Ethiopia.

Bradford, M. M. (1976): A Rapid & Sensitive Method for the Quantification of microgram quantities of protein using the principle of protein-dye binding. In: Fagbemi (1995): Purification of fasciola specific antigens and their use for the Immunodiagnosis of Fasciolosis in ruminants. *Annals of Biochem*. **72**:248-254.

Briskey, D. W. (1998): Diagnosis of Liver Fluke infections in Cattle. Merial, Vet Bulletin: 1-4.

Cervi, L., Rubinsteni, H and Masih, D. T. (1996): Involvement of excretion-secretion products from *Fasciola hepatica* inducing suppression of the cellular immune responses. Cordova, Argentina, *Elev, Vet Parasitol,* **61**(1-2): 97-98.

Charles, E. Ophard. (2003): Diagnostic Serum Enzymes Review Metabolism Energy Summary Elmhurst College. Virtual Chem.

Coles, Embert. H. (1986): Veterinary Clinical Pathology W. B. Sounders Company Kansas Fourth Edition Pp125-128.

Cornelissena, Jan. B., Cor, W. J., Gaasenbeeka, P., Fred, P. H., Borgsteedea, H. M., Wicher, G., Holland, b. C., Michiel, M., Harmsena, Wim., Boersm, J. A. (2001): Early Immunodiagnosis of fasciolosis in Ruminants using recombinant *Fasciola hepatica* cathepsin L-like proteaseq *International. J. for parasitol*. **31** (2001) 728-737.

CSA (2001): Central Statistical Authority, Federal democratic Republic of Ethiopia, Central Statistical investigatory, Statistical abstract, 2001.

Dagne, M. (1994): Survey on Prevalence and Economic Significance of Bovine Fasciolosis in Debre Berhan region. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Daniel, F. (1995): Economic Importance of organs condemnation due to Fasciolosis and Hydatidosis in cattle and sheep slaughtered at Dire Dawa Abattoir. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University Debre Zeit, Ethiopia.

Dinka, A., (1996): Preliminary study on Prevalence of Fasciolosis in small ruminants. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Fagbemi, B. O., Guobadia, E. E., Mbuh, J. V. (1995): Purification of fasciola specific antigens and their use for the Immunodiagnosis of fasciolasis in ruminants. In:Dioulasso: Parasitology research in Africa. University of Ibadan, Nigeria. Pp 209-217.

Fekadu, R. (1988): A preliminary Survey of Bovine fasciolasis around Bahir Dar and Evaluation of the fluckicidal activity of rafoxanide and closantel preparations. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Ferre, I., Barrio, J. M., Gonzalez-Gallego, J., Rojo-Vazquez, F. A.1994: Appetite Depression in sheep Experimentally infected with *Fasciola hepatica. Vet. Parasitol.* **55**, 71±79.

Ferre, I., Lopez, P., Gonzalo-Orden, M., Julian, M. D., Rojo, F. A., Gonzalez-Gallego, J. (1995a): The Effects of subclinical fasciolosis on hepatic secretory function in sheep, *Parasitol. Res.* **81**,127±131.

Ferre, I., Lopez, P., Rojo-Vazquez, F., Gonzalez-Gallego, J. (1996): Experimental ovine fasciolosis: Antipyrine clearance as Indicator of liver damage. *Vet. Parasitol.* **62**,93±100.

Ferre, I., Ortega-Mora, L. M., Rojo-Vazquez, F. A. (1995b): Seroprevalence of *Fasciola hepatica* infection in sheep in northwestern Spain, *Parasitol. Res.* **81**,137±142.

Ferre, I., Ortega-Mora, L., Rojo-Vazquez, F. A. (1997): Serum and Bile antibodies responses (IgG and IgA) during sub clinical *Fasciola hepatica* infection in sheep. Spain, *Elev, Vet Parasitol,* **68**(3): 261-267.

Galtier, P. L. G., Tufenkji, A. E., Franc, M., 1986: Incidence of Experimental fasciolosis, on the activitie of drug metabolizing enzymes in lamb liver. *Drug metab. Dispos.* **14**,137-141.

Galtier, P., Coulet, M., Sutra, J. F. Biro., Souvour, B., Aluinerie, M. (1994): *Fasciola hepatica*: Mebendazole and Thiabendazole Pharmacokinetics in sheep. *Parasitol.* **79**,166-176.

Getu, D. (1987): A study on the Incidence and Economic Significance of Fasciolasis in Woliata Awraja. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Girmay, W. (1988): Prevalence of Fasciolasis in Kalu Province. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Hailu, D. (1995): Bovine Fascioliosis at Awassa municipal slaughter house Prevalence and Economics loss. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Haroun, E. M., Elsan houri, A. A. and Gameel, A. A. (1989): Response of goats to repeated Infections with *Fasciola gigantica*. Faculty of Veterinary scince. Universty of Khartoum, Sudan, *Elev*, *Vet Parasitol,* **30**(4): 287-296.

Hawkins, C. D. (1984): The use of Haemoglobin, packed cell volume and Serum sorbitol dehydrogenase as Indicators of the Development of Fasciolosis in sheep. Universty of Melbourne, Australia. *Vet Parasitol,* **15**(2): 125-133.

Hendrix, C. M. (1998): Diagnostic Veterinary Parasitology. Second edition Auburn University Alabama Mosby Pp. 45-46.

Hillyer, G. V. (1999): Immuno diagnosis of Human and Animal fascilosis. In: Dalton, J. (Ed), Fasciolosis ,13. CAB international, Pp. 435-447.

Hillyer, G. V., Soler de Galanes, M., Buchon, P., Buorland, J. (1996): Herd evaluation by enzyme-linked immunosorbent assay for the determination of *Fasciola hepatica* infection in sheep and cattle from the Altiplano of Bolivia. *Vet. Parasitol.* **61**, 211–220.

Hunter, A. (1994): Fasciolosis. In: Animal health. Specific Diseases in the Tropics. First edition. CTA. Vol. 2 Macmillan, London, Basingstoke, Pp. 149-154.

Hymanot, A. (1990): Preliminary Survey of Bovine Fasciolosis in Eastern Hararghe Administrative region. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Ibarra, F., Montenegro, N., Vera, Y., Boulard, C., Quiroz, H., Flores, J. and Ochoa, P. (1998): Comparison of Three ELISA tests for Seroepidemiology of Bovine fascioliosis. Mexico, *Elev, Vet Parasitol*, **77**(4): 229-236.

Jorgen, H. and Brian, Perry. (1994): The epidemiology, Diagnosis and Control of helminth Parasites of ruminants. Food and Agricultural Organization of the United Nations, Italy, ILRAD. Pp 31-37.

Kara, M. and Kircal, F. (2004): Comparation of crude and excretory / secretory antigens for the Diagnosis of *Fasciola hepatica* in sheep by western blotting. Turk *J. Vet Animal sci,* Tubitak, 943-949.

Kaufmann, J. (1996): Parasitic Infections of Domestic Animals, a diagnostic manual, Berlin Germany, Ciba-Geigy Pp 6-8.

Levieux, D., Levieux, A., Mage, C. and Garel, J. P. (1992): Immunological Detection of Chemotherapeutic success in bovine fasciolosis using the specific antigen F2. France, *Elev, Vet Parasitol* **45**(1-2): 81-88.

Malone, J. B., Gommes, R., Hansen, J., Yilma, J. M., Slingenberg, J., Snijders, F., Nachet, O. F. and Ataman, E. (1998): A Geographic Information System on the potential Distribution and abundance of *Fasciola hepatica* and *F. gigantica* in East Africa based on food and agriculture organization databases, *Elev, Vet Parasitol*, **78**: 87-101.

Manyazewal, Z. A. (1995): A study of Bovine Fasciollosis in Western Ethiopia. Bedelle, Regional Veterinary Laboratory, I*n*: Dioulasso: Parasitology Research in Africa. Burkina Faso, Pp208-207.

Marin, M. S. (1992): Epizootiologia de la Fasciolosis bovina en Asturias. Identificación y expresión de un antigeno unitario. Phd thesis. Facultad de Biologia, Universidad de Oviedo.

Martinez, A., Martinez-Cruz, M. S., Martinez, F. G., Gutierrez, P. N. and Hernandez, S. (1996): Detection of Antibodies to *Fasciola hepatica* Excretory- Secretory Antigens in Experimentally Infected goats by Enzyme Immunosorbent Assay. Cardova, Spain, *Elev, Vet Parasitol,* **62**(3-4): 247-252.

Martinez-Moreno, A., Jimenez, A., Lugue, V., Moreno, T., Redondo, E. S. H., Martin M. J. and Perez J. (1999): Liver pathology and immune response in experimental *Fasciola hepatic* infections of goats. *Elev, Vet Parasitol,* **82**(1): 19-33.

Merck, & Co. Inc (2006): Merck Veterinary manual, White house station, M. J. USA, merial.

Merck, & Co. Inc. (1998): Merck Veterinary manual, Inc, Philadelphia eight edition. USA, Pp. 2192-2193.

Mesfin, A. Y. (1999): Study on the Prevalence of Bovine Fasciolosis and Morphometric Analysis of Liver fluke Population in North Gondar area, Ethiopia. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University Debre Zeit, Ethiopia.

Mezgebu, B. (1995): A survey on Ovine Fasciolosis and Lung worm Infestation in Addis Ababa and the Surrounding highland areas. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Moges, E. (2003): A study on bovine fasciolosis and hydatidosis at Jimma abattoir, Ethiopia. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Mulugeta, T. (1993): Prevalence and Economic Significance of Bovine fasciolosis at the Sopral Kombolcha meat factory. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

O'Neill, S. M., Brady, M. T., Callaghan, J. J., Mulcahy, G., Joyce, P. Mills, K. H. G. & Dalton, J. P. (2000): *Fasciola hepatica* infection down regulates th1 responses in mice. *Parasite immunology*, 147–155.

Ogunrinade, A. 1 and Ogunrinade, B. 2(1980): Economic Importance of Bovine Fasciolosis in Naigeria, Bull Animal Heal production. Africa.

Payne, W. J. A. (1990): An Introduction to Animal Husbandry in the tropics Fourth edition Black Well Scince. Oxford. London, Pp 47-74.

Perez, J., Ortega, J., Bravo, A., Diez-Banos, P., Morrondo, P., Moreno, T., Martinez-Moreno, A. (2005): Phenotype of Hepatic Infiltrates and Hepatic limph nodes of Lamb primarily and challenge infected with *Fasciola hepatica* , with and without triclabendazole treatment. *Vet. Res.* **36**, 1-12.

Rahmeto, A. (1992): Fasciolosis Clinical occurrences, Coprological, Abattoir and Snail survey in and around Wolisso. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Ramnic Sood, (1999): Medical Laboratory Thechnology Methods and Interpretations fifeth edition New Delhi Jaypee Brothers Pp 480-529.

Reinecke, R. K. (1983): Veterinary Helminthology. Butterworths, Durban. Professional publishers, PTY. Ltd. Pp. 250-258.

Rickard, L. G. (1995): Development and Application of a dot- ELISA test for the Detection of serum antibodies to *Fasciola hepatica* antigens in llamas. Oregon State University, USA, *Elev, Vet Parasitol,* **58**(1-2): 9-15.

Rokni, M. B., Massoud, J. and Kia, E. B. (2003): Comparison of adult somatic and cysteine Proteinase antigens of *Fasciola gigantica* in Enzyme Linked Immunosorbent Assay for Diagnosis of bovine Fasciolosis, OIE seminar on biotechnology, Tehran, Iran. Proc, Nov 9-13.

Roman, T. (1987): Study on Economic Significance of Bovine Fasciolosis and Hydatidosis at Gonder Abattoirs. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Sanchez-Andrade R, Morrondo P, Lopez C, Pandero R, Diez P (1995): Evaluation of *Fasciola hepatica* infection prevalence in cattle in Galicia (north west Spain) by enzyme linced immunosorbent assay *res Rev parasitol*, **55**:103-107.

Sanchez-Andrade, R., Paz-Silva, A., Suarez, J. L., Pandero, R., Pedreira, J., Lopez, C., Diez-Banos, P., Morrondo, P. (2002): Influence of Age and Breed on Natural Bovine Fasciolosis in an Endemic area (Galicia, NW Spain). *Vet. Res. Commun.* **26**, 361-370.

Seyoum, S. (1987): Incidence of Bovine Fasciolosis in Buno Province. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Sloss, M. W., Kempt, R. L., Anne, M. Z. (1978): Veterinary Clinical Parasitology. fifth edition, ISBN, Pp. 20-21.

Soulsby, E. J. L. (1982): Helminthes, Arthropods and Protozoa of Domesticated Animals, Seventh Edition. Balliere Tindall, London, UK, Pp.40-52.

Takele, A. (1995): Bovine Fasciolosis: Prevalence and Economic Impact at Mekele municipal abattoir. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Thrusfield, M. (1995): Veterinary Epidemiology second edition, University of Edinburgh, Black well science (180-188).

Troncy, P. M. (1989): Helminths of livestock and Poultry in Tropical Africa. In: Fischer. (1989). Manual of tropical veterinary Parasitology. CAB International, UK. Pp.63-73.

Tsegaye, T. (1995): Epidemiology of Bovine Fasciolosis and Hydatidosis in Debre Berhan Region, Ethiopia. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Ueno, H., Gutierres, V. C., Mattos, M. J. T., and Muller, G. (1982): Fasciolosis problems in ruminants in Riogrande dosul, Brazil, *Elev*, *Vet Parasitol,* **11**(2-3): 185-191.

Urquhart, G. M., Duncan, J. L., Armour, J., Dunn, A. M., Jenning. (1996): Veterinary Parasitology. Second Edition. Blacwell Scince, UK. Pp. 103-113.

Van-Tiggele, L. J. and Over H. J. (1976): Serological Diagnosis of Fasciolosis. Central Veterinary Institute, The Netherlands, *Elev. Veterinary Parasitol,* **1**(3): 239-248.

VEIN 2004: Sheep Health and Production. Postgraduate foundation in veterinary science of University of Sydney. Pp 508.

Wassie, M. (1995): Prevalence of Bovine and Ovine Fasciolosis: A preliminary Survey in Nekemte and its Surrounding Areas. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

William, F. J. (1997): Veterinary Parasitology Reference Manual. fourth edition Washington State University. Pp. 5-7.

Wondwosen, A. (1990): Prevalence of Bovine Fasciolosis in Arsi Administrative Region. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Yadeta, B. (1994): Epidemiology of Bovine and Ovine Fasciolosis and Distribution of its Snail intermediate Host in Western Shoa. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Yilma, J. M. and Malone, J. B. (1998): A Geographic Information System forecast model for Strategic control of fasciolasis in Ethiopia, Faculty of Veterinary Medicine, Addis Ababa University. Ethiopia *Elev. Vet Parasitol*, **78**: 103-123.

Yohannes, T. (1994): Bovine Fasciolosis: Prevalence and Economic Importance Assessment trial on cattle slaughtered at Bahir Dar municipal abattoir. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Yosef, S. (1993): Prevalence of Ovine Gastrointestinal Helminthes in and around Asella. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

Zerfu, M. (1991): Prevalence of Gastrointestinal helminthes of Cattle in Chilalo Awraja, A Administrative Region. DVM Thesis, Faculty of Veterinary Medicine, Addis Aba University. Debre Zeit, Ethiopia.

Zewdu, B. (1991): Prevalence and Economic Analysis of Liver Fluke Infestation in Cat Slaughtered at Jimma Municipal Abattoir. DVM Thesis, Faculty of Veterina Medicine, Addis Ababa University. Debre Zeit, Ethiopia.

8. ANNEXES

Annex 1. The simple sedimentation method (McMaster),(Antonia *et al.*, (2002).

1. Ten grams of feces were mixed with 60ml of a 5% water detergent solution in a large test tube and stirred well with a stirring rod.
2. The sample was strained through a large mesh sieve to a conical 1000 ml flask, washed copiously and fills up with tap water.
3. The sample was allowed to stand for 10 min and then the supernatant discarded and re-suspended the sediment in tap water. The sedimentation process was done four times.
4. After the last sedimentation and decantation the sediment was recovered in to a test tube and fill up to 50 ml volume with tap water.
5. The tube was agitated to re-suspend the sediment. With a Pasteur pipatte filled bothe chambers of a McMaster slide.
6. A McMaster slide (Urquhart *et al.*, 1996) with two chambers of 0.15 ml each was used and all the *F.hepatica* eggs present on the bottom of the chambers were counted.

Annex 2 Parasites recorded other than fasciolosis in the study areas

Trematodes	Cestodes	Nematodes
Schistosoma	*Monezia*	*Strongyloides*
Paramphistomum	*Stelasia*	*Toxocara*
	Hideatic ciste	*Trychostrongyle*
	Cysticercus	*Trichuris*

Annex 3 Procedure for enzyme (LDH) analysis

1. Working reagent, samples and controls were preincubated to reaction temperature 30/37^{0}C
2. The photometer was adjested to 0 absorbance with distilled water.
3. Pipetted in to a cuvette:
   . Working reagent------1.0 ml
   . Sample or control-----20ul
4. Gently mixed by inversion, inserted cuvette in to the cell holder and start stopwatch.
5. Incubated for 30 seconds and recorded initial absorbance reading.
6. The absorbance reading repeated exactly after 1, 2 and 3 minutes.
7. The difference was calculated between absorbances.
8. The mean was calculated of the results to obtain the average change in absorbance per minute.

Annex 4. Procedure for enzyme (GGT) analysis:

1. Working reagent, samples and controls were preincubated to reaction temperature.
2. The photometer was adjested to 0 absorbance with distilled water.
3. Pipetted in to a cuvette:
   Working reagent------1.0 ml
   Sample 100ul
4. Gently mixed by inversion. Inserted cuvette in to the cell holder and start stopwatch.
5. Incubated for one minute and recorded in initial absorbance reading.
6. The absorbance reading was repeated exactly after 1, 2 and 3 minutes.
7. The difference between absorbances was calculated.
8. The mean of the results was caculated to obtain the average change in absorbance per minute.

Annex 5. Materials required for serum enzyme analysis.

-Photometer with a thermostatted cell compartment set at 25/30/37^{0}C. Capable to read at 405 nm.

-Stop match. Strip-chart recorder or printer.

-Cuvettes with 1-cm pathlength.

-Micropipettes to measure reagent and samples.

Annex 6. Reagent composition for LDH:

R1.LDH substrate. tris buffer 100 mmol/l ph 7.5. pyruvate 2.75 mmol/l. sodium chloride

R2. ldh coenzyme. nadh 1.55 mmol/l.

Annex 7 Reagent composition for GGT :

R1 buffer/ glycylglycine. tris 133 mmol/l. glicylglycine 138mmol/l.

R2 substrate/glupa-c l-y-glutamil)- 3-carboxyl -4-nitroanilide 23mmol/l.

Annex 8. Normal value of animals serum enzymes (Merck & Co, 1998)

Enzyme	Cow	Sheep	Goat
GGT	4.9 –25.7	19.6 – 44.1	20 - 50
LDH	308.6 - 938	83 – 475.6	78.5 – 265.3

Annex 9 Enzyme Linked Immunosorbent Assay (ELISA) test protocol.

1. The wells of the polystyrene micro plate were precoated with an immunocaptured "f2" antigen (only the wells of even-numbered columns were coated with f2 antigen).

2. Serum samples to be tested were diluted and incubated in the wells for an hour at 37^0C. Antibodies specific to "f2" antigen present in the serum form an "f2" antigen antibody immune-complex and remain bound in the wells.

3. After a washing step (three washes), an anti-bovine immunoglobulins monoclonal coupled to peroxydase was added to incubate for half an hour. This conjugate binds to the immune-complex.

4. After a washing steps (three times), the substrate (TMB) was added to the conjugate, the plats were incubated for 20 minutes at 21^0C away from the light forming a blue compound becoming yellow after blocking. The intensity of the color was a function of the rate of antibodies present in the serum sample. The different classes of seropositivity were determined according to the results obtained from the control sera provided in the kit. These controls must be added to each microplate.

Annex 10. Materials required for diagnosis of fasciolosis by ELISA method.

1) Microplates reader
2) Centrifuge
3) Centrifuge tubes
4) Vortex
5) Microplate washing system that distributes 300 µl per well
6) Precision Micropipettes and Multi-dispensing micropipettes (The precision required must be lower or equivalent to 5% for all the volumes indicated)
7) Disposable pipette tips
8) Distilled water: the water used for the reconstitution of the controls, the wash solution can come from a conventional distillation system or any other high-performance water purification system (reverse osmosis, resin or activated charcoal purification.).
9) Microplate covers aluminium foil or adhesive.
10) Incubator at +37°C (±3°C)
11) Photometer

Kit contents

Coated micro plates 5
Concentrated wash solution (x 20) 1x 100 ml bottle
Dilution buffer **2** light green (for samples) 1 x 120 ml bottle
Dilution buffer **1** light blue (for conjugate) 1 x 120 ml bottle
Positive control (liquid) negative control (*liquid*) 1 x 0.5 ml bottle
Anti-ruminant Igg / peroxidase conjugate 1 x 0,75 ml bottle
Revelation solution **3** (tmb) *ready to use* 1 x 60 ml bottle
Stop solution (h2 so4 0,5m solution) *ready to use* 1 x 60 ml bottle

Annex 11. Instructions for diagnosis of fasciolosis by ELISA method.

1) DEPOSIT OF THE SERA

a) Treatment of controls:
Controls are diluted to 1/20 by using the following method (see note 2 and 3)
- Dispense: - 190 µl of "Dilution buffer **2** » per well
- 10 µl of undiluted negative control in A1 and A2.
- 10 µl of undiluted positive control in B 1 and B 2.

b) Treatment of serum samples:
Sera or pools of sera are diluted to 1/20 by using the following method (see note 2 and 3)
Dispense: - 190 µl of "Dilution buffer **2** » per well
- 10 µl of each serum (individual serum or serum pool) in one coated well (even-numbered columns)
- 10 µl of each serum (individual serum or serum pool) in one uncoated well (odd-numbered columns)

Analyses
Homogenize the contents of the wells by gently shaking the plate (see note 1).

Cover the plate (with a lid, aluminium or adhesive foil) and leave to incubate for **1 hour** (± 5 min.) **at 37°C** (± 3°C).

2) WASHING

a) Dilute a bottle of "Concentrated Wash Solution (20x)" in 1900 ml of distilled water. This solution is
Hereafter called the "Wash solution". The dilution can be carried out before the elimination of crystals, which appeared at +5°C (±3°C), in condition that the whole 100 ml vial is used.
b) Empty the content of the plate by "flick-off" or better by an automatic method.
c) Fill all the wells of the plate with the wash solution; then empty them again.
d) Repeat the step **c)** twice (a total of 3 washes).

3) DEPOSIT OF THE CONJUGATE

a) Dilute the conjugate to 1/100 with the "Dilution buffer 1" and dispense 100 µl per well.
b) Cover the plate (with a lid, aluminium foil or adhesive) and incubate for 30 min. (± 3 min.) **at 37°C** (± 3°C).

4) WASHING

a) Empty the content of the plate by "flick-off" or better by an automatic method.
b) Fill all the wells on the plate with the wash solution; then empty them again.
c) Repeat the step b) twice (total of 3 washes)

5) REVELATION

a) Dispense 100 µl of "Revelation Solution 3" ready to use per well
b) Incubate the plate at 21° (± 5°C) for 20 minutes (away from the light).
c) Dispense 100 µl of "Stop Solution" per well.

d) Shake gently the plate until the coloured solution is homogenized. Wipe carefully the underside of the plate*:*

6) READING

a) Read the optical densities at 450 nm (OD.450). The photometer must first be blanked on air.

b) Calculate the corrected OD.450 for each serum sample: Subtract the OD 450 value obtained from an uncoated well from the OD.450 from a coated well.

INTERPRETATION

Calculate for each sample, the ratio S/P:

S/P = corrected OD450 value of the sample / corrected OD450 value of the positive control x 100

S/P % of the sample Infestation within the herd	results and the level of correlation between test	
%E/P > 150%	+++	Strong Infestation
	(>50% of infestation	
S/P included between 80% and 150%	++	Medium Infestation
	(between 20% and 50% of infestation)	
30<%E/P<80%	+	Low Infestation
	(<20% of infestation)	
S/P<30%	0	No infestation

9. CURRICULUM VITAE

PERSONAL INFORMATION:

Name	Meskerem Adamu Chere
Sex	Female
Date of birth	1967
Place of birth	Harar
Nationality	Ethiopian
Marital status	Married
Number of children	Two
Religion	Christian (Orthodox)

EDUCATIONAL BACKGROUND

ELEMENTARY SCHOOL

Place	Harar
Name of school	Aboker Elementary school
Attended	1973-1980

SECONDARY SCHOOL

Place	Harar
Name of school	Harar Senior Secondary School
Attended	1981-1982
Place	Cuba
Name of school	ISLA, 21
Attended	1982-1984

HIGHER EDUCATION LEVEL

FIRST DEGREE

Place	Cuba, Havana
Name of school	ISCAH University
Attended	1985 - 1990
Field of study	Veterinary Science

WORK EXPERIENCE

1- 1991– 1993: A field Veterinarian SNNRS Agri. Devt. Breau, Yeky (teppi)

2- 1994 – 1999 Animal health department head

3- 2000--2004. Head of animal & fish development and protection

RESEARCH PAPERS

1. The Assessment of Sanitary Quality of Leg Ham DVM thesis (1990), Havana, Cuba.
2. Study on comparative diagnostic techniques of fasciolosis in slaughtered ruminants at Elfora Export Abattoir Debre Zeit, Ethiopia

ADDITIONAL TRAINING

1. Training on public health (Refreshment kours for one month)
2. Seminar on various titles, such as tsetse fly control and tripanosomiasis, animal health information system, National animal disease surveillance system etc.
3. Since I am a member of Ethiopian Veterinary Association I am attending annual workshop on animal health aspects.

ADDITIONAL INFORMATION

LANGUAGES

	Listening	Speaking	Writing	Reading
Amharic	Excelent	Excelent	Excelent	Excelent
English	Excelent	Very good	Excelent	Excelent
Spanish	Excelent	Excelent	Excelent	Excelent
Oromiffa	Good	Good	Good	Good

CONTACT ADDRESS

Teppi, Sheka zone yeki woreda P.O.Box 107, Tel 0475560007

Addis Ababa Tel 0911731735

10. SIGHNED DECLARATION SHEET

I, the under signed, declare that this thesis is my original work and has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledged.

Name Meskerem Adamu Chere

Signature ____________________

Date of submission June 23, 2006

This thesis has been submitted for examination with my approval as an academic advisor.

Dr Getachew Tilahun(Associate Professor) ____________________

2005/MES
AUTHOR Meskerem adamu
TITLE Diagnostic approaches to Fasciolosis
DATE DUE | BORROWER'S NAME

1116
MES
2006

Study On Comparative Diagnostic Techniques Of Fascilosis In Slaughtered Ruminants At Elfora Export Abattor Debre Zeit Ethiopia.

Meskerem Adamu

C-1