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A CLINIC-PATHOLOGICAL STUDY, LESION CHARACTERIZATION AND FINANCIAL LOSS DUE TO FASCIOLOSIS AND *HEPATIC NECROBACILLOSIS* IN CATTLE SLAUGTHERED AT THREE MUNICIPAL ABBATOIRS OF CENTRAL ETHIOPIA

MSc THESIS

By

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A CLINIC-PATHOLOGICAL STUDY, LESION CHARACTERIZATION AND FINANCIAL LOSS DUE TO FASCIOLOSIS AND *HEPATIC NECROBACILLOSIS* IN CATTLE SLAUGTHERED AT THREE MUNICIPAL ABBATOIRS OF CENTRAL ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Pathology

BY

Wondimu Hika Uma

June, 2020

Bishoftu, Ethiopia

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As member of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by: **Wondimu Hika Uma** titled as"***A clinic-pathological study, lesion characterization and financial loss due to fasciolosis and hepatic necrobacillosis in cattle slaughtered at three municipal abattoirs of central Ethiopia*****"** and recommend that it be accepted as fulfilling the thesis requirement for the degree of master of science in Veterinary Pathology.

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Final approval and acceptance of the dissertation is contingent upon the submission of its corrected Copy to the CGC through the concerned departmental graduate committee. I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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3. Dr Bersisa Kumsa Department chairperson	----------	----------

DEDICATION

My dedication is to almighty God for His nurturing, guiding and growing me to this day. He loved me, help me and success me to this time. I declare also to my Mother Belayinesh Tolosa, again my lovely wife Tewabech Hika and my Childrens Ararsa Wondimu and Bilisuma Wondimu who were patient when I'm out of my home.

STATEMENT OF THE AUTHOR

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	Signature	Submission date
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LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
GOT	Glutamic Oxalacetic Transaminases
GPT	Glutamic Pyruvic Transaminase
RBC	Red Blood Cell
WBC	White Blood Cell
PCV	Packed cell volume
TEC	Total Erythrocyte Cell Count
DLC	Differential Leukocyte Count
MCV	Mean corpuscular volume
TLC	Total leukocyte count
Hgb	Hemoglobin
NAHDIC	National Animal Health, Diagnostic and Investigation Center
LPS	Lipopolysacharide
NTD	Neglected Tropical Disease

ABSTRACT

The study was conducted from October, 2019 to May, 2020 by using cross-sectional study design to assess a clinic-pathological, lesion characterization, and financial loss due to fasciolosis and hepatic necrobacillosis in cattle slaughtered at three municipal abattoirs of central Ethiopia. Sixty cattle were selected using systematic random sampling for this study, from which blood samples and liver tissue of the same animals were collected before and after slaughter, respectively. Tissue sampling for bacterial culture and histopathology were collected from the infected liver. Post-mortem inspection results, 41.6% (25/60) gross pathologic lesions, of these, 20% (12/60) were mixed infection of liverfluke and *Fusobacterium necrophorum* while 8% (5/60) and 13.3% (8/60) of the infections were due to *Fusobacterium necrophorum* and liverflukes alone, respectively. The histopathologic examination of the affected livers indicated heavy infiltration of inflammatory cells, biliary cirrhosis and extensive fibrous of connective tissue proliferation in the hepatic capsule. The hematological assay results indicated that PCV, Hb, and RBC's were lower in the infected cattle while, the WBC's (Eosinophilia, Neutrophilia, Monocyte, and Lymphocyte) were higher. The biochemical analysis of blood samples from the infected cattle showed that the liver enzymes AST, ALT, and ALP were significantly higher in animals with hepatocyte degeneration. The estimated financial losses due to liver condemnation by fascioliasis and hepatic necrobascillosis were 1,747,200 ETB/ ($56361.3) annually. Therefore, the study concluded that the liverflukes and infections due to *Fusobacterium necrophorum* are the major problems of cattle slaughtered at the selected abattoirs of the study areas. Thus, corrective measures and further investigations are recommended to lessen economic loss from fasciollosis caused liver condemnation.

Keywords: Abattoir; Cattle; *Fusobacterium necrophorum*; Liverfluke; Ethiopia.

1. INTRODUCTION

Ethiopia is known by its huge livestock population leading the different African countries. Although it is the leading country by the largest population of cattle, the needed production and productivity expected from them is very low. This is done due to currently known public health and veterinary importance diseases. Liverfluke is one of these neglected zoonotic diseases in livestock. An infection of liver has great economic impact from the numbers of livers condemned in the slaughterhouses (Tomate, 1973; Sayed, *et al.,* 2008; Eman, *et al.,* 2016; Beesley, *et al.,* 2018). Bovine fasciollosis (*Fasciolla gigantic,* prevalent in Africa) is among the common liverfluke causes liver condemnation by causing expensive tissue damage as they migrate through the liver **(**Zachary, 2017).

Economically important parasitic disease, bovine fasciolosis is very common in the tropical countries. Infestation with Fasilolosis is usually associated with grazing wet land and drinking from the snail infesting watering places (Payne, 1990; Eman, *et al.,* 2016). There are two phases' developmental stages of diseases in the definitive host. A migratory phase is starts with penetration of intestinal wall by incited youngflukes. Following the penetration of the intestine, flukes travel within the abdominal cavity, go through the liver or other organs, and cause lesion. The secondary phase starts by entering of parasites into ducts of the bile, liver and flukes mature, feed on blood, and produce eggs (Behm and Sangsten, 1999; Eman, *et al.,* 2016).

Lesions of liver are the most common and economically important and predispose to *Fusobacterium-necrophorum* infection in cattle. Whenever there is marshy and wetland areas at grazing points of animals, liver lesions due to fasciolla infection is a must. The effect of liver lesion in cattle resulted in reduce feed intake, weight gain, feed efficiency, and dressing percentage so it was suggested that a relationship exists between the severity of liver abscesses and animal performance. The most liverfluke induced abscessation includes well-encapsulated, possessing thick fibrotic walls and chirrotic outlines. During digestion of grain, the starch granules promoted the pace of ruminal fermentation of the starch so it increased the probability of acidosis and liver abscesses (Nagaraja *et al.,* 2005).

The ruminal wall infection from the grain indigestion by passes the fasciolla eggs and secondary bacteria followed by liver abscesses can develop. Because of the close correlation between the incidence of ruminal pathology and liver abscesses in cattle, the term 'rumenitis-liver abscess complex' is commonly used. Even though the clear-cut mechanism is not predictable, it is established that rapid fermentation of grain by ruminal microbes and the consequential gathering of organic acids (volatile fatty acids and lactate) result in ruminal acidosis (acute or subacute). Acid-induced rumenitis and damage of the protective surface often aggravated by foreign objects (sharp feed particles, hair, etc.) predispose the ruminal wall to invasion and colonization by *Fuobacteriumnecrophorum* (Oelke *et al.,* 2005).

Entry of the organisms into blood or causes ruminal wall abscesses, will later shed bacterial emboli into the portal circulation. Thus, it will result in the infection and abcess formation of liver and filters bacteria from the portal circulation. The liver is a vascular, therefore richly oxygenated, and a highly defended organ because of its numerous phagocytic cells (leukocytes and Kupffer cells). Therefore, *Fusobacteriumnecrophorum* has to defeat both high oxygen concentrations and phagocytic mechanisms in order to stay alive, propagate and begin abscess formation. Then they can protect themselves from phagocytosis by producing lipopolysacharide called the leukotoxin and endotoxin. Additionaly, they release cytolytic products of lysosomal enzymes and oxygen metabolites, which have detrimental effect on the liver paranchyma (Oelke *et al.,* 2005).

The diagnosis of Fasciolosis would not be direct. The tentative and symptomatic diagnosis may be established based on prior knowledge of epidemiology of the disease in a given environment, surveillance of clinical sign, grazing history, seasonal occurrence, and identification of snail habitats and dropping of adult flukes examined from feaces of animals respectively. The postmortem examination and examination of feaces at laboratory confirms fasciolla species prescence in animals. The direct, reliable and cost-effective diagnosis of fasciollosis is through post-mortem examination of liver from slaughtered animals (Urquhart *et al.,* 1996).

Several studies associated to lesions characterization based on gross pathological studies and economic loss due to fasciollosis has been reported in several places of the country (Yusuf and Alkabi, 2016). The only pathological and biochemical changes in liver infected with fluke on ruminants at ELFORA export

abattoir in Bishoftu, Ethiopia, 10km away East of our study area was done by Belina *et al*. (2012). However, Fasciolosis, its predisposing factors, evaluation of haematological and serum biochemical changes and complication take place subsequent to infestations by fasciolosis; *Hepatic necrobacilisis* in cattle slaughtered at Gelan, Dukem and Akaki Manucipal abattoirs was not conducted so far.

The study was therefore conducted with planned main and specific objectives below:

The main objective:

- To Characterize pathological changes caused by Liverflukes and *Hepatic Necrobacilosis* from cattle slaughtered at selected municipal abattoirs.

The specific objectives of the study were:

- To identify the major species of liverflukes from slaughtered cattles in the study area.
- To characterize gross and microscopic lesions and asses changes in hematological and serum biochemical parameters of infected cattles.
- To determine the association of isolated liverflukes and hematological and serum biochemical parameters in slaughtered cattles from selected abattoirs.
- To isolate *Fusobacteriumnecrophorum* complicated by fasciolosis causing liver abscess and related risk factors that complicated the diseases.
- To calculate the financial losses on the condemned liver due to the effect of the Fasciollosis and *Fusobacterium Necrophorum* in the study area.

2. LITERATURE REVIEW

2.1. Etiology and morphology of fasciolosis

Among the species of fasciolla, *Fascila hepatica* and *Fasciola gigantic*a are most common in the country and causing Fasciolosis in domestic animals mainly ruminants due to their indiscriminate feeding habit. The development of disease has four different stages; an early incubation phase of between a few days up to three months with little or no symptoms; a persistent phase that may be known by general clinical signs and later on changed to a latent phase with less known clinical sign and then month's to year later, ultimately changed to a unending or chronic obstructive phase. The inflammation of bileduct, and gall bladder developed at chronic state may causes gallstones and fibrosis (Rahman *et al.,* 2017).

The morphology of fasciola is helping us to classify them at the species level. There are different structures found between species of fasciolla being, *Fasciola hepatica* is a leaf shaped, with broad and cone shaped anterior projection. The tegument is armed with sharp spines. The young fluke at the time of entry in to the liver is 1-2 millimeter (mm) in length and lancet like when it has become fully mature in the bile ducts. The eggs have an indistinct operculum and develop only after the eggs have been laid (Michael, 2004). The eggs of fasciola have yellowish brown shell with a small knob at their posterior ends. *Fasciola gigantica* is larger than *Fasciola hepatica* and can reach up to 7.5cm length. It has leaf like, the anterior end with very short conical shape. *Fasciolla gigantica* eggs are larger than those of *Fasciola hepatica*, measuring 190x100 micrometer (µm) as from measured report of Taylor *et al.* (2007).

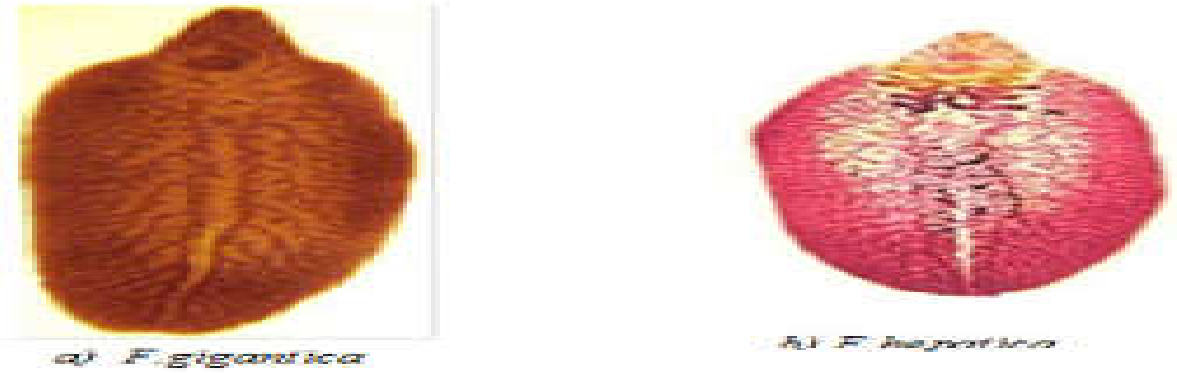

Figure 1: Adult stages of Fasciola spp. Source: (Michael, 2004).

Fusobacteriumnecrophorum is the most common causes of liver abscesses. It is a Gram-negative, pleomorphic, filamentous, obligate anaerobe that is a normal flora of the alimentary tract, respiratory tract, and reproductive tract. According to the report of Tadepalli *et al*., (2009), the bacteria start growing by attaching the wall of rumen and develop lesions at the luminal epithelium from which it migrate through portal blood and admitted to the liver. Degradation and utilization of protein in the ruminant digestion is undertaken by the presence of bacterial normal floura *Fusobacteriumnecrophorum,* which is found in higher concentration in grain-fed than forage-fed cattle (Smith, 2015).

2.2. Epidemiology

Fasciolosis is found in marshy areas and causes limiting factor for bovine and ovine production. *Fasciolla hepatica*, which is a temperate species, is the mostly important trematode of domestic ruminant and common causes of liver fluke disease in temperate areas of the world. Thus, it is found in Southern and Northern America, Europe, Australia and Africa. While, *Fasciolla gigantica* is very common and economically important widely distributed in and around different tropical countries. The development of *Fasciola hepatica* and *Fasciola gigantica* eggs, larval stages and its intermediate host snails in the environment are highly dependent on geo-climatic, ecological and anthropogenic factors such as elevation, rainfall, temperature, evapo-transpiration, moisture, vegetation and soil type (Brown *et al*., 2017).

The variations, prevalence, epidemiology and ecology of Fasciola species involved were reported by different authors in Ethiopia. Terefe *et al.* (2012) reported the attribution to the variation in different eco-climatic conditions like, altitude, rainfall, temperature and livestock management system. His work also identified the outstanding change that occurs during *Fasciola hepatica* infection in all host species. Availability of suitable snail habitat is the main predisposing factor for distribution of fasciollosis. Habitats very crucial for the intermadiate host of fasciolla is mainly environment containing the wet mud to free water and they reside permanently in the stream, ditches and at the edge of small ponds. Fields with clumps of rushes are often suspect the site. Through a slightly acid pH environment is optimal for Lymnaeidae truncatula, excessively acid pH levels are detrimental (Wakuma, 2009).

Abessetion of liver have no age and sex category in catle, but the abscesses of significant economic impact occur in feedlot cattle. *Fusobacteriumnecrophorum* is found as normal floura of ruminant animals and have different concentration in differet feeds of animals. The concentration in the rumen ranges from 10^5 to 10^6/g of ruminal contents, and is influenced by the diet and certain antimicrobial feed additives. Zhang *et al*. (2006) reported the number of *Fusobacteriumnecrophorum* cells in the rumen is at least 10-fold higher in grain-fed cattle compared to forage-based diet (>10/g vs. $<10^5$/g of ruminal contents. This bacteria uses lactate as a major substrate instead of sugars, its population is increased in cattle fed high-grain diet probably due to turnover of lactates in the rumen. The ruminal concentration is not affected by the inclusion of the ionophore, monensin, but is significantly reduced by tylosin and virginiamycin (Zhang *et al.,* 2006).

2.3. Clinical signs

The clinical sign exhibited by animals infected by fasciola will not be indicated only by external observations. The clinical features of Fasciolosis may vary depending on forms of the disease (acute, sub-acute and chronic). Acute Fasciolosis occur rarely in cattle and is less common than the chronic form and causing hepatitis by simultaneous migration of immature flukes. It is responsible for wide spread morbidity and mortality in cattle characterized by weight loss, anemia and hypoproteinemia. Edema of lower jaw, ematiation and pale mucous membrane is typical sign. Depending on the severity of the disease, death in untreated animals follows in about two to three months although many survive longer than this and may eventually recover if not re-infected. Diarrhea may develop in chronic form duration of six month in cattle (Cullen and Stalker, 2016). The pressure caused by load of parasites and lesions found in liver results in pressing the vital organ of thoracic cavity and grunting sound may appear upon animals moving downward.

2.4. Transmission and pathogenesis

The infestations and pathogenesis of fasciola is clearly indicated by following its developmental stages. According to Zachary (2017), when *Fasciola hepatica* metacercariae are ingested, they migrate to the liver and then take up residence within the bile ducts. The residents of mature flukes in the outside of larger hepatic and bile ducts causes cholengitis and ectasia respectively. Adult *Fasciola hepatica* is leaf-

shaped flukes that inhabit the biliary system; their eggs pass via the bile into the intestinal tract and eventually are passed in the feces (Zachary, 2017). The knowledge of relationship between the intermediate host and parasites will make bridge for the knowing transmission methods. It is also important to understand how events in the snail influence genetic diversity of parasites in the mammalian host (Jones *et al.,* 2015). The main tissue residence for *F.hepatica* is liver and some times have attachments to the wall of other important vital organs.

The intermediate hosts of *F. hepatica* are freshwater snails from family Lymnaeidae and the intermediate host for *F.hepatica* and *F.gigantica,* snails from family Planorbidae act as an intermediate host very occasionally. Human infected by ingestion of aquatic plants that contain the infectious larvae of different stages. Experimental study reported by Duménigo *et al.* (2000), suggested that humans consuming raw liver dishes from fresh livers infected with juvenile flukes could become infected.

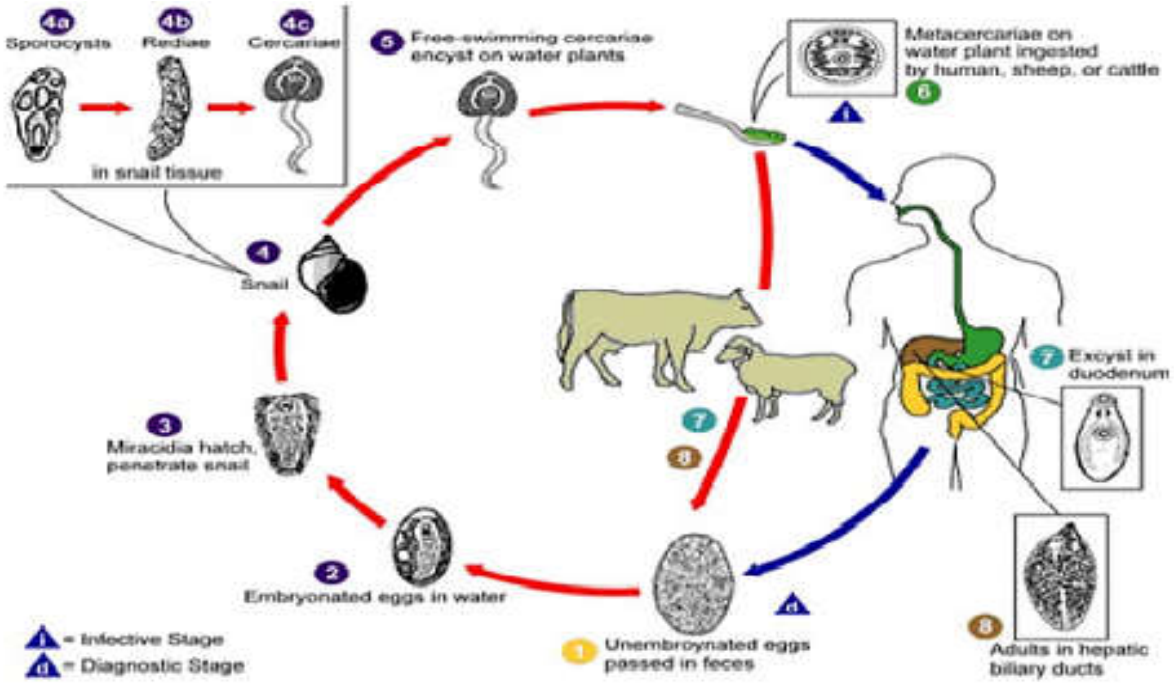

Figure 2: Life Cycle of Fasciola. Source: Michael (2004).

The most common stimulating factors for *Hepaticnecrobacilosis* include ruminal acidosis in cattle fed at grain (feedlot-feeder) (navel ill) in calves and lambs. *Fusobacteriumnecrophorum* possessed proteolytic enzymes, which were bound to the cell wall. They also observed that the proteolytic activity (inferring

production) was related to the amount of glucose in the medium, reducing when high concentrations were added (Wahren *et al.,* 1971*;* Jonathan, 2014).

2.5. Diagnosis

Diagnosis of fasciollosis is not only depending on single laboratory unit or history of the patients. Examination and identification of eggs from feaces of animals by coproscopic examination, detection of specific anti-body by ELISA or Westernblot techniqiues at 24hours post-infection. Accordingly these methods are used for early detection of parasites and as screening test for diagnostic and treatment purpose (Esteban *et al.,* 1998). However, epidemiological diagnosis from the surveilence of snail distribution, common clinical sign and postmortem examination will used for screening of diseased animals. The most important diagnostic methods to allocate the increaments of leukocytes and development of fibrogenesis are by measuring serum concentration and haematological methods. (Rubarth, 1960; Jonathan, 2014).

2.6. Treatments

The only drug used for effective treatment of fasciollosis in the domestic animals is triclabendazole. Many drugs have some drawbacks. Some are effective against young liverfluke and and others are effective for both young and adult flukes. The preferred floucocidal drugs triclabendazole is effective at killing flukes of any age. Chlortetracycline and tylosin appear to be useful against *Fusobacteriumnecrophorum* infections in animals. However, since liver abscesses and foot rot remain significant diseases, these antibiotics are far from satisfactory. The typical in vitro activity of penicillin against *Fusariumnecrophorum* is interesting considering that the organism is gram negative. It is possible to protect cattle from developing liver abscesses by using a toxoid prepared from the cytoplasmic fraction of sonically treated *Fusarium necrophorum* cells. The amount of cytoplasmic toxoid administered appeared to be critical (Garcia *et al.,* 1973; Jonathan, 2014).

2.7. Prevention and control

The controlling of fasciollosis in domestic animals is started from controlling intermediate host. The best long term method for reducing mud snail population such as *Lymnaeidae truncatula* ensures permanent

destruction of snail habitat. Controlling and removing of marshy areas will control snails. The possible ways of controlling the snail is by using niclosamide, cupper sulphate and seasonal methods. However, all of these methods are limited due to cost effective, environmentally not feasible and rapid multiplications of snails. Since ponds, watercrest, marshy and muds are targeted sounrce of snails the control methods should be forwarded to control all the factors for growth of snails (Marquardt *et al.,* 2000).

The main important control measure is by managing feeding of animals the have low acidity and preventing acidosis in animals. Increasing roughage and decreasing concentrates with increasing transition periods from roughage to finisher can reduce ruminal lesion developments and reduces distribution of *F.necrophorum*. Feeding animals with high roughage content feed to increases rumastication to increase saliva flows in regurgitation that buffers the ruminal contents which reduces the acidity of intraruminal concentrations and lowers rminal lesions, and finally inferior the abcessation of liver (Jonathan, 2014).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Akaki, Dukem and Gelan Municipal Abattoir which is located 30km east of Addis Ababa at 9°N latitude and 40°E longitudes, with in an altitude of 2300 meter above sea level in the central Oromia. The mean annual maximum and minimum temperatures are 26 and 14°C, respectively, with mean relative humidity level of 61.3% (NMSA, 2016).

3.2. Study Design and Study Population

The study was conducted from October, 2019 to May, 2020 by using cross-sectional study design to assess a clinico-pathological, lesion characterization, and financial loss due to fasciolosis and hepatic necrobacillosis in cattle at Akaki, Gelan and Dukem Municipal abattoirs. The systemic random sampling was used for selection of animals and the animals were categorized depending on their ages and sex following the procedures used by Johnson (1998) and Belina *et al.* (2012). Liver tissue and blood samples of the same animals were collected. The study animals comprised male indigenous Zebu cattle that were presented for slaughter from caffee donsa, wolayita, Seqa coqorsa and Abba Samuel to the Gelan, Dukem and Akaki Municipal abattoir. A total of 60 cattle were selected and were examined following ante-mortem and post mortem inspection procedure.

3.3. Ante-mortem Inspection and Post mortem examination

The identification mark at antemortem were the marking tagged on animals and eartags found on their ears. Attention was given to the factors such as age, body condition, and origin of the animals to determine the impact of these factors on the disease picture, however; almost all cattle that was presented for slaughter was male local breeds. During the post mortem examination, infected liver of bovine was collected and ordered according to the animal code. At the same time, presence of hepatic necrosis was recorded carefully. Fashiollosis that appeared in the liver tissue were collected, recorded and preserved by 4% buffered formalin for further identification. Gross pathological lesion characterization was done

by appropriately examining the livers and gall bladders for the presence of Fasciola, Fasciola spicies and lesions induced by *Hepatic*n*ecrobacillosis.*

3.4. Sample collection and processing

Following recording of the parameter of animals at ante mortem, samples of blood were collected and post-mortem inspection of livers of the same animals were undertaken for existences of Fasciola species, liver abscesses and gross liver pathology by using systematic random sampling. The specimens for the bacterial culture and histopathology were collected from affected livers and transported to laboratory by sterile saline water and 10 neutral buffered formalin respectively. Gross and histopathological lesions were assessed accordingly and serum was collected to analyse biochemical alterations following roughtine pathological procedures.

3.5. Tissue processing and Examination

Liver tissue sample collections were done from the randomly selected cattle slaughtered in the abattoirs to identify presence of parasite or bacteria in the damaged liver that could be suspected as the causal agents for the liver damages. Accordingly, about 60 liver samples were collected from slaughtered cattle for histopathological, serum biochemical and culturing for bacteriological study. During the post mortem, examination liver of bovine was collected and ordered according to the animal code (Belina *et al,* 2012). At the Sabeta national animal health, diagnostic and investigation center (NAHDIC) laboratory, tissues were processed by automated tissue processor machine with its increasing alcohol concentration, cleared by xyline and embedding was done accordingly and tissue blocks were sectioned at 5µm. The sections were dewaxed, rehydrated and stained using haematoxyline and eosin (H & E) stain. The slides was mounted with Dibutyl phthalate xylene (DPX) and allowed to dry before examination under a light microscope following procedures of Bancroft and Gamble (2002).

3.6. Haematological studies and serum biochemical analysis

3.6.1. Blood sample collection

Eight milliliter of blood was collected from jugular vein using sterile plain vacutainer (clot activator) (for serum) and EDTA coated vacutainer tubes (for complete blood count) was labeled according to the ear tag of animals. Upon arrival at the laboratory, blood samples were rendered to stand at room temperature for three hours to allow serum separation and the blood with anticoagulant was used for Giemsa staining following procedures used by Hodzic *et al.* (2013).

3.6.2. Blood Sample Processing

Hemoglobin determination: The Hgb concentration was evaluated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobin meter according to the methods described by Dein (1984). Diluted (0.1N) hydrochloric acid is mixed into a graduated cylinder with 20ml of blood sample and distilled water was added until the color of the diluted blood sample matches the glass standard. The dilution was determined by the Hemoglobin level of the blood sample following procedure by Philippe (2009).

Total Erythrocyte count (TEC): TEC was performed in 1:200 dilution of blood in Haym's solution. Blood was taken up to 0.5 marks in a RBC diluting pipette and the solutions were taken upto 101 marks. Mechanically the pipette was shaken thoroughly by holding the pipette in between the index finger and thumb and the drop of first come was discarded and the second drop was placed on the counting chamber. The cells were stabilized for 1-2 minutes and total red blood cells in each mm area were counted under high magnification (40x); and the total red blood cells was determined by manual method using hemocytometer according to Dein (1984).

Total leucocyte count (TLC): TLC was also determined by taking the fresh blood up to 0.5 levels in a WBC diluting pipette and 0.1N HCl was sucked upto 11 marks. Mechanically the pipette was rotated gently by holding the pipette in between the index finger and thumb. The Total WBC were counted under low magnification (10x); and determined by manual method using haemocytometer according to the procedures set by Dein (1984).

Packed Cell Volume (PCV): PCV was measured using microhaematocrit reader from microhaematocrit (75x16 mm) capillary tubes were filled with blood and centrifuged at 12,000 rpm for 5 min and the percentage of RBC was recorded by hematocrit reader by comparing the value with normal value of the bovines according to Ibrahim (2012).

Differential leukocyte counts (DLC): Blood smear was made and air-dried after preparation. The smear was fixed in methanol for 5 minutes and was stained with working Giemsa solution for 35 minutes, was washed with tap water, blotted and examined under the microscope for differential leukocyte counts using 100x microscopy. Each cell (Neutrophil, Basophils, Eosinophils, Monocytes and Lymphocytes) was counted until 100 white cells were counted and the percentages of each WBC were determined.

3.6.3. Indole Test

Sterilized test tubes containing 4ml of tryptophan broth was taken and the growth culture incubated for 24hrs in the tube was aseptically inoculated. In addition, the tube was incubated at 37°C for 24 hours. The presence or absence of ring was observed by following previous procedures of Josue *et al.* (2015).

3.6.4. Gram's staining

Gram's staining method was done by preparing a smear, heated gently to fix, flood the slide with 0.5% crystal violet and leave for 30sec. The slide was tilted and rinsed gently with water, fluided with iodine solution and remain for 30 seconds. Decolourization was done with 95% ethanol until colour ceases to run out of the smear. Then the slide was rinsed with water and was flooded with 0.1% counterstain safranin and leave to act for about 30sec. Finally, cleaned was examined using an oil immersion (100x) objective to observe cell morphology and Gram reaction as following Wilson and Miles (1975) procedures.

3.6.5. Microbiological Culture

The specimens from liver lesions for bacterial culture were collected by icepack and bottles filled with saline water. Then, taken into laboratory and it was added to the sterile Petri-dish containing egg yolk agar

for bacterial culturing. The identification of bacterial colonies was conducted after 48 hour incubation anaerobically at 37°C. The fusobacterium egg medium we used contains vancomycin, neomycin, josamycin, and egg yolk. All species of fusobacteria grew were assessed to see the minimal inhibition by these antimicrobial disc. After incubation 3 drops of Methyl red was added to a liquot following procedures by Athavale *et al.* (2002) and color change was observed.

3.7. Analysis of economic losses

Economic losses due to this parasites and its secondary bacterial complications in Akaki, Gelan and Dukem slaughter house was predicted based on condemned organs under the study. The financial losess due to the affected and condemned organs were assessed by requesting through dirct interview and datas found at district clinics and the calculated median values of one liver was three hundred fifty Ethiopian birr. The calculated value was adopted from the formula developed by Ogunrinade and Ogurinade (1980). From the calculated values of the liver condemned due to fasciolosis and *Hepaticnecrobacilosis* infected liver during the study period was: $EL = \Sigma CS * Coy * Roz$; The definition of these letters are; Annual loss estimated due to liver condemnation (EL), annual slaughter rates at the abattoir (estimated from retrospective abattoir record (ΣCS), Average cost of each cattle liver (Coy) and Condemnation rates of cattle liver due to Fasciolosis and *Hepatic necrobacilosis* (Roz).

3.8. Analysis of data and statistical methods

Collected and recaped data was intered into MicrosoftExcel spreadsheets and analyzed using STATA version14. Descriptive statistics (frequency and percentages) was analyzed. The association of age, origin and body condition with Fasciolosis infection and *Hepatic necrobacilosis* in the liver was assessed by Chisquare (X^2) test). The statistical analysis system (SAS, 2000) was used to determine the mean, range and standard deviation of hematological data. The level of the mean values of the infected and none infected was determined using t-test and a $P<0.05$ was considered as significant.

4. RESULTS

The result shown by the current study reported the prevalence of cattle liver infected by both Fasciolosis and *Hepaticnecrobacilosis* 41.6% (25/60). From these results liver lesions infected with only liverfluke was 13.3% (8/60) in which greater leasions by *Fasciolla hepatica*, and half result of reported lesions by mixed infection of *Fasciolla gigantica* and *Fasciolla hepatica* (table 1). The *Fusabacterium-necrophorum* (liver abscesses) result shows that 8% (5/60) and mixed infection of Fasciolosis and *Hepaticnecrobacilosis* was 20% (12/60).

Table 1: Comparision of liver lesion according to Liverfluke species in examined cattles.

	Fasciola Infected (8/60) 13.3%			Infected by Liver abscess only	Infected by both liverfluke and liver abscess
	Fasciolla hepatica	*Fasciolla Gigantica*	Mixed Infection *F. hepatica* and *F.gigantica*		
Identified lesions	4	2	2	5	12
%	6.6	3.3	3.3	8	20

4.1. Post Mortem Gross Pathology Examination

The characteristic gross pathological lesions found at postmortem include firm, pale, swollen and irregularly outlined liver with tough texture. Adult liverfluke and calcification was observed up on insized bile duct and liver paranchyma. From 60 livers grossly examined, 17 of them have been showed *Hepaticnecrobacilosis* from these lesion mixed infection of fasciolosis and *Hepatic Necrobacilosis* (12/60) and single infection of *Fusobacterium necrophorum* was (5/60) with various sizes of nodular gowth which dotted on the exterior hepatic areas and maximum sizes of large nodules measured 3cm. Upon insized liver parenchyma, oozing of fluids containg adult flukes was observed.

Among the examined organ, there were an enlargement with rounded edges and dark yellow nodules of two inches in diameter and a clay inconsistency are usually scattered throughout the organ (fig 3).

Emphysema at the peritoneal areas, distension of the gall bladder with bile from pressure exerted by the nodules, necrotic foci in the pleura, diaphragm and heart were the lesions found at postmortem.

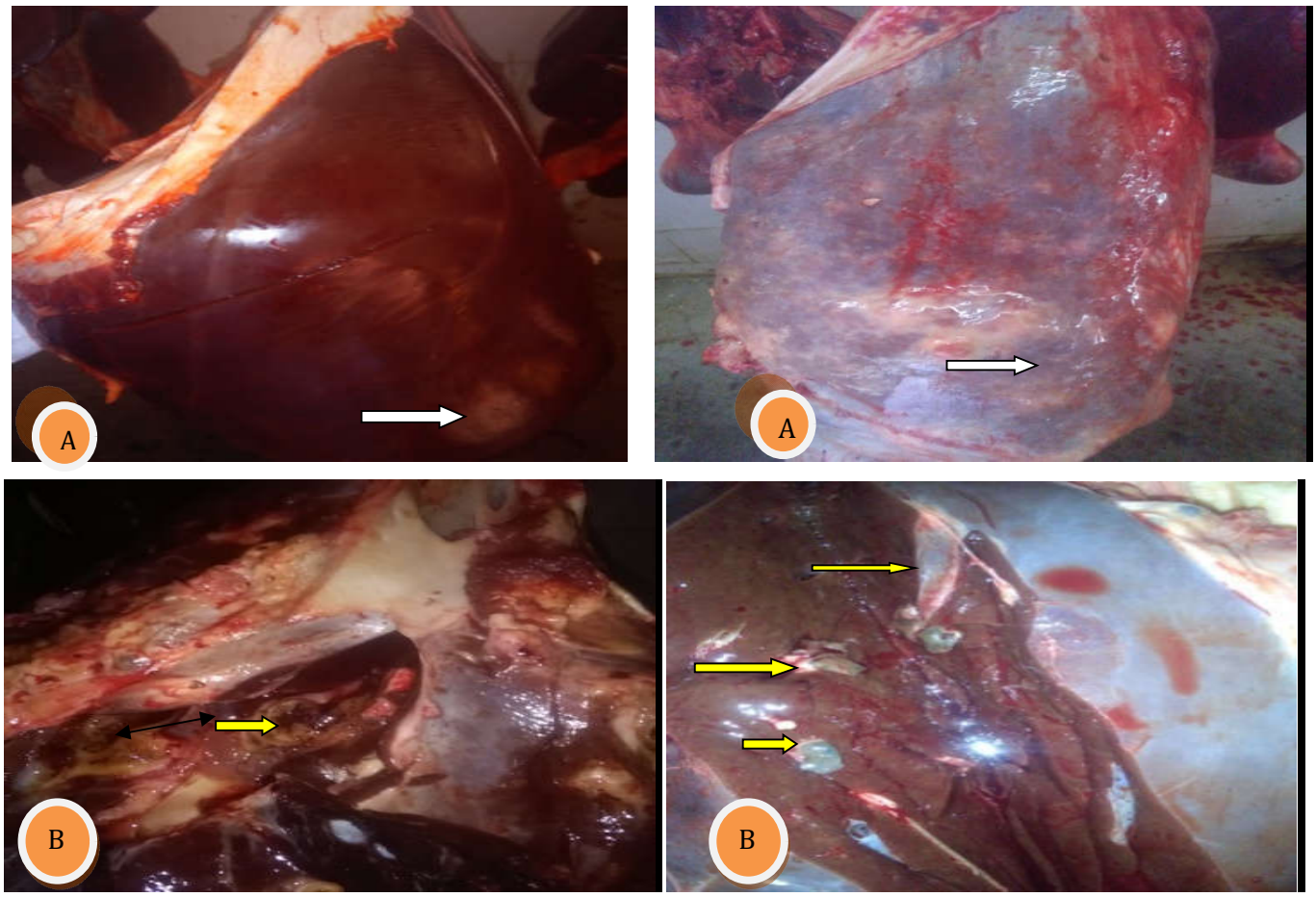

Figure 3: A) Bovine liver showing (nodules) abscess formation (white Arrows). B) Incised same bovine liver infected by Fasciolosis (adult Fasciolla (yellow arrows) and *Fusobactriumnecrophorum*, abscess formation and cholangitis (black arrow).

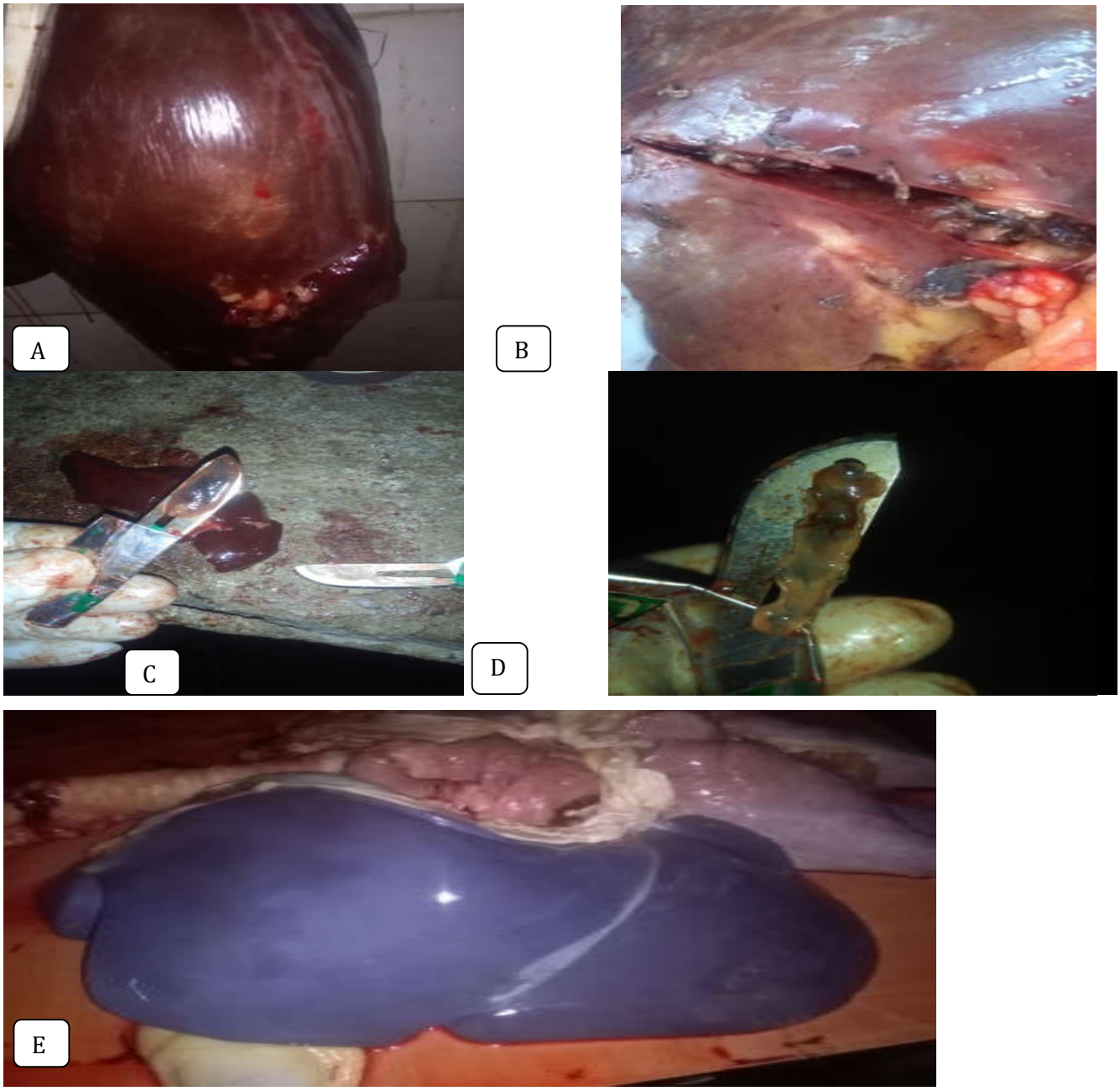

Figure 4: A) Bovine liver infected by fluke and *Fusobacteriumnecrophorum*, B) Incised bovine liver showing fluke infestation C) Adult *Fasciola hepatica*, D) Adult *Fasciola gigantica* and E) Slightly normal bovine liver.

4.2. Histopathological Results

From the representative samples collected from liver, histopathological lesions showed normal hepatic cord arrangement and normal hepatic lobulation areas, with few infiltration's of inflammatory cells. The characteristic histopathological lesions found of mixed infection of fasciolosis and secondary bacterial infection composed of severely congested blood vessels in portal area, huge number of infiltration of inflammatory cells especially macrophages and lymphocytes (fig 5). Inflammatory cells releaced in response to fasiolosis, eosinophilic had shown in greater number then other leukocytes. Epithelial lining of the billiary epithelial ducts shown as hyperplastic and developed through the paranchymal areas of liver (fig 6 & 7).

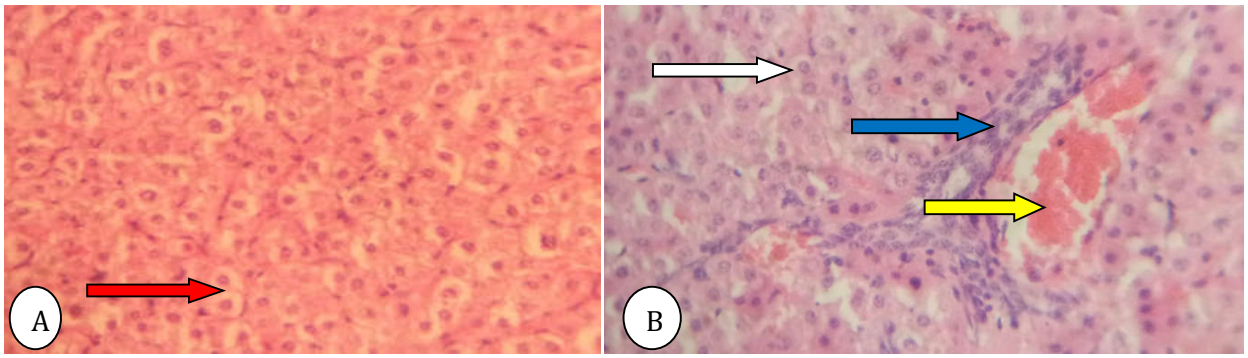

Figure 5: A) Collapse and clapse hepatocytes and separation of the hepatic cells (red arrow), (HE x40). B) Strictly congested blood vessels (yellow arrow), infiltration of inflammatory cells (blue arrow) and separated hepatic cells (white arrow).

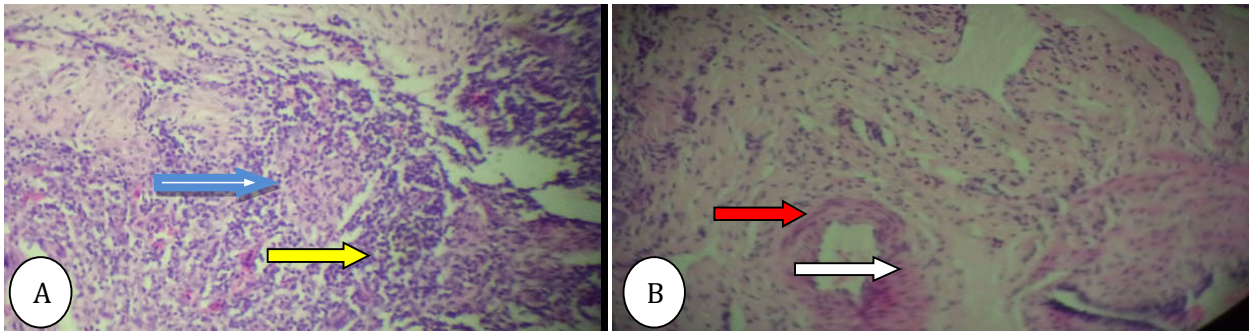

Figure 6: A) Infected liver, showed centrilobular immense coagulative necrotic hepatocytes (blue arrow) and massive infiltration of inflammatory cells (yellow arrow) (HE x40). B) The concentric areas filled with eosinophlic cytoplasms (white arrow) hepatocyte vacuolations around central veins (red arrow).

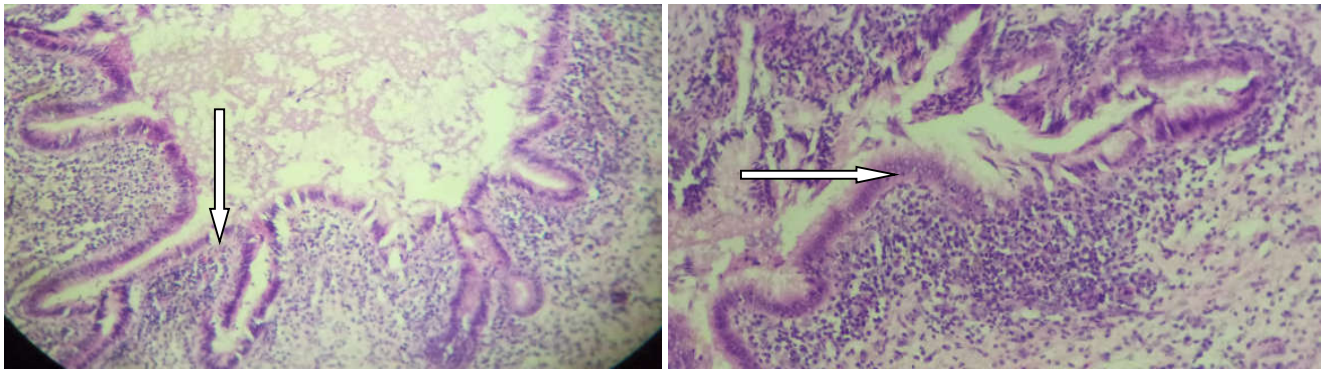

Figure 7: Proliferation and hyperplastic areas of the luminal epithelium at the main divisions of bileducts with papilar projections, within the lumen and distributions of inflammatory cells (HE x10).

The distribution of differenct inflammatory cells within abscessed liver with rod shaped bacteria resides in the connective tissue triads, the viscible vaculated mascular layers are fount along the chirrotic bileducts (fig 8).

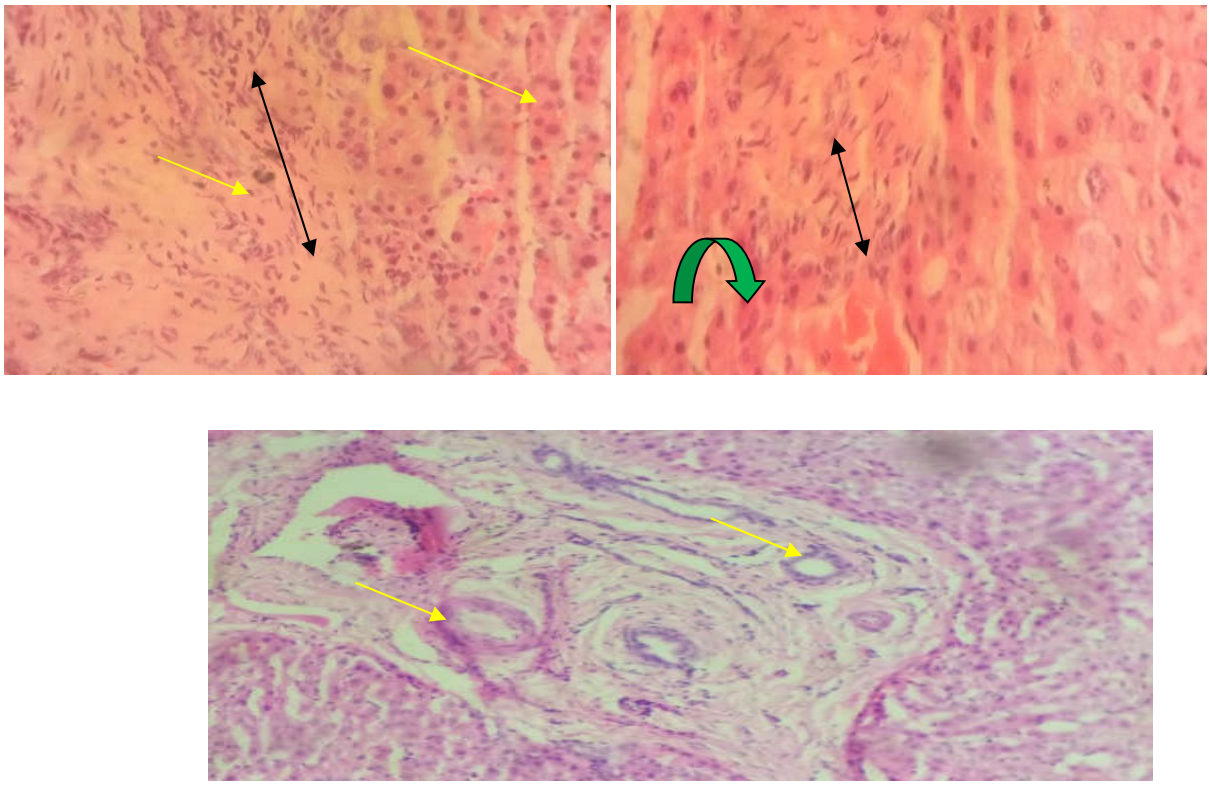

Figure 8: Pyogenic membrane surrounding abscessed liver (black arrow) with infiltrated inflammatory cells; kupfer cells and eosinophils (yellow arrow) and congested blood vessel (green arrow).

The epithelial linings of bileducts are rectotized and disquameted with evidence of large areasn of abscessed hepatic paranchyema (fig 9).

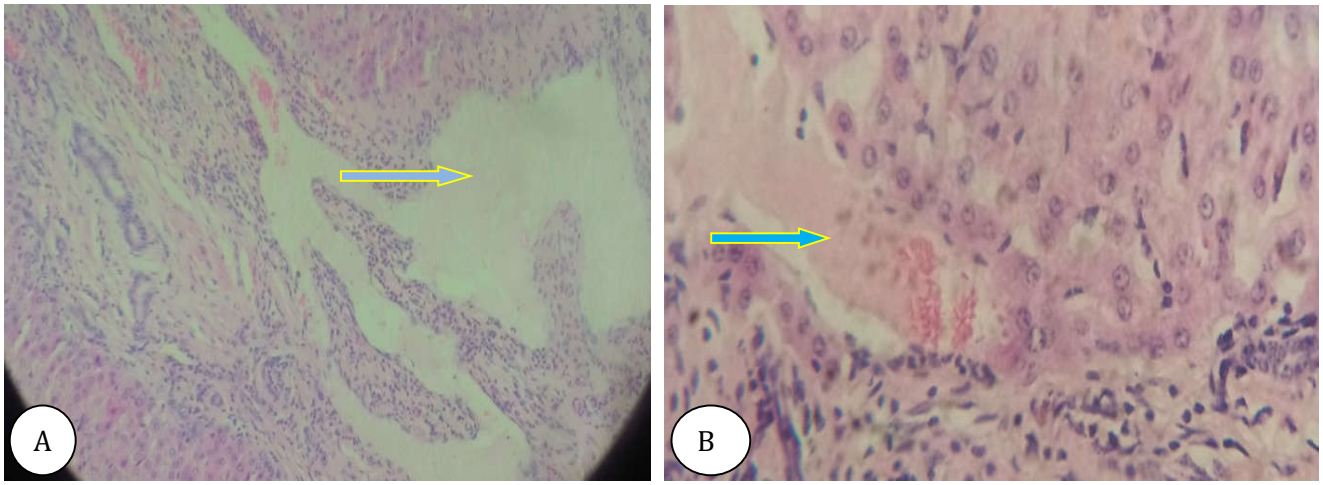

Figure 9: Disquamated and abscessed hepatic parenchyma and massive infiltration of inflammatory cells (lymphocytes, macrophages, neutrophils and eosinophils) (HE x10 and x40 (A&B respectively)).

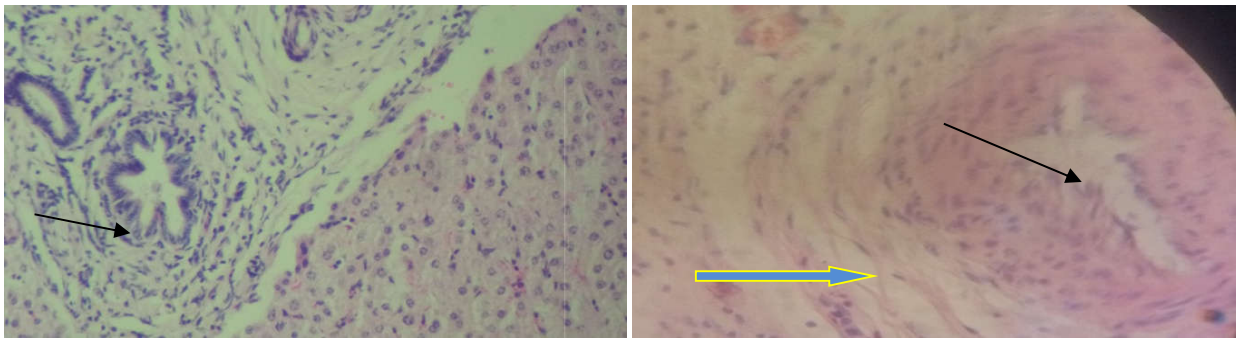

Figure 10: Liver infected by fasciolla spicies and *Fusobacteriumnecrophorum* results cirrhotic bileducts, widespread fibrous connective tissue creation around the intrahepatic bile ductules (black arrow) and hyperplasia of fibrocytes (blue arrow) (HE x40)

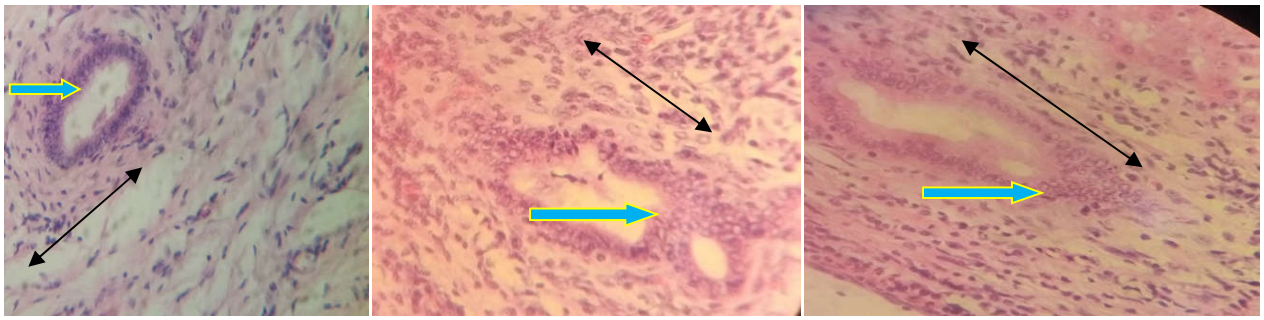

Figure 11: Metaplastic columnar epithelial cells to cuboidal epithelial cells (blue arrows), necrotized hepatocytes (black arrows) and infiltration of inflammatory cells (HE x40).

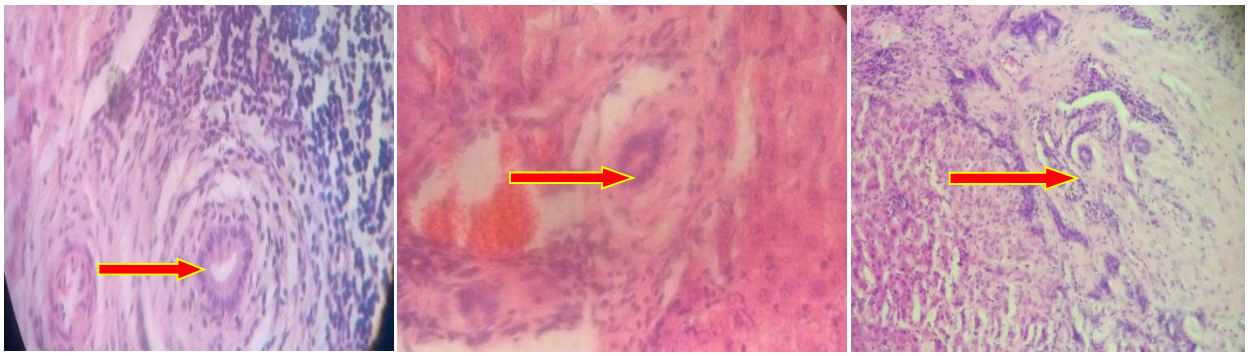

Figure 12: Chirrotic bileducts, proliferation of connective tissue and infiltration of inflammatory cells surrounding portal triads (arrows) (HE x40 & x10).

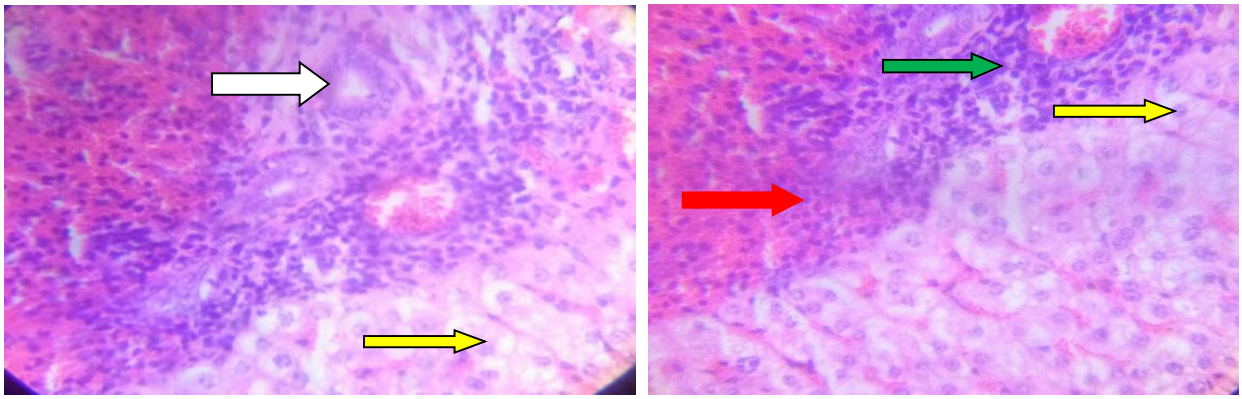

Figure 13: Vacuolar degeneration of hepatocytes with dilated hepatic sinusoids (yellow arrow), inflammatory cells (green arrow), pyogenic membrane consisting proliferation of fibrous connective tissue (red arrow), and metaplastic columnar epithelial cells (white arrow) (H and E stain).

4.3. Biochemical Analysis Result

The results were obtained by measurement of the concentration of all proteins present in serum excluding clotting factors. Albumin and the immunoglobulins (IgG, IgA and IgM) were the major results.

Table 2: Comparisons of serum biochemical analysis in infected and non-infected liver by Fasciolosis and liver abscess.

No	Liver condition	AST (u/l)	ALT (u/l)	ALP (u/l)	Tota protein (g/dl)	Albumin (g/dl)
1	Infected	84±6.64	37.2±3.64	109±2.34	4.66	4.88
2	Non infected	45.55	20±2.68	69.41	8.22	8.11
	P value	0.0032	0.0034	0.0055	0.0321	0.0067

Table 3: Compared the serum liver enzymes collected from different ages of animals infected with mixed infection of Fasciolosis and *Fusobacteriumnecrophorum*.

Age	**Status**	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**TotalProtein (g/dl)**	**Albumin(g/dl)**
Young	Infected	82.44±4.65	36.81±9.22	112.97±11.76	4.76	4.90
	Non infected	46.43± 3.23	15.72±5.45	77.32±4.55	6	6.01
	P value	0.0041	0.0032	0.0521	0.0022	0.0031
Adult	Infected	85.76±4.76	38.11±4.12	107.75±7.66	4.44	4.7
	Non infected	45.35±13.56	15.57±5.44	61.76±7.98	5.96	6.12
	P value	0.0275	0.0043	0.0581	0.0231	0.0337

4.4. Haematological profile

The results obtained hematological analysis results determined for Fasciolosis and *Hepatic necrobacilosis* in infected and non-infected liver were present in table 4 and the differential leukocyte count results were revealed by table 5. The result indicated that PCV, Hb and RBC were lower in the infected liver than in

non-infected. However, WBC count was higher in the infected than in non infected liver of cattle.

Table 4: Hematological test results of liver of cattle slaughtered at sellected abattoirs infected by Fasciolosis and *Fusobacteriumnecrophorum*.

Hematological parameters	**Infected**	**Uninfcted**	***P value***
Hgb (g /dl)	6.94	10.94	0.0043
PCV (%)	22.1	35.54	0.0127
RBC (cells/mm3) x 10^6	$44.3*10^6$	$59.3*10^6$	0.0338
WBC (cells/mm3) x10^5	$10.27*10^5$	$9.3*10^5$	0.0357

Table 5: The average values of the differential leukocyte count of Fasciola and *Fusobacterium-necrophorum*.

Differential counts	**Infected**	**Uninfcted**	***P value***
Neutrophils	18	13	0.0033
Lymphocytes	52	61	0.0156
Monocytes	6	7	0.0077
Eosinophis	24	16	0.0259
Basophils	00	00	0.000

4.5. Indole Test

The test result was displayed by decomposition from bacterial actions and presence of amino acid accumulations in the medium. The formation of a pink to red color (“cherry-red ring”) in the reagent layer on top of the medium within seconds of adding the reagent was observed.

Figure 14: Biochemical characterization of *Fusobacteriumnecrophorum* with Indole test formation of pink to red colour ("cherry-red ring") (black arrows) showing positive result.

4.6. Gram's staining

The characteristic rod-shaped bacteria resulted from gram stain have been the typical character of *Fusobacteriumnecrophorum.* The morphology of stained slide showed that rod-shaped long, nonbranching and flamentous pleomorphic bacilli with blunt ended parallel sides.

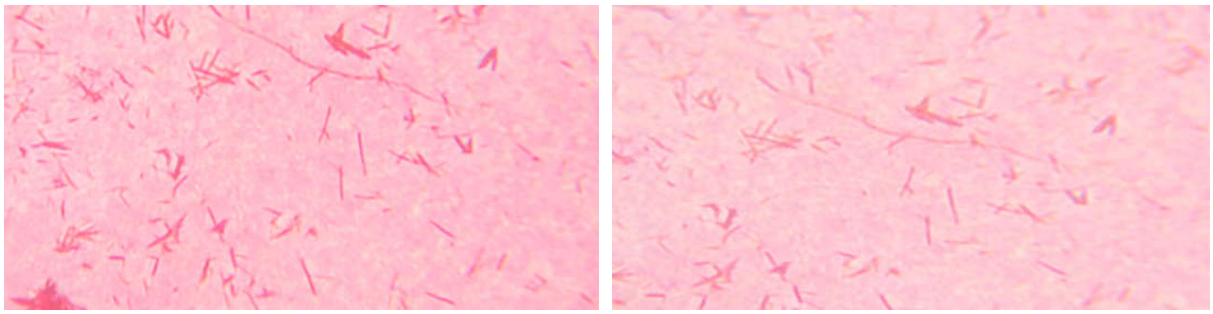

Figure 15: Characteristic *Fusobacteriumnecrophorum* shown by Gram stain (100x) oil immersion.

4.7. Microbiological culturing

The result obtained by bacteriological culture of necrotized liver for a periods of 48 hours anaerobically at room temprature, the colony of observed bacteria were appeared as round, grey, and shiny in appearance having diameter of about 1-5 µm (fig 16).

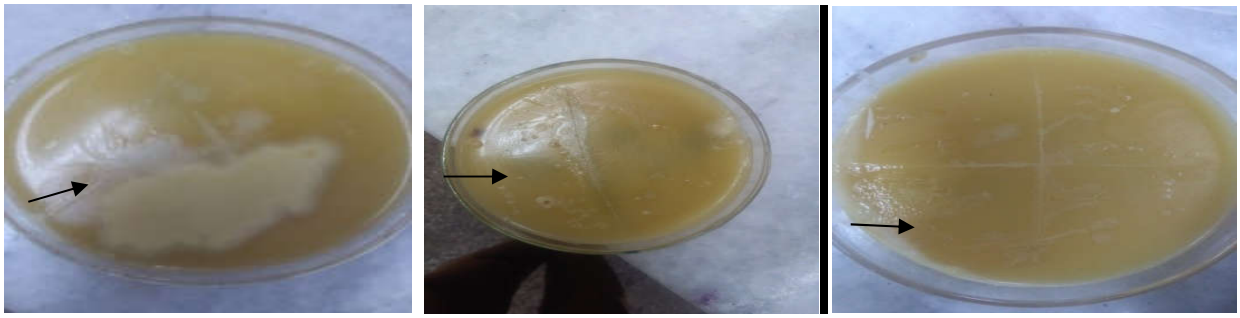

Figure 16: Colony of *Fusobacteriumnecrophorum* appearing round, grey, and shiny in appearance aproximately having a diameter of 1-5 µm (arrows).

3.7.1. Methyl red

Adding 3 drops of metyle red (MR) to the broth, the yellow color was observed which indicated negative result. So that this MR test again realized that the bacteria that was isolated from fresh liver, tissue for bacterial isolation was *Fusobacteriumnecrophorum* and was MR negative.

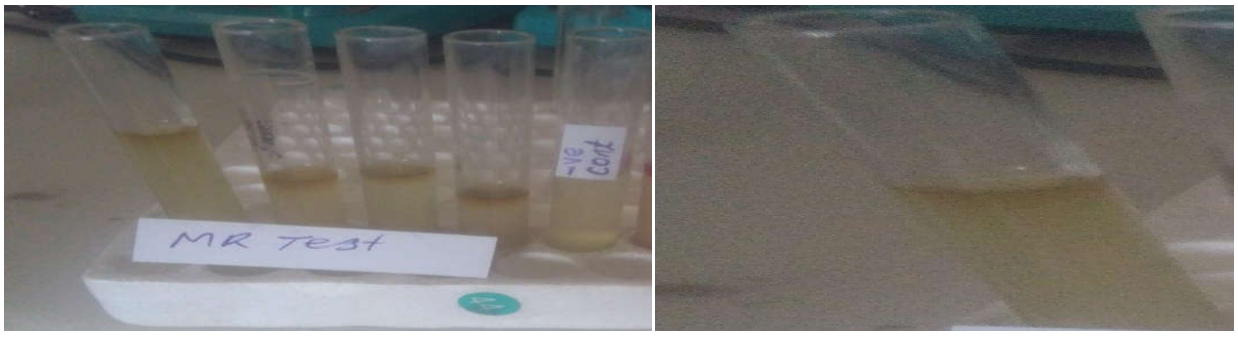

Figure 17: MR test for *Fusobacteriunnecrophorum* sub-spies necrophorum isolation showing negative result (no colour change).

4.8. Occurrence of liverflukes and associated risk factors

From the present result, origion has significant effect on the prevalence of bovine fasciolosis; being higher in those came from wolayita Sodo, South Nations and Nationalities of Ethiopia (table 6). Younger animals were highly infected by fasciollosis and *Hepatic necrobascilosis*. Moderete animals were more affected than excellent body conditioned animals (table 7).

Table 6: Occurrences of bovine fasciolosis and *Hepaticnecrobacilosis* based on origin

No	Origin of the animals	Number of examined	Infected	%	X^2	P value
1	Caffe donsa	14	5	36		0.001014
2	Wolayita	15	9	60		
3	Seka coqorsa	14	5	36		
4	Abba samuel	18	6	33		

Table 7: Association of age and body conditions with occurrences of fasciollosis.

Variables	Levels	Nº Of Examined	Nº of Positive(%)	P Value
Body Condition Score	Moderate	20	14	0.0337
	Excellent	40	11	
Age	Young	35	17	0.0001054
	Adult	25	8	

4.9. Financial loss analysis

The economic significance of Fasciolosis and *Fusobacteriumnecrophorum* in ths study was calculated from on the information obtained from data recording sheet, during postmortem assessment and interview with local butchers in the selected towns. The average annual cattle slaughtered at these three selected municipal abattoirs was estimated to be 14000 cattle as I obtained this data from records of these selected abattoirs, and the mean retail price of bovine liver in selected towns was estimated to be 300 ETB and the prevalence of Fasciolosis and *Hepatic necrobacilosis* in selected municipal abattoirs shows that 41.6%. Therefore, the estimated annual loss from organ condemnation was calculated. Yearly losses dueto liver condemnation= ΣCS*Coy*Roz= 14000*300*41.6% = 1747200ETB ($49920).

5. DISCUSSION

Fasciolosis is a negleced parasitic diseases causing growth retardation, economic losses and its transmission in attribution to environmental and climatic conditions of the locations that favors the survival of the intermediate host, the *Lymnaea* snail (Magaji *et al*., 2014). In this study, sixty (60) bovine livers were examined and 41.6% (25/60) of liver samples showed gross and histopathological lesions of Fasciola species and *Fusobacteriumnecrophorum* infection. The identified lesions were caused by single or mixed infestations of *Fasciola hepatica* and *Fasciola gigantica.* The complications of the fasciollosis was resulted in lever abscessations from underlined bacterial infection which is in agreement with the report of Sohair *et al.* (2009). The result may be due to pathophysiology of the bacterial floara from grain overload and its migration to liver through ruminal epithelial lumen. In parallel to this assumption, Al-Khafaji and Rhaymah (1993) was reprted the same statement as the secondary complications of liverflukes will result in bacterial infection.

The current study resulted the combination of fasciolosis and underlined causes of lesions by bacteriosis resulted, 20% (12/60) mixed infection, 8% (5/60) *Fusobacteriumnecrophorum* and 13.3% (8/60) shows lesion caused by liver flukes only. The same result was reported by Sohair *et al.* (2009), were it is aimed to isolate *Fusobacteriumnecrophorum* complicated by fasciolosis causing liver abscess and this result was extremely far from result obtained by Al-Mahmood and Al-Sabaawy (2019) that they were recorded about 4% of fasciolosis. The difference in these results occurred due to difference in weather condition and climate of our study areas.

The result of liver abscess during this study was found to be 28.3% (17/60), this was in line with the results reported by Yousif (2016) 32% prevalent in Karbala province of Iraq and higher than 0.7% report by Hussein *et al.* (2016) in Asella, Ethiopia. Post-mortem gross pathology of examined liver shown, hard up on palpation and incision, firm and tough in consistency. The similar results of fibrosis, enlarged and cordlike in structure was reported by Jones *et al*. (1997) and Sayed *et al*. (2008). These changes were very similar to findings described by Ahmed *et al.* (2005).

The histopathologic resulted in current study in bovine liver infected with *Fusebacteriumnecrophorum* were found surrounding the nonuniform eosinophilic core incircled by inflammatory cells and fibrous

connective tissue capsule. This result is inagreement with the report of Darwish (1996) mentioned that there was a synergistic relationship between *Fusobacteriumnecrophorum* and it is substance called leukotoxin that have antigenic properties to the tissues of liver and also have strong association with the result reported by Sayed *et al.* (2008).

The cirrhotic bile duct, containing protrution of epithelial lining and newly formed bileducts within the adenomatous arrangment has hyperplastic proliferation (fig 10). The presence of mature worms causing necrosis and disquamation of ductal epithelium was inagreement with Sayed *et al*. (2008) who reported toxin released from *Fusobacteriumnecrophorum* induced necrosis of hepatic cells and Darwish (1996) from liver of Camel.

The isolated bacteria *Fusobacteriumnecrophorum* was assumed to causes the necrosis of ductal epithelium with complication of liver flukes and produces potent leukotoxins. The predisposing factors for these infections may be resulted from concentration of *Fusobacteriumnecrophorum* in ruminal contents which is higher in grain-fed than forage-fed cattle and ruminal acidosis. This may resulted from the damaged ruminal wall allows entry from the rumen and colonization of the ruminal wall by *Fusobacteriumnecrophorum,* which travels via portal blood to liver to set up the infection. Then the bacteria use a variety of means to create anaerobiosis and evade host defensive mechanisms in invading and colonizing the ruminal wall and reaching the liver to cause abscessation (Ahmed and Mohammed, 2014).

The heamatological examination resulted, PCV, Hb and RBC were found minimum in the abscessed liver however, eosinophil, neutrophil, monocytes and lymphocytes were found maximum in the affected liver. Total protein and albumin in this study is lower in infected liver of cattles. The damage and/or necrosis, degeneration and ploriferation of hepatocytes in this study were due to increased plasma concentration and highly increased clncentration of ALT level in cytoplasm. The present report is inagreament with the result reported by Hodzic *et al.* (2013) and Mbuh and Mbwaye (2005) who reported highly destruction of hepatocytes by increased level of ALT. This is because, ALT is the predominant enzyme found in liverand its increament is associated with high deaths of hepathocytes due to liverfluke and *Fusobacteriumnecrophorum,* which in inagreement with report of Kilad *et al.* (2000) supposed increase in ALT is due to hepatocyte death from liverfluke infection.

There were strong association between age and *Hepatic necrobcilosis,* being higher in young animals than adult in this findings. The problems may be due to feeding habit of young who feed mainly protein diets and poorly developed immunity against the infection that agree with report by Radostits *et al.* (2007) showing as the higher the resistence aginist the liverfluke, the low prevalence found in the animals.

From the present work the body condition has significant association with the occurrences of fasciollosis and its preceedings *Fusobacteriumnecrophorum*. It was higher in medium sized body condition than that of the animal having higher body conditions. Economic losses reported from condemend liver in one year of present finding was 1,747,200 ETB. The higher and lower economic lesses were reported by different authors in different places. The discripancy between the report will be due to difference in the study sites and climatic conditions and poor recording system of organ condemined or false data reported during the study.

6. CONCLUSION AND RECOMANDATIONS

The present study was carried out to enumerate the gross and histopathological lesions of liver induced by liverflukes infestation with the complicated bateria and to detect the relationship between them. The study confirmed that fasciolosis and *Hepatic necrobacilosis* were the main diseases of cattle affecting their liver and reduces weight gain. This may be due to the fact that the origins of animals have suitable ecological condition to the existence and multiplication of the intermediate host snail (*Lyminidae truncatula*). The study was concluded that the liver abscess causes severe economic losses from reduced carcass weight and condemned organ and *Fusobacteriumnecrophorum* subspecies necrophorum, causes related pathological lesions in liver and surrounding organs in cattle.

Therefore, based on the concluded results, the following recommendations are forwarded:

- ➔ Management and strict biosecurity from the prevalence of the intermediate host prevalent.
- ➔ The mixing and providing of food of animals with different fluococidal drugs.
- ➔ deworming practice should be there regularly in the study area for parasitic control,
- ➔ increasing the roughage diets in their feeds and increasing feeding times
- ➔ increasing mastication and saliva flows that neutralize the contents of acidic rumen that fascilitates metabolic activities.

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8. ANNEXES

Annex 1 : Histopathological procedures (Takulder, 2007)

1. Tissue fixation by fixative agent, mainly by 10% neutral buffered formaldehyde

2. Trimming part of the tissue in a way that the lesion we require be included or not missed and tofit standard histological processing tissue cassettes (5mm thickness).

3. Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax. Formalin-I 2hr, Formalin-II 2hr , 70% Alcohol 1hr , 95% Alcohol, 100% Alcohol-I 1hr, 100% Alcohol-II 2hrs , 100% Alcohol-III 2hrs, Xylene-I 1:30hrs, Xylene-II 1:30hrs, Xylene-III 1:30hrs, Paraffin-I 2hrs and Paraffin-II 3hrs.

4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60^{o}c) is poured and allowed to settle and solidify.

5. Sectioning: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put an Incubator over night.

6. Staining: Hematoxyline eosine stain procedure

a. The slides should be deparaffinized by xylene by passing two times for five minutes

b. Hydrate slides in 3 changes of 100% alcohol each for 3minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes

c. Wash in distilled water until the repellent disappears

d. Mayers hematoxyline will be place for minimum of 10 minutes.

e. Wash in running water until clear drop will seen

f. Decolorize in the 1% acid alcol and see for some times to see what a nucleic should be distinct; cytoplasm should be uncolored.

g. Wash in the running water.

h. Stain in eosin, 3 dips.

i. Wash in running water until clear color will see.

j. Again dehydraye in the ascending alcohol.

k. Bypass three times in xylene.

l. Mount cover glass with DPX.

m. Finally test the ready slides under microscope.

Annex 2: The procedures of blood haemoglobine determination (Ibrahim, 2013).

1. Take 0.1N HCl (1%) into central graduated tube up to mark

2. Blood will be sucked upto 20(20µl) mark of Sahli's pipette.

3. Transfer the blood from pipette to central graduated tube of the hemometer.

4. Mix it well with the help of stirrer or rod and allow it to react for two minute.

5. Make up with distilled water by adding drop by drop until the color matches with the Standard comparator tube and mix well.

6. When the color matches take out and record the values on the side as gm/100ml and or in percentage.

7. Repeat 5 to 6 times and take the average value.

Annex 3: The procedures of blood PCV determination (Bancroft and Gamble, 2002; Ibirahim, 2013).

1. The blood is filled in to a micro hematocrit tube to (3/4th) and sealsit with sealer.

2. Centrifuge the filled hematocrit tube in a hematocrite centrifuge at 12000 rpm for 4-5 Minutes.

3. Use haematocrit reader to read and record the result.

Annex 4: The procedures of the total RBC count (Ibrahim, 2013).

1. Take the blood in to RBC pipette up to 0.5 marks

2. The RBC diluting fluids will be sucked upto 101 marks emmediately.

3. Rotate the pipette between thumb and other fingers with finger. This gives a dilution of 1:200.

4. The haemocytemeter should be cleaned and covered with cover slip.

5. Place the cover slip in position over the counting chamber by gentle pressure

6. Expel a drop of blood on to the counting chamber by holding the pipette at an angle of 45º.

7. Allow the hemocytometer for 2-3 min to Settle down the RBC in counting chamber

Counting rules

- Count under 40 x objectives under microscope
- Don't C unting the cell touching the bottom and right lines
- Count first from left to right directions and then vice verse
- Counting the cell touching the left and tope lines

Annex 5: Dilusions of common laboratory reagents during study

1 5% Formalin

Formaline, 33%	50.0ml
Distilled water	950.0ml

2 Buffered Formaline (10%)

Sodium hydrogen monobasic	4.5 gm

Sodium hydrogen dibasic	6gm
Formalin, 37%	100ml
Distilled Water	900ml

3. Normal Physiological saline (0.9%)

Nacl	9gm
Distilled water	1000ml

Annex 5:

Akaki manucipal abattoir slaughter house (Some Photographs of cattles in the fence)