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A Clinico-Pathological Study, Lesion Characterization and Financial Loss Due to Fasciolosis and Hepatic Necrobacillosis in Cattle Slaughtered at Municipal Abattoirs of Central Ethiopia

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Abstract

Fasciolosis also called liver flukes is a highly pathogenic parasitic disease of humans and the livestock caused by flatworms of the genus *Fasciola*. Its pathology and pathogenicity is more of in liver organ and results in tissue destruction, inflammation, local or systemic toxic/allergic reactions, internal bleeding and it leads to secondary bacterial complications. A cross-sectional study was conducted from October 2019 to June 2020 to assess a clinico-pathological study, 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 and liver tissue of the same animals were collected before and after slaughter. 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 liver fluke and *Fusobacterium necrophorum* while 8% (5/60) and 13.3% (8/60) of the infections were due to *Fusobacterium necrophorum* and liver flukes alone. The histopathologic results indicated heavy infiltration of inflammatory cells, biliary cirrhosis, extensive fibrous connective tissue proliferation in the hepatic capsule, and periportal infiltration of inflammatory cells. The hematological results indicated that PCV, Hb, and RBC's were lower in the infected cattle while, the WBC's were higher. The biochemical analysis of blood samples showed that the liver enzymes AST, ALT, and ALP were significantly higher in animals with hepatocyte degeneration. The calculated financial losses due to liver condemnation by fascioliasis and hepatic necrobascillosis were 1,747,200 ETB/($56361.3) annually. The study concluded that the liver flukes 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 • Fasciolosis and hepatic necrobacillosis

Abbreviations: AHI: Animal Health Institute; ALP: Alkaline Phosphatase; ALT: Alanine Transaminase; AST: Aspartate Transaminase; DPX: Dibutyl Phthalate Xylene; Hb: Hemoglobin; NMSA: National Metrology Service Agency; PCV: Packed Cell Volume; RBC: Red Blood Cell; WBC: White Blood Cell

Introduction

Liver is considered the most important organ for animal health production and reproduction. Many of the metabolic activities of the body occurred in the liver. Liver infection is an important disease that affects all kinds of meat producing animals, leading to great losses to livestock production and national income due to condemnation of great numbers of livers in the slaughterhouses [1].

Bovine fasciolosis is an economically important parasitic disease of cattle in tropical and subtropical countries. Infestation with fasilolosis is usually associated with grazing wet land and drinking from the snail infesting watering places [2]. The development of infection in definitive host is divided into two phases; migratory phase and the biliary phase [3]. The parenchyma phase begins when encysted juvenile flukes penetrate the intestinal wall. After the penetration of the intestine, flukes migrate within the abdominal cavity, penetrate the liver or other organs, and cause lesion. *Fasciola*

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hepatica has a strong predilection for the tissues of the liver and cause severe intensity of liver lesion, second phase (the biliary phase) begins when parasites enter the biliary ducts of the liver and flukes mature, feed on blood, and produce eggs [4].

Abscessetion of liver are the most common and economically important *Fusobacterium necrophorum* infection in cattle. Liver abscesses occur at all ages and in all types of cattle, but the abscesses of significant economic impact occur in feedlot cattle [5]. Liver abscesses in cattle 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. Abscesses found in the liver at the time of slaughter or necropsies often are well-encapsulated, possessing thick fibrotic walls. Histologically, a typical abscess is pyogranulomatous, with a necrotic center, encapsulated and often surrounded by an inflammatory zone [6]. Grain digestion, particularly gelatinization of the starch granules promoted the rate of ruminal fermentation of the starch so it increased the probability of acidosis and liver abscesses [7].

Liver abscesses are secondary to the primary foci of infection on the ruminal wall. 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. Although the precise mechanism is not recognized, it is accepted that rapid fermentation of grain by ruminal microbes and the consequent accumulation 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 *Fuobacterium necrophorum* [8].

The organism then gains entry into the blood or causes ruminal wall abscesses, which subsequently shed bacterial emboli into the portal circulation. The liver, resulting in infection and abscess formation, 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, *Fusobacterium necrophorum*, as an anaerobe, has to overcome both high oxygen concentrations and phagocytic mechanisms in order to survive, proliferate and initiate abscess formation. The leukotoxin and endotoxic lipopolysacharide of *Fusobacterium necrophorum* may protect it from phagocytosis. In addition, the release of cytolytic products such as lysosomal enzymes and oxygen metabolites, because of destruction of phagocytes, has a detrimental effect on the liver parenchyma [9].

A tentative diagnosis of fasciolosis may be established based on prior knowledge of epidemiology of the disease in a given environment, observation of clinical sign, information on grazing history, seasonal occurrence, and identification of snail habitats. Confirmatory diagnosis however, is based on demonstration of Fasciola species eggs through standard examination of feces in the laboratory, post mortem examination of infected animals. Even though it is impossible to detect Fasciola in live animals, liver examination at slaughter or necropsy is the most direct, reliable, and cost effective technique for the diagnosis of fasciolosis [10].

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 [11]. The only pathological and biochemical changes in liver infected with fluke on ruminants at ELFORA export abattoir in Bishoftu, Ethiopia, 10 km away East of our study area was done by Belina et al. However, Fasciolosis, its predisposing factors, evaluation of haematological and serum biochemical changes and complication subsequent infestations by fasciolosis; *Hepatic necrobacilisis* in cattle slaughtered at Gelan, Dukem and Akaki Manucipal abattoirs was not conducted so far.

The aim of this study was to evaluate the occurrences of Fasciolosis and liver abscesses succeeding in cattle at Gelan, Akaki and Dukem Municipal abattoirs and to isolate the *Fusobacterium necrophorum* subspecies *necrophorum*, and pathological lesions associated with the occurrence of the cases. The study also determined the association of isolated liver flukes and hematological and serum biochemical parameters in slaughtered cattle. We hypothesized that the lesions developed by fasciolosis would be proceeding by bacteria *Fusobacterium necroforum* and *abscessetions* would be the commonest lesions developed by bacteria.

Materials and Methods

Study area

The study was conducted in the areas of very near to Addis Ababa, Akaki, Dukem and Gelan Municipal Abattoir which is located 30 km 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. Once you leave the city of Addis Ababa borders, the first town you will come across will be Akaki-Gelan, and further East will be the town of Dukem. The area has an annual rainfall of 86.6 mm, of which 84% is in the long rainy season (June to September). The mean annual maximum and minimum temperatures are 26ºC and 14ºC, respectively, with mean relative humidity level of 61.3% national metrological service agency [12].

Study design and study population

A cross sectional study on bovine Fasciolosis and Hepatic necrobacilosis was conducted from October 2019 to June 2020 at Akaki, Gelan and Dukem Municipal abattoirs. Animals were included in the study using systematic random sampling method where only the first animal was chosen randomly. After such selection animals were grouped in to young and adult ages and moderate and excellent body condition scores [13]. Liver tissue and blood samples of the same animals were collected using systematic random sampling. The study animals comprised male indigenous Zebu cattle that were presented for slaughter from east and south-east areas of the country to Gelan, Dukem and Akaki Municipal abattoir. A total of 60 cattle were selected and examined following ante-mortem and post mortem inspection procedure.

Ante-mortem Inspection and post mortem examination

Each animal was identified based on the enumerate marks on its body tagged before slaughter and assessment of body condition was carried out using a modified method described by Nicholson and

Butterworth. Attention was given to the factors such as age, body condition, feeding sources 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. Then the livers were examined for presence of fasciollosis infestation and gross pathological changes. 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 hepatic necrobacillosis. Liver and bile duct were inspected for the presence of *Fasciola* species by applying pressure, palpation and incisions, of the routine internal organ inspection procedures. When the evidence of fascioliasis was found, they were classified as mature or immature and gross lesions were characterized following procedures reported by Sohair and Eman.

Accordingly the primary examination involves visualization and palpation of the organs; secondary examination involves more incision of liver; opening of bile duct and hepatic lymph nodes. For generalized liver fluke infestations (Fascioliosis), incision was made in different parts of the liver to check the presence of fluke in the parenchyma. The cut liver was pressed to squeeze out flukes from the tissue and smaller bile ducts. The gross pathological changes of hepatic lymph nodes as well as the lesion of liver abscesses and distribution of the lesion to hepatic lobes was also thoroughly examined and recorded according to Elshraway and Mahmoud. Then these were collected in universal bottles to identify the involved *Fasciola* species. Species identification of the recovered Fasciola was performed (based on the morphological feature of the parasite) and was classified into *Fasciola hepatica*, *Fasciola gigantica*, mixed and immature liver flukes [14].

Sample collection and processing

Blood sample was taken during ante mortem. During post-mortem, livers of the same animal were appropriately inspected for presence of *Fasciola* species and liver abscesses and gross liver pathology after slaughter. Based on gross pathological examination study animals were grouped into four: Group-A (showed no visible gross lesion (none infected groups), group-B (confirmed with *Fasciola*) and *Fasciola* indicative lesion and, group-C (co-infected) (Viscible adult *Fasciola*, *Fasciola* indicative lesion and Hepatic necrobacilosis) and group–D (presence of hepatic necrobacilosis only). The specimens for suspected hepatic necrobacillosis were collected from affected livers and transmitted to the laboratory under anaerobic conditions then it was added to the sterile petri-dish containing egg yolk agar for bacterial culturing. Gross and histopathological lesions and serum biochemical alterations was assessed. Liver tissue sample collections were done from the randomly selected cattle slaughtered in the abattoir. This is just 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 tissue samples were collected from different animals among those samples collected.

Tissue processing and examinations

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 [15]. Those parasites that appeared in the liver tissue were collected and recorded. At the same time presence of any pathological changes were recorded carefully [16].

After recording the gross changes, pieces (5 mm) of liver from the infected cattle were collected and fixed in 10% buffered formalin. Histopathology processes was done at the Animal Health Institute (AHI), Sebeta, Ethiopia. The fixed tissue sample was trimmed and processed in an automatic tissue processor in different chambers containing different alcohol concentrations (70%, 80%, 95% and 100%, 100%, 100%, 100%), cleared in xylene and embedded in paraffin for preparation of fine blocks. Blocks were sectioned at 5 µm. The sections were dewaxed, rehydrated and stained using Haematoxyline and Eosin (H and 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.

Haematological studies and serum biochemical analysis

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 eartag of animals and was taken to laboratory. 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.

Total protein determination: This is a quantitative measurement of the concentration of all proteins present in serum (note that this excludes clotting factors). The major proteins are albumin and the immunoglobulins (principally IgG, IgA and IgM). Total protein is measured in serum to give an indication of total immunoglobulin concentration since (total protein-albumin)=globulins of which the major component is immunoglobulins. Total protein is used in the 'liver function tests'; some chronic liver diseases cause increases in immunoglobulins, which increases total protein (though this may be offset by a decrease in albumin). The most widely used a method is the biuret reaction, in which an alkaline copper (II) solution reacts with peptide linkages to form a complex that absorbs light at wavelength 540 nm.

Serum biochemical analysis: Collection of blood was carried out from jugular vein of the animal and about 8 ml of blood, was taken and serum was separated after centrifugation at 3,000 rpm for 5 min and sera were transferred in to 2 ml of tubes and were stored at -20^{o}C until used. The serum total protein was measured using burette method. Analysis of samples was performed after bringing the samples to room temperature [17]. The sera samples were analyzed for ALT, AST and ALP commercially available respective enzyme

working reagent of the test kits, using humastar 80 chemistry analyzer at Fitsum clinic in Addis Ababa Sub-City.

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. The Sahli method is based on converting haemoglobin to acid haematin (brown colour) and then visually matching its colour against a solid glass standard. Diluted (0.1 N) hydrochloric acid is mixed into a graduated cylinder with 20 ul 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.

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. Haym's solution was suck up to 101 marks shifting the blood from the stem to the bulb of the RBC pipette. Mechanically the pipette was shaken thoroughly by holding the pipette in between the index finger and thumb. On a clean Neubar haemocytometer counting chamber, a drop of diluted blood was placed. 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.

Total Leucocyte Count (TLC): TLC was also determined by taking the fresh blood up to 0.5 levels in a WBC diluting pipette. Diluted 0.1 N hydrochloric acid was suck up to 11 marks shifting the blood from the stem to the bulb of the WBC pipette. Mechanically the pipette was rotated gently by holding the pipette in between the index finger and thumb. On a clean Neubar counting chamber, a drop of diluted blood was placed. The cells were stabilized for 1-2 minutes and total white blood cells in each mm area was counted under low magnification (10x); and the total white blood cells were determined by manual method using haemocytometer according to the procedures set by Dein.

Packed Cell Volume (PCV): PCV was measured using microhaematocrit reader from microhaematocrit (75 mm × 16 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.

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 by calculations used by Jain.

Indole test

Sterilized test tubes containing 4 ml of tryptophan broth was taken and the growth culture incubated for 24 hrs in the tube was aseptically inoculated. In addition, the tube was incubated at 37ºC for 24 hrs. 0.5 ml Kovac's reagent was added to the broth culture. The presence or absence of ring was observed following the procedures previously used by Josue et al.

Gram's staining

Gram's staining was done by preparing a smear and heated gently to fix and the slide was flooded with 0.5% crystal violet and leave for 30 sec. The slide was tilted and rinsed gently with water. The slide was flooded on sufficient (1%) (Gram's iodine) to rinse off excess water and covered with fresh iodine and allow to remain for 30 sec and the slide was tilted to wash off the iodine with water. Decolourization was done with 95% ethanol until colour ceases to run out of the smear. The slide was rinsed with water and was flooded with 0.1% counterstain safranin and leave to act for about 30. It was washed briefly with water and blotted to dry and the slide was examined using an oil immersion (100x) objective to observe cell morphology and gram reaction [18].

Microbiological culture

The specimens for hepatic necrobacillosis were collected from affected livers and transmitted to the laboratory under anaerobic conditions. Then 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 37ºC. *Fusobacterium* egg yolk agar is a new medium selective for *Fusobacterium* species and differential for *Fusobacterium necrophorum*. The medium contains vancomycin, neomycin, josamycin, and egg yolk. All species of fusobacteria grew with only minimal inhibition. Most other organisms were significantly inhibited by the medium [19].

Metylene Red (MR) test: The MR test detects the production of sufficient acid during the fermentation of glucose and the maintenance of conditions such that the pH of an old culture is sustained below a value of about 4.5, as shown by a change in the color of the methyl red indicator which is added at the end of the period of incubation. Some bacteria have the ability to utilize glucose and convert it to a stable acid like lactic acid, acetic acid or formic acid as the end product. The medium was allowed to equilibrate at room temperature. Pure culture was inoculated in medium and incubated aerobically at 37ºC for 24 hour and after 24 hour 1 ml of broth was added to clean and sterilized test tube and re-incubated for 24 hours. After incubation 3 drops of methyl red was added to a liquot following procedures by Athavale et al. and color change was observed [20].

Financial loss analysis

The total financial loss incurred due to fasciolosis and hepatic necrobacilosis in Akaki, Gelan and Dukem municipal abattoir was estimated based on liver condemnation. The economic loss due to liver condemnation was estimated through interview made with local butcher men in these selected towns and the average price of each cattle liver was calculated to be 350.00 Ethiopian Birr. The direct loss was thus calculated according to the formula adopted by Ogunrinade. Using the market price of a bovine liver, the monetary loss occasioned by condemnation of

Fasciola and hepatic necrobacilosis infected livers was calculated as follows: EL=ΣCS X Coy X Roz; where:

EL: Annual loss estimated due to liver condemnation.

ΣCS: Annual slaughter rates at the abattoir (estimated from retrospective abattoir record).

Coy: Average cost of each cattle liver.

Roz: Condemnation rates of cattle liver due to fasciolosis and hepatic necrobacilosis.

Statistical data analysis

The data collected was entered in to Microsoft excel spreadsheets and analyzed using STATA version 14 statistical software's. 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 *Chi-square (χ^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.

Results

The current study showed that the prevalence of cattle liver infected by fasciolosis and *Fusabacterium necrophorum* shows 41.6% (25/60). From these results liver lesions infected with only *fasciolla* species was 13.3% (8/60); where 6.6% (4/60) *Fasciolla hepatica*, 3.3% (2/60) *Fasciolla giantica*, 3.3% (2/60) mixed infection of *Fasciolla gigantica* and *Fasciolla hepatica*. The lesions having only *Fusabacterium necrophorum* (liver abscesses) result shows that 8% (5/60) and mixed infection of Fasciolosis and *hepatic necrobacilosis* was 20% (12/60) (Table 1).

	Fasciola infected			Infected by liver abscess	Infected by both liverfluke and liver abscess	Total
	Fasciolla hepatica	*Fasciolla Gigantica*	Mixed Infection *F. hepatica* and *F. gigantica*			
Idenfied lesions	4	2	2	5	12	25
%	6.6	3.3	3.3	8	20	41.6

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

Gross pathology lesion characterization

Gross lesions found at postmortem include firm, pale, swollen and irregularly outlined liver with tough texture. When a section of the bile duct was cut through, there was aberrant migration of flukes and in many cases, calcification was observed. Thickened, distended bile ducts containing adult flukes, decomposed materials and cholangitis were also observed at post mortem. Hepatic necrobacilosis and infection of *Fusobacterium necrophorum* were seen with multiple nodules varies in size which scattered on the hepatic surface as large nodules of about 0.75 cm-3 cm. The nodules were surrounded by thick membrane containing thick creamy, white-green pus, small abscess scattered in hepatic surface and within the parenchyma of liver. These nodules sized about 0.3 cm in diameter which filled with white-yellow pus. Hard, dark and brown color liver with multiple soft abscesses surrounded by hyperemic zone on the surface were noted and up on cutting section, a viscous yellow material oozed from the cut ends.

An enlarged liver with rounded edges and dark yellow nodules of two inches in diameter with a clay consistency are usually scattered throughout the organ. Sub-peritoneal emphysema, distension of the gall bladder with bile from pressure exerted by the nodules, necrotic foci in the pleura, diaphragm and heart were lesions seen at surrounding organs.

Histopathological results

In uninfected cattle's liver, Histopathological lesions showed normal hepatic cord arrangement and normal hepatic lobulation areas, with few infiltrations of inflammatory cells especially macrophages around the portal area, which associated with congestion in less notifiable hepatic sinusoids. The histopathological lesions of mixed infection of fasciolosis and *Fusobacterium necrophprum* composed of severely congested blood vessels in portal area, huge number of periportal infiltration of inflammatory cells especially macrophages and lymphocytes areas of vacuolar degeneration and coagulative necrosis around central vein with fibrosis in portal areas and hyperplasia of bile ducts showed a finger like projection into lumen leads to stoppage of bile pigment flow. The necrotic lesions emphasized by deeply eosinophilic cytoplasm with karyorhexis and karyolysis of their nuclei.

Moreover, the degenerative changes manifested by vacuolation of the hepatocytes particularly around central vein. Hyperplasia of the biliary epithelium where the epithelial lining of the bile ducts thrown as papillomatous projections into the lumen were also seen.

The histopathological examination of abscessed livers have been revealed the lesion was characterized by abscess surrounded by the pyogenic membrane as well as marked neutrophils infiltration surrounded by the pyogenic membrane consisting from proliferation of fibrous connective tissue with neutrophils in the lumen of blood vessels. Abscess with liquefactive necrosis showed in its center surrounded by marked thickness pyogenic membrane. The most important liver tissue alterations consisted of increased fibrous connective tissues proliferation within the portal triads with concentric arrangement

(portal cirrhosis), around hepatic blood vessels which revealed vacuolation of its muscular layer and around small biliary cirrhosis were. Necrosis and desquamation of the epithelial lining of the bile ducts were evident in many instances and large abscess in hepatic parenchyma.

Biochemical analysis result

The results were obtained by measurement of the concentration of all proteins present in serum excluding clotting factors. Many other proteins are included in the measurement but individually none contributes more than 5% of the total, and most much less (Tables 2 and 3).

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 2. Comparisons of serum biochemical analysis in fasciolosis and liver abscess in infected and non-infected liver of cattle.

Age	Status	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/dl)	Albumin (g/dl)
Young	Infected	82.44 ± 4.65	36.81 ± 9.22	112.97 ± 11.76	4.76	4.9
	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

Table 3. Serum liver enzymes mixed infection of Fasciolosis and *Fusobacterium necrophorum* and non-infected cattle's in age wise comparisons.

Haematological profile

The mean values of the haematological profiles determined for fasciolosis and hepatic necrobacilosis infected and non-infected liver of cattle are present in Table 4. 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 5).

Hematological parameters	Infected	Uninfcted	P value
Hgb (g /dl)	6.94	10.94	0.0043
PCV (%)	22.1	35.54	0.0127
RBC (cells/mm^3) × 10^6	44.3*10^6	59.3*10^6	0.0338
WBC (cells/mm^3) ×10^5	10.27*10^5	9.3*10^5	0.0357

Table 4. Hematological profile of cattle slaughtered at sellected abattoirs infected by Fasciolosis 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	0	0	0

Table 5. The average values of the differential leukocyte count of fasciola and *Fusobacterium necrophorum* infected and the uninfected cattle.

Indole test

The teat was performed by a chain of a number of different intracellular enzymes, a system generally referred to as "tryptophanase. Sterilized test tubes containing 4 ml of tryptophan broth was taken and the growth culture from 18 to 24 hrs in the tube aseptically inoculated. The tube was incubated at 37ºC for 24 hrs-28 hrs. 0.5 ml Kovac's reagent was added to the broth culture. 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.

Gram's staining

The staining of smears by gram stain have been showed the characteristic form of *Fusobacterium necrophorum* it was observed as gram-negative, long, non-branching filamentous pleomorphic bacillus with parallel sides and blunt or tapering ends.

Microbiological culturing

Fusobacterium necrophorum isolation from liver abscess incubated for 48 hours anaerobically at 37ºC. The colony of *Fusobacterium necrophorum,* sub-necrophorum were appeared as round, grey, and shiny in appearance have diameter 1-5 µm. The biochemical test to isolate of *Fusobacterium necrophorum* was similar to standard procedure.

Methyl Red (MR test)

Adding 3 drops of 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 *Fusobacterium necrophorum* and was MR negative.

Occurrence of liver flukes and associated risk factors

The result of present study showed that origin has significant effect on the prevalence of bovine fasciolosis; being higher in those came from wolayita, South Nations and Nationalities of People of Ethiopia (Table 6). Younger animals were highly infected by fasciollosis and hepatic necrobascilosis. Moderete body conditioned animals were more affected than excellent body conditioned animals (Table 7).

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 6. Occurrences of bovine fasciolosis and hepatic necrobacilosis based on origin of the animals.

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	0.000105
	Adult	25	8	

Table 7. Prevalence of liver by only fluke in age and body condition score among cattle.

Financial loss analysis

The economic significance of fasciolosis and *Fusobacterium necrophorum* was analysed based on the information obtained during postmortem examination 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's and the mean retail price of bovine liver in selected towns was 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 is=1747200 ETB and the annual loss due to liver condemnation=ΣCS × Coy × Roz=14000 × 300 × 41.6%=Ethiopian Birr ($56361.3s).

Discussion

In this study, sixty (60) bovine livers were examined and 41.3% (25/60) of liver samples showed gross and histopathologica lesions of Fasciola species and *Fusobacterium necrophorum* infection. All other studies strongly implicated both *Fasciola hepatica* and *Fasciola gigantica* as well as bacteriological studies as causative agent of liver abscess and Fasciolosis in cattle which is in agreement with the report of Sohair et al. This may be attributed to that bacteria were acquired by the flukes in the small intestine of ruminants and during migration that may suggest that the flukes can spread highly pathogenic bacteria.

The result of mixed infection of fasciolosis and Hepatic necrobacilosis in present study were inagreement with the report of Al-Mahmood and Al-Sabaawy that they were recorded about 4% of fasciolosis. The difference in these results occurred due to difference in climate, sample size and study methodology.

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 study conducted in Karbala province of Iraq which shown the prevalence of liver abscess 32% by Yousif et al. and higher than 0.7% report by Hussein et al. in Asella, Ethiopia.

Fusobacterium necrophorum that was isolated as a single anaerobic bacteria evidence exists for pathogenic *Fusobacterium necrophorum* produces a potent leukotoxin, a fact that may explain some of these observations by Hofstad and Blood and Radostitis also stated that Fusobacterium necrophorum was considered to be one of the most common causes of hepatic abscesses.

Hepatic abscesses appeared in the current study in bovine liver infected with *Fusebacterium necrophorum* were consisted of homogenous structurless eosinophilic core surrounded by inflammatory cells mainly lymphocytes, histiocytes, eosinophiles and polymorphnucealr cells and bounded by fibrous connective tissues capsule. Similar results were observed by Darwish mentioned that there was a synergistic relationship between *Fusobacterium necrophorum* and its leukotoxin substance were antigenic to the hepatic tissue. The histopathological changes revealed cirrhosis which appeared as portal, multilobular, biliary, pericellular and Glissonian's according to the fibrous connective tissues distribution. The same findings was also reported by Sayed et al. The biliary cirrhosis associated with papillomatous protrusions of the epithelial lining of the intrahepatic bile ducts with hyperplastic proliferation of the ductul epithelium and newly formed bile ductules in adenomatous arrangement were observed in this results. These findings were agreed with Sayed et al. Darwish from liver of camel. The hyperplasia of the bile ducts is an attempt to regenerate hepatic parenchyma when the parenchymal cells have lost their capacity to regenerate themselves. Also, Popper and Hutterer mentioned that the hyperplasia of the ductul epithelium occurs as a result of fluke toxic products which cause changing in the structural integrity of the ductul cells in non-specific and potentially destructive manner.

The ductul epithelium revealed necrosis and desquamation with presence of mature worms within its lumina were appeared in the current study. These findings were attributed to the effect of toxic products elaborated by *fasciola* worms repoted by Sayed et al. also reported that invasion of the liver by migrating immature liverfluke damages the tissue and results in reduction of the oxygen tension (anaerobic condition), that allowed the germination and proliferation of *Fusobacterium necrophorum* with release of its toxins and induce hepatocellular necrosis.

Gross examination of *fasciola* infested livers revealed that the liver were hard, firm and tough in consistency and the cut section showed large whitish areas of fibrosis while the affected ducts were thickened, enlarged and cordlike in structure. Similar findings were observed by Jones et al. and Sayed et al. The results of histopathological changes of liver abscess was revealed that the abscess surrounded by the pyogenic membrane, marked neutrophils infiltration and RBCs replacement sinusoids surrounded by pyogenic membrane consisting from proliferation of fibrous connective tissue. These changes were very similar to findings described by Ahmed and Mohammed. Also, agreed with Raji et al. who reported the histopathological examination for 268 abscessed livers showed well-circumscribed area of liquifactive necrosis surrounded by leucocyte infiltration and fibrosis.

The result of the current study showed that age has significant effect on the prevalence of bovine fasciolosis and hepatic necrobcilosis being higher in young animals than adult. There was a decrease in infection rate (prevalence) as age increased. This may be due to the result of acquired immunity with age which is manifested by humoral immune response and tissue reaction in bovine liver due to previous challenge that agree with report by Radostits et al. who reported as increased resistance against fasciolosis (low prevalence) with age is most likely related to the high level of tissue reaction seen in bovine liver. Liver fibrosis which impedes the passage of immature flukes acquired thickening, stenosis and calcification of bile ducts, assumed unfavorable site for adult parasites and consequently fasten their expulsion and which confirmed the occurrence of higher infection rate in younger animals.

The results of the present study indicated that body condition of the animal has significant association with the occurrence of fasciolosis. The prevalence was higher in moderate body condition animals than that of excelent body conditioned animals was.

The result indicated by heamatological examinations, PCV, Hb and RBC were lower in the infected live than in uninfected and on the other hand, the WBC (Eosinophilia, Nuetrophilia, Monocytopaenia and Lyphocytopaenia), were higher in the infected cattle than in un-infected. Total protein and albumin in this study shows decreament in infected cattle. ALT is present in high concentration in the cytoplasm of hepatocytes and is inagreament with the consideration of its liver specific in small animals and Camel. Its plasma concentration increases with hepatocellular damage/necrosis or degeneration and hepatocyte proliferation. The previous findings of Mbuh and Mbwaye, also detected the raise of ALT in fasciolosis may be due to hepatocyte destruction since ALT is primarily found in the liver parenchyma. On the other hand, the raise in mean value of ALT in this study may be due to hepatocyte death from liver fluke and *Fusobacterium necrophorum* infection causing complete or partial bile ducts obstruction and then returning of bilirubin to hepatocyte, which was agreement with report of Kilad et al. who found as increase in ALT may be due to hepatocyte death from liver fluke infection.

The total financial loss encountered due to condemnation of infested liver from one year data recorded from the three abattoirs in this study was 1,747,200 ETB per annum and this finding was higher than the results reported by Adem and Daniel, who reported a total financial loss of about 154,188 and 215,000 ETB per annum in cattle due to fasciolosis at Ziway and Dire Dawa municipal abattoir respectively. The variation of the results may be due to the ecological and climatic difference between the two localities.

Conclusion

In conclusion the present study was carried out to enumerate the gross and histopathological hepatic lesions induced by liver flukes infestation with complicated bateria and to detect the relationship between *Fusobacterium necrophorum* infections and liver fasciolosis. The study confirmed that fasciolosis and hepatic necrobacilosis is an important disease entity causing considerable loss of income due to condemnation of affected liver and carcass weight reduction at Akaki, Gelan and Dukem municipality abattoir. 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 financial losses and *Fusobacterium necrophorum* subspecies necrophorum, causes liver abscess in cattle. Generally understanding the type of liver lesions in case of fasciolosis and hepatic necrobacilosis will help in finding ways for the preventive and therapeutic measures, increasing roughage in the ration and multiple daily feedings increase the time of mastication and saliva flow which in turn lowers the number of ruminal lesions and, indirectly, the number of liver abscesses.

Ethics Approval and Consent to Participate

All animals were managed following standard ethical principles. Not applicable to obtain ethical certificate since we have collected only post-mortem samples.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available in the main text and from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

“WH, AF, and JS designed the study. WH performed the data collection and prepared the manuscript. JS, YH and DT analyzed the data. JS, AF and DT revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version”.

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