Hematobiochemical Alterations and Lesion Characterization Caused by Fasciolosis in Cattle Slaughtered at Gondar ELFORA Abattoir, Ethiopia

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Abatract

Fasciolosis is a neglected zoonotic parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, which presents significant threats to livestock and human health. An abattoir-based cross-sectional study was conducted from January to September 2023 at Gondar ELFORA abattoir, Ethiopia, to assess hematobiochemical alterations and lesion characterization caused by fasciolosis in slaughtered cattle. The study involved 100 apparently healthy male local breed cattle, evenly split into 50 infected and 50 non-infected individuals, using a purposive sampling technique. Findings showed reductions in HGB, PCV, TEC, lymphocytes, monocytes, total protein, albumin, and glucose levels in infected cattle. Elevated TLC, eosinophils, neutrophils, AST, ALT, and ALP levels were noted. Liver changes included hepatomegaly with juvenile flukes in acute cases and smaller, firmer livers with bile duct

engorgement in chronic cases. Microscopic findings revealed eosinophil infiltration, hemosiderin pigmentation, and fibrous tissue proliferation with epithelial cell metaplasia. The results of hematobiochemical alterations were consistent with gross and microscopic findings, indicating a significant impact on liver physiology and histology, leading to substantial losses in meat and milk production.

Keywords**:** *Abattoir; Cattle; Fasciolosis; Hematobiochemical alterations; Lesion characterization*

1. Introduction

Fasciolosis is a neglected zoonotic parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, which presents significant threats to livestock and human health [43]. Fasciola, commonly known as liver fluke, induces acute and chronic inflammation of the liver. Subsequent symptoms include submandibular edema and cirrhosis, leading to weight loss, anemia, hypoproteinemia, and anorexia. The larger species, *F. gigantica*, particularly suppresses the host's immune system, exacerbating its pathogenicity and lethality [6, 42]. All mammals can be infected by these species, but cattle are particularly vulnerable to these parasites [7].

Acute fasciolosis involves immature flukes causing severe liver damage, hemorrhage, and fibrinous deposits before migrating to bile ducts. Chronic phases feature fluke settling in bile ducts, causing anemia, hypoproteinemia, and cirrhosis, with ducts often enlarging, becoming cystic, and calcifying in cattle [12]. Hematological assessments are vital for diagnosing systemic diseases [33]. Fasciolosis impairs liver metabolic functions, altering biochemical markers like glucose, liver enzymes, and serum proteins due to hepatocyte damage [17, 41]. Evaluating these alterations is crucial for assessing hepatic damage caused by the disease [1, 9, 17, 41].

Abattoirs provide essential epidemiological data on diseases through meat inspection procedures, including antemortem and postmortem examinations. Backyard settings in Ethiopia are significant sites for slaughter [27].

Recent studies emphasized gross pathological examination to characterize lesions and quantify economic losses from fasciolosis [13, 26]. Integrative studies linking hematobiochemical changes with lesion characterization in bovine fasciolosis are limited [23]. Therefore, the present study was conducted to characterize gross and microscopic lesions in cattle infected with fasciolosis and to determine and compare hematobiochemical profiles between Fasciola-infected and non-infected cattle.

2. Materials and Methods

2.1. Study area

The study was conducted from January to September 2023 at Gondar ELFORA abattoir, a privately owned facility for sheep, goats, and cattle in Gondar, the capital of the central Gondar administrative zone in the Amhara regional state. Gondar is located at latitude 12° 36' N and longitude 37° 28' E, with an altitude ranging from 1800 to 2220 meters above sea level, is approximately 727 km from Addis Ababa. The area receives 880-1172 mm of annual rainfall, mostly during the wet season from September to May. The average temperature is 20.3°C with 55.7% relative humidity [10]. The abattoir, which primarily serves the local market and University of Gondar, slaughters 25-30 cattle weekly from various districts within the central Gondar zone.

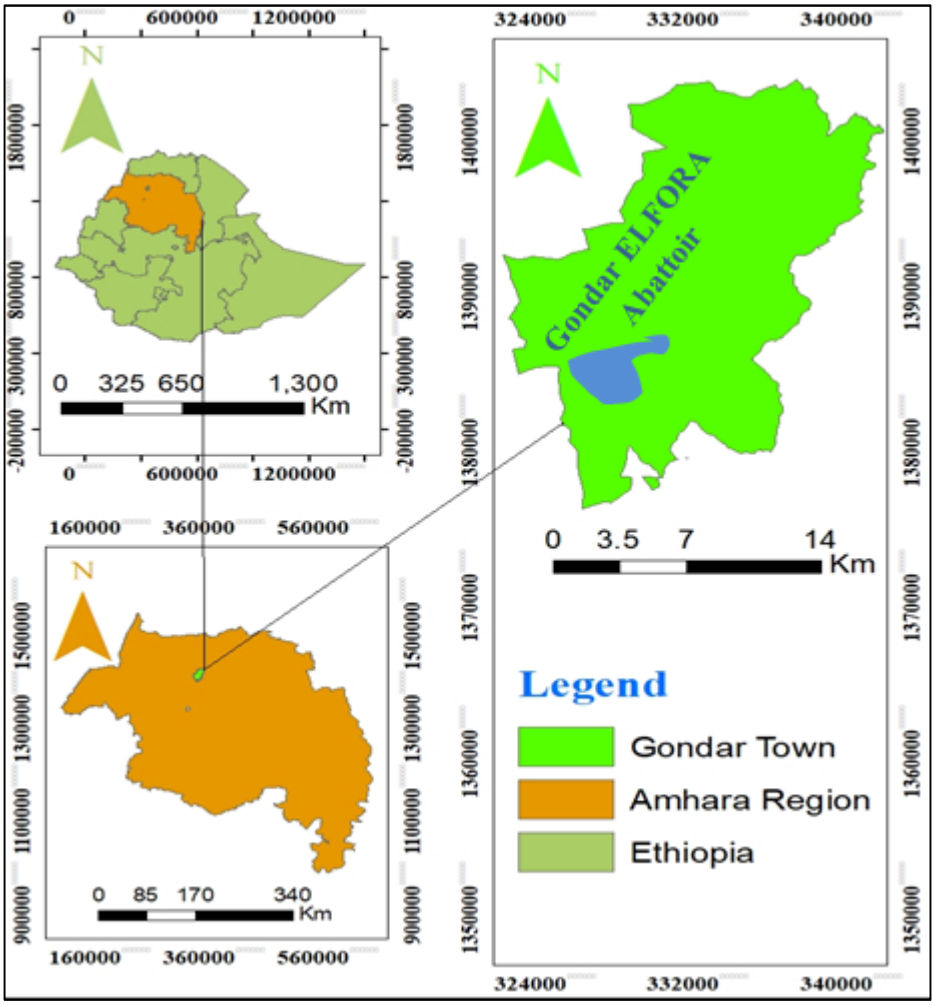


Figure 1: Map of the study area

2.2. Study animal

The study animals were male, apparently healthy, local breed cattle brought for slaughter for human consumption at Gondar ELFORA abattoir. These cattle were raised in an extensive farming system, transported from their origin to the abattoir by trucks, and kept in lairage for one day.

2.3. Study design and sampling method

An abattoir-based cross-sectional study was conducted from January to September 2023 to assess the hematobiochemical alterations and lesion characterization caused by fasciolosis in cattle slaughtered at the Gondar ELFORA abattoir. A total of one hundred male, apparently healthy, local breed cattle (50 fasciola-infected and 50 non-infected) were selected from the abattoir using the purposive sampling method.

2.4. Antemortem and postmortem inspection

Cattle brought for slaughter were subjected to antemortem inspection in the abattoir during the study period. Blood samples were collected for hematology and biochemical analysis, and fecal samples were taken for furthers examination. After postmortem inspection, blood samples were categorized into infected and control groups. Postmortem inspection involved visual inspection, palpation, and systematic incision of the liver, bile ducts, and gallbladder. Livers with fasciolosis lesions and liver flukes were selected for histopathological examination, and bile samples were collected. Fasciola specimens from bile ducts and gallbladders were harvested in normal saline as described by [21].

2.5. Sample collection and transportation

A total of 200 blood samples were collected from cattle: 100 from fasciola-infected animals (50 for hematology and 50 for serum biochemical analysis) and 100 from non-infected controls (50 for hematology and 50 for serum biochemical analysis). Each blood sample, comprising 5 mL, was drawn from the jugular vein following antemortem inspection. Additionally, 20 liver tissue samples were collected from 50 fasciola-infected cattle after postmortem examination. Blood for hematological analysis was collected in EDTA-coated tubes, while serum samples were collected in plain tubes and kept on ice packs. Liver tissue samples showing gross lesions were fixed in 10% neutral buffered formalin for histopathological examination following the method described by [5]. Finally, all samples were transported to the veterinary pathology laboratory at the University of Gondar.

2.6. Sample processing

2.6.1. Hematobiochemical analysis

Hematology analysis utilized EDTA-coated blood samples stored at +4°C. Parameters including PCV, HGB, TEC, TLC, DLC, and calculated values MCV, MCH, and MCHC were measured using a Sysmex automated hematology analyzer. For biochemical analysis, anticoagulant-free blood samples were allowed to clot and serum was stored at -20°C. Serum levels of ALT/GPT, AST/GOT, ALP, total protein, albumin, and glucose were analyzed using a clinical chemistry analyzer and commercial test kits from Human GmbH (Wiesbaden, Germany).

2.6.2. Histopathology examination

Tissue samples were processed by dehydration in alcohol, clearing with xylene, and embedding in paraffin wax. Sections cut at five micrometers using a microtome were stained with hematoxylin and eosin (H&E), mounted with dibutyl phthalate xylene (DPX), and coverslipped. Slides were dried thoroughly and examined under a light microscope (10X to 100X magnification) to assess microscopic alterations; identify lesions, and record findings. Photographic documentation followed the method outlined by [5].

2.7. Analysis of data and statistical methods

The data obtained were arranged, checked, coded, and entered into an Excel spreadsheet (Microsoft Office Excel 2010). They were then analyzed using STATA version 16. Significant differences between the hematological and biochemical parameters of the Fasciola-infected and non-infected samples were determined by t-test. Differences were considered significant when $P<0.05$, and the results were expressed as means ± SE.

3. Results

3.1. Hematobiochemical findings

The mean hematology and serum biochemical parameters of Fasciola-infected and non-infected cattle are compared in Tables 1 and 2. Infected cattle showed lower mean values for hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), total erythrocyte count (TEC), lymphocytes, monocytes, total protein, albumin, and glucose. Conversely, they had higher mean values for mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), neutrophils,

eosinophils, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Basophil levels were similar in both groups. Non-infected cattle's parameters were within reference ranges. Significant differences (P<0.05) were found in HGB, PCV, MCV, TEC, TLC, lymphocytes, monocytes, neutrophils, eosinophils, AST, ALT, ALP, total protein, albumin, and glucose between infected and non-infected cattle, while MCH and MCHC showed no significant differences.

Table 1: Hematological parameters of fasciola-infected and non-infected cattle

Parameters	**Mean ± SE**		**Range**	**t-*value***	***P*-value**
	Infected (n=50)	**Non-infected (n=50)**			
HGB (g/dL) *	4.76 ± 0.18	9.76 ± 0.19	8-14	20.34	0.000
PCV (%) *	15.96 ± 0.62	34.98 ± 0.81	24-42	19.52	0.000
MCV (fL)*	49.10 ± 2.23	56.42 ± 1.59	40-60	2.87	0.005
MCH (pg)^{NS}	15.05 ± 0.88	15.78 ± 0.44	11-17	0.74	0.457
MCHC (%) ^{NS}	33.18 ± 2.23	28.89 ± 1.07	28-36	2.00	0.0509
TEC ($\times 10^6$/µL) *	3.37 ± 0.10	6.29 ± 0.11	5-10	18.17	0.000
TLC ($\times 10^3$/µL) *	14.35 ±0.18	6.41 ± 0.22	4-12	29.09	0.000
LYM ($\times 10^3$/µl) *	1.66 ± 0.05	3.17 ± 0.17	2.5-7.5	8.79	0.000
MON (x10^3/µl) *	0.019 ± 0.001	0.79 ± 0.08	0.02-0.85	9.60	0.000
NEU (x10^3/µl) *	4.35 ± 0.21	2.52 ± 0.14	0.6-4	8.33	0.000
EOS (x10^3/µl) *	3.25 ± 0.135	0.53 ±0.037	0-2.4	18.19	0.000
BAS (x10^3/µl) ^{NC}	0 ± 0	0 ± 0	0-0.2	-	-

The reference range adopted from [37]; HGB=Haemoglobin; PCV=Packed cell volume; TEC=Total erythrocyte count; MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration; TLC=Total leukocyte count, LYM=Lymphocytes; MON= Monocytes; NEU=Neutrophils; EOS=Eosinophils; BAS=Basophils, *Significant at *P*<0.05, ^{NS}Non significant at *P*<0.05, ^{NC}No change between groups.

Table 2: Biochemical parameters of fasciola-infected and non-infected cattle

Parameters	Mean ± SE		Range	t-value	*P*-value
	Infected (n=50)	Non-infected (n=50)			
AST (U/L) *	244.78 ± 5.63	108.52 ± 3.31	46-176	20.37	0.000
ALT (U/L) *	61.12 ±1.96	21.46 ± 0 .86	5-35	17.43	0.000
ALP (U/L) *	195.7 ± 3.11	69.14 ± 5.02	18-153	23.90	0.000
TP (g/dL) *	3.92 ± 0.17	6.51 ± 0.057	6-7.5	13.84	0.000
Albumin (g/dL) *	2.43 ± 0.086	3.79 ± 0 .035	3.4-4.3	14.03	0.000
Glucose (g/dL) *	25.14 ± 0.71	55.74 ± 1.42	37-79	19.22	0.000

The reference range adopted from [37]; ALT = Alanine aminotransferase; AST= Aspartate aminotransferase; ALP =Alkaline phosphatase; TP=Total protein, *Significant at $P<0.05$

3.2. Gross and microscopic lesions characteriztion

Gross Lesions

Acute case**:** The liver was enlarged (hepatomegaly) with rounded edges due to inflammation of the parenchymal layer, and pinpoint hemorrhages were present on the parietal surface. Some areas of the parenchyma were pale due to necrosis. The capsule was firm, rough, and thick with whitish or reddish discoloration within the parenchyma. A juvenile fluke was observed in the cut section (Figure 2A). There was also congestion and engorgement of the gall bladder with bile, and upon dissection, a blackish brown exudate was found. The mucosa was normal, and there were no flukes or eggs in it (Figure 2B).

Chronic case**:** The liver was small in size and firm in consistency with a corrugated capsule (Figure 3A). The bile duct was engorged (Figure 3B). Upon dissection, numerous mature and adult twisted flukes were found along with blackish brown exudate, giving the liver a pipe stem appearance. The gall bladder was decreased in size, and its mucosa was thick and filled with flukes and ova (Figure 3C). The flukes collected from the bile ducts and gall bladder were identified as *Fasciola hepatica* and *Fasciola gigantica* (Figure 3D).

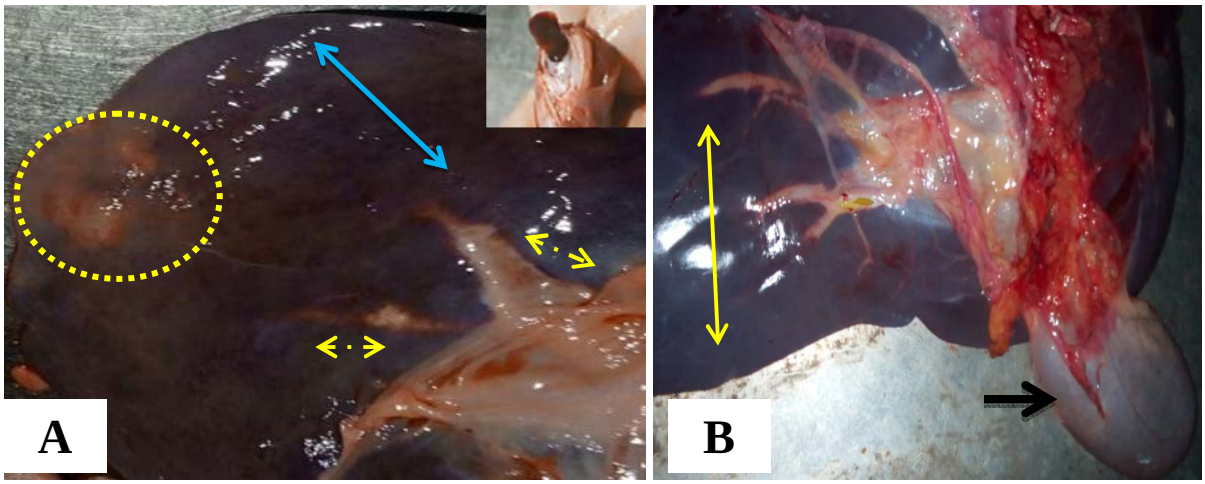


Figure 2: Gross lesion of acute fasciolosis: A) Hepatomegaly with rounded edges and pinpoint hemorrhages (blue arrows) on the parietal surface, paleness in some areas (dotted arrows), a firm, rough, and thick capsule with whitish or reddish discoloration within the parenchyma (dotted circle), and juvenile fluke (inset) seen at the cut section. (B) Congestion (yellow arrows) and engorgement of the gall bladder with bile (black arrow).

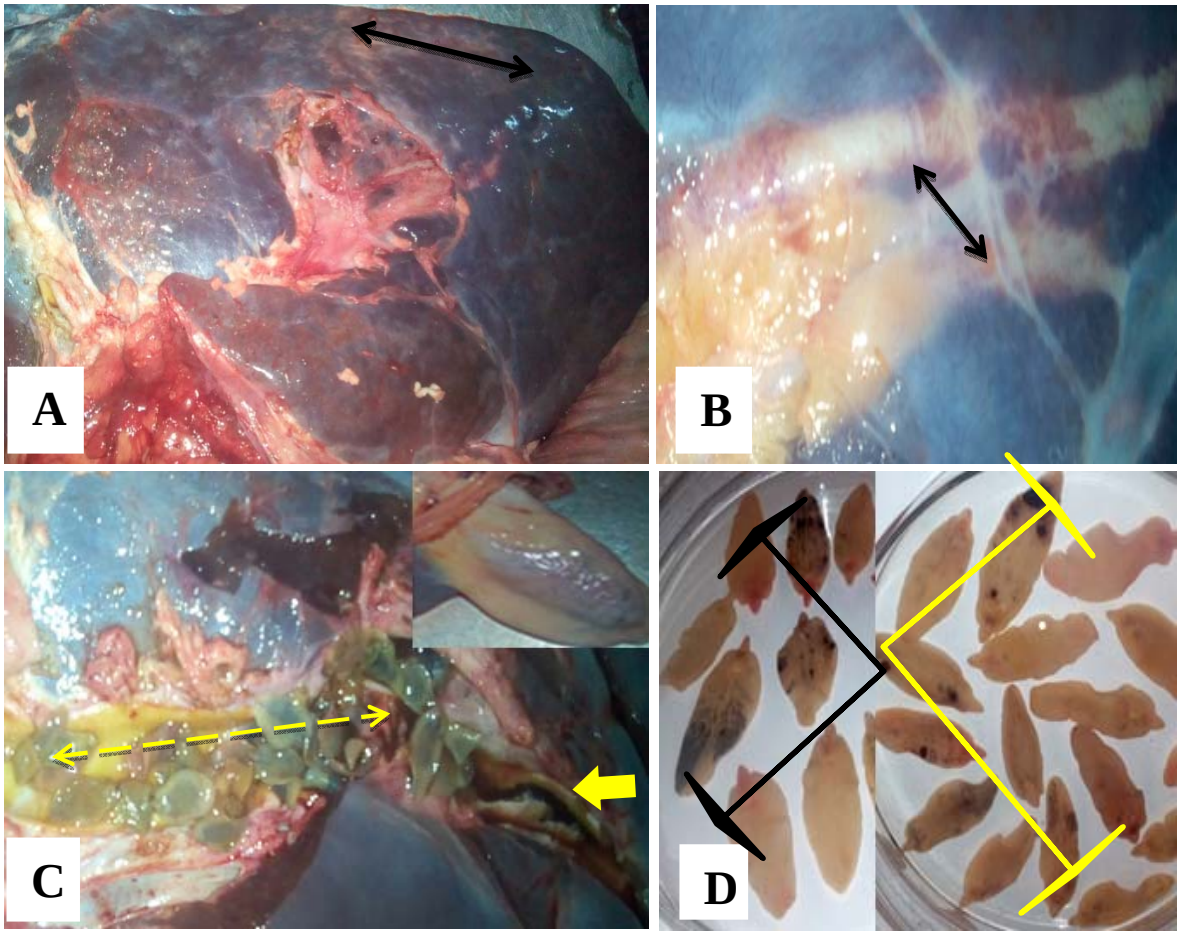
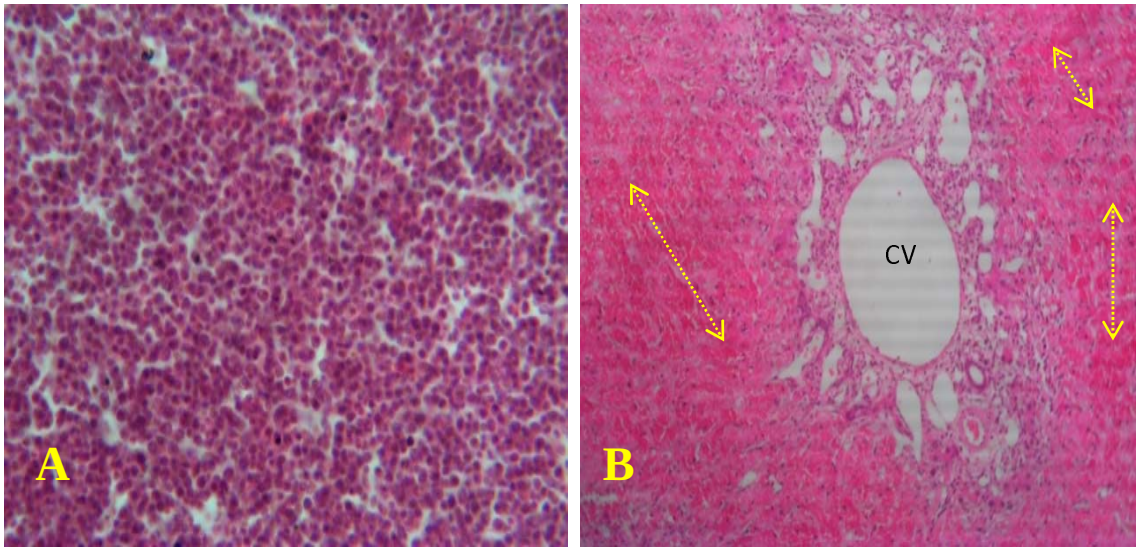


Figure 3: Gross lesion of chronic fasciolosis: (A) the liver was small in size and firm in consistency with a corrugated capsule (arrows). (B) Engorged bile ducts (arrows). (C) Numerous mature and immature twisted flukes (lined arrows) along with blackish-brown exudate (yellow arrow), and the gall bladder (inset) was decreased in size. (D) Identification of *F. hepatica* (black image) and *F. gigantica* (yellow image).

Microscopic Lesions

Acute case: Excessive eosinophil infiltration was observed in migratory tracts (Figure 4A), along with congestion around the central vein due to dilation of central vein and sinusoids engorged with a large number of RBCs (Figure 4B). Hemosiderin pigmentation was also noted (Figure 4C). A necrotic area surrounded by degenerating swollen hepatocytes and inflammatory cells, with RBCs engorgement, was identified (Figure 4D). Coagulative necrosis was observed in the migratory tracts, surrounded by large clear vacuoles, and accompanied by pyknosis, karyolysis, and karyorrhexis within the cytoplasm (Figure 4E). Swollen hepatocytes with collapsed cytoplasm, hyperplasia of fibrocytes, and congested blood vessels at the portal area were also recorded (Figure 4F).

Chronic case: Neutrophil infiltration was observed within the sinusoidal capillaries and among necrotic hepatocytes (Figure 5A). There was proliferation of fibrous connective tissues with fibrosis and hemorrhage (Figure 5B). Fatty changes were noted along with metaplasia of columnar epithelial cells and bile duct proliferation (Figure 5C). Metaplasia of columnar to cuboidal epithelial cells, along with metaplasia of columnar epithelial cells, was also observed (Figure 5D).



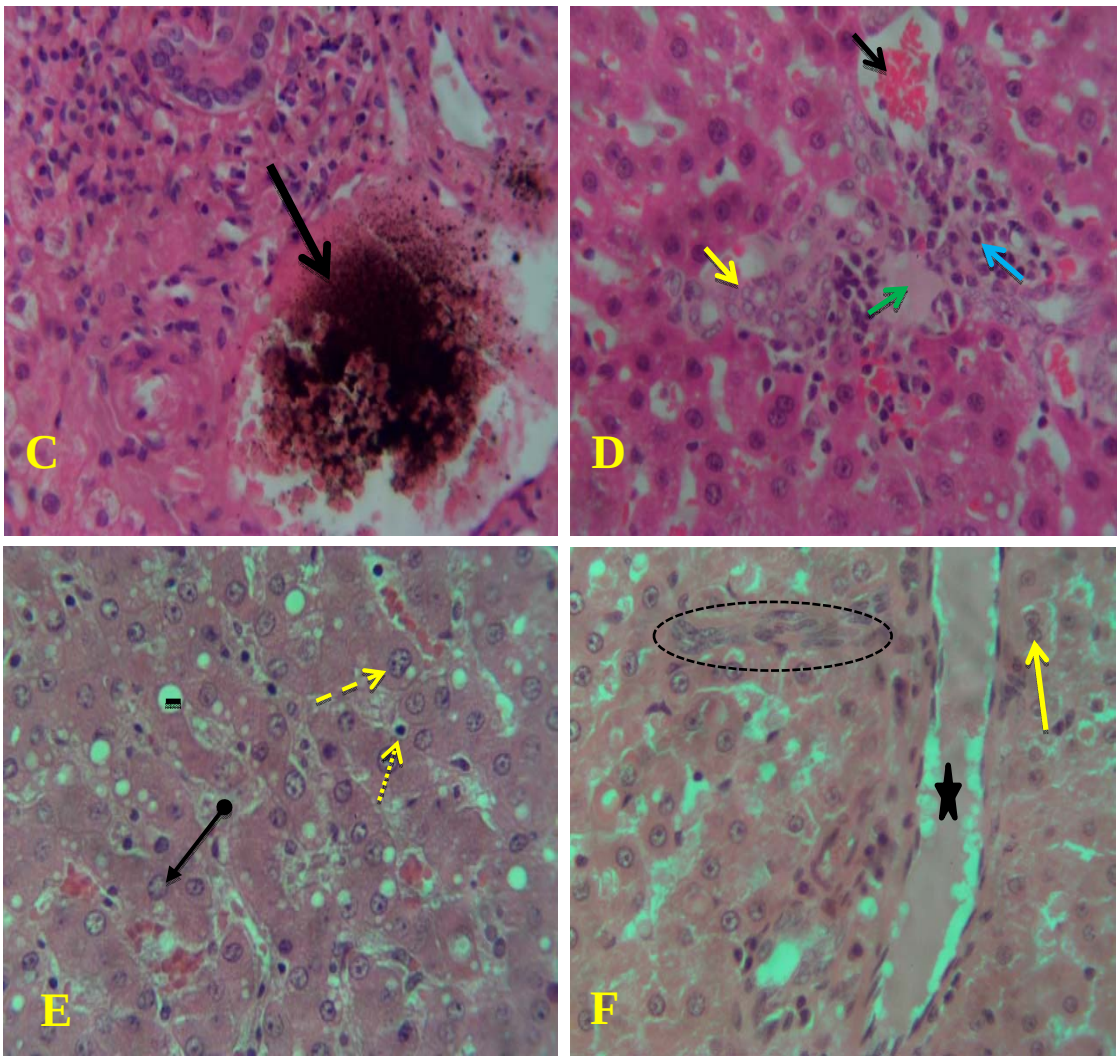


Figure 4: Microscopic lesion of acute fasciolosis: (A) Excessive eosinophil infiltration in migratory tracts. (B) Congestion around the central vein (CV) with engorgement of red blood cells (dotted arrows), 10X magnification. (C) Hemosiderin pigmentation (arrow). (D) Necrotic area (green arrow) with engorgement of red blood cells (black arrow), surrounded by degenerating swollen hepatocytes (yellow arrows) and inflammatory cells (blue arrow). (E) Coagulative necrosis (arrow tail) in the migratory tracts, surrounded by a large clear vacuole (line), pyknosis (dotted arrow), karyolysis (arrowhead), and karyorrhexis (lined arrow) within the cytoplasm. (F) Swollen hepatocytes with collapsed cytoplasm (arrow), hyperplasia of fibrocytes (circle), and congested blood vessels around the portal area (star). H&E. 40X.

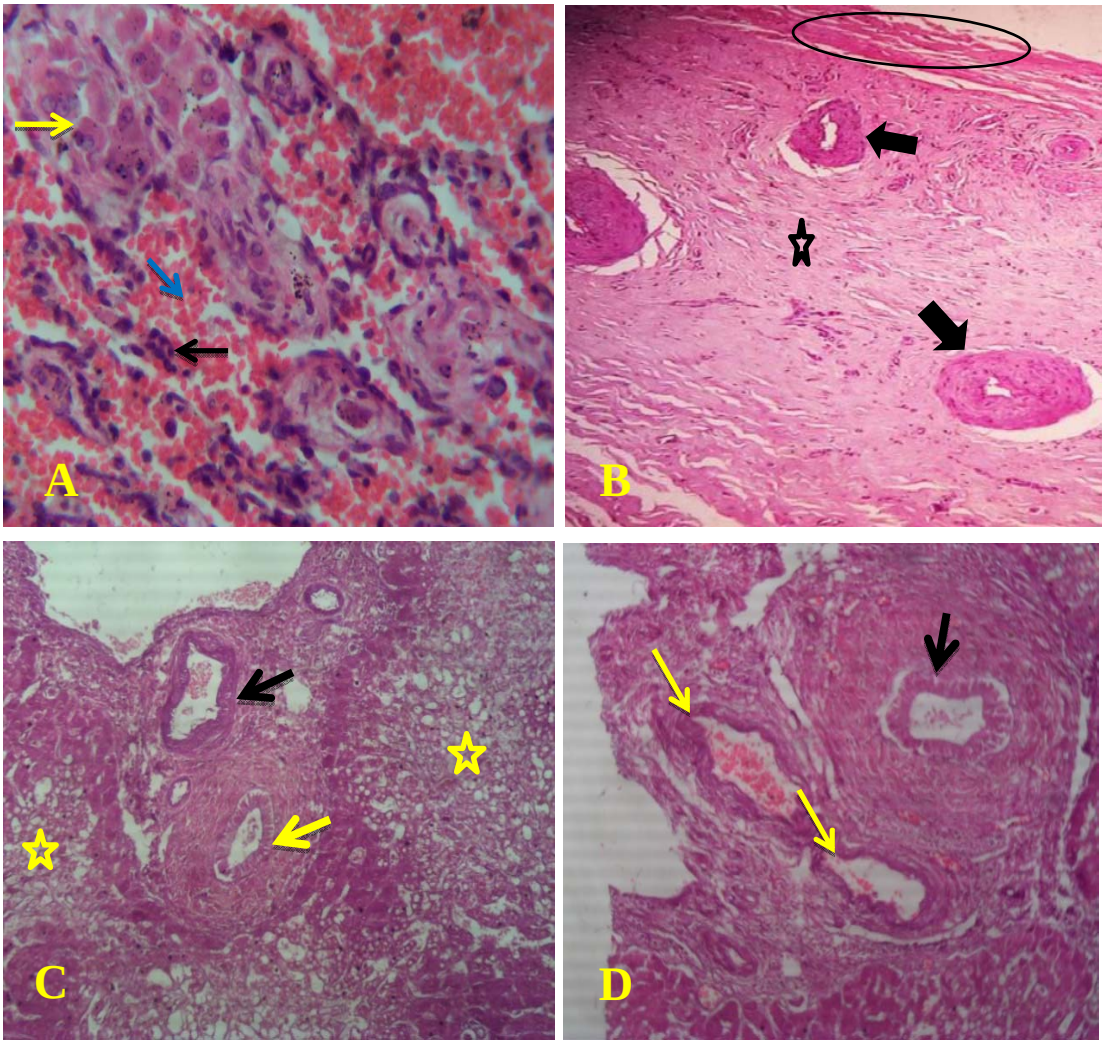


Figure 5: Microscopic lesion of chronic fasciolosis: (A) Neutrophil infiltration (black arrow) within sinusoidal capillaries (blue arrow) and among necrotic hepatocytes (yellow arrow). (B) Proliferation of fibrous connective tissues (arrows) with fibrosis (star) and hemorrhage (circle). (C) Fatty changes (stars) along with metaplasia of columnar epithelial cells (black arrow) and bile duct proliferation (yellow arrow). (D) Metaplasia of columnar to cuboidal epithelial cells (yellow arrows) and metaplasia of columnar epithelial cells (black arrow). H&E. 40X.

4. Discussion

The diagnosis of bovine fasciolosis was based on hematobiochemical findings and gross and microscopic lesions. Significant reductions were found in hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), total erythrocyte count (TEC), lymphocytes, monocytes, total protein, albumin, and glucose in Fasciola-infected cattle. Conversely, total leukocyte count (TLC), eosinophils, neutrophils, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and

alkaline phosphatase (ALP) were elevated. Basophil levels were similar in both infected and non-infected cattle, with the latter's parameters within reference ranges. These results align with findings from other studies [1, 9, 17, 19, 36].

Reductions in PCV (Packed Cell Volume), TEC (Total Erythrocyte Count), HGB (Hemoglobin), and MCV (Mean Corpuscular Volume), along with elevated MCHC (Mean Corpuscular Hemoglobin Concentration), indicate microcytic hyperchromic anemia likely due to blood loss from hemorrhages caused by migrating immature flukes and blood-sucking adult flukes, as noted by [9]. [24] also observed significant reductions in PCV, HGB, and TEC due to liver fluke infestation and related hepatic damage. Similar findings were reported by [9, 14, 17, 30, 40]. Additionally, [3, 25, 41] observed significant reductions in these values in Fasciola-infected sheep. The significant reduction in MCV, non-significant reduction in MCH, and elevated MCHC values align with [39], who found non-significant changes in these parameters in Fasciola-infected cattle. [41] reported significant reductions in MCV, MCH, and MCHC in infected sheep. [11] noted a non-significant reduction in MCV and elevations in MCH and MCHC in infected cattle. These differences may be due to ecological and nutritional factors affecting hematological profiles [41].

Elevations in TLC, eosinophils, and neutrophils in infected cattle indicate leukocytosis, eosinophilia, and neutrophilia, while reductions in lymphocytes and monocytes indicate lymphocytopenia and monocytopenia. These findings align with other studies [9, 14, 39]. [16] reported significant leukocytosis, neutropenia, and lymphocytopenia, with non-significant eosinophilia and monocytosis in infected sheep. [41] observed significant leukocytosis and eosinophilia, with non-significant neutropenia, lymphocytosis, and monocytopenia in sheep. [15] also found significant elevations in leukocytosis, eosinophilia, and neutrophilia in infected cattle. The elevation in TLC (Total Leukocyte Count) observed in response to combat liver flukes [24]. Eosinophilia which may arise from defense mechanisms against parasitic infections [41] or the release of cytotoxic molecules from the parasite's surface [32]. Neutrophilia, another common finding, can be attributed to secondary bacterial infections resulting from fluke migration through the biliary parenchyma [39] or the phagocytic activity of neutrophils degrading the parasite's cuticle. Additionally, lymphocytopenia observed in these infections could be associated with immune responses and the concurrent increase in neutrophil counts [22].

Elevations in AST, ALT, and ALP levels, alongside reductions in TP, albumin, and glucose levels observed in Fasciola-infected cattle, are indicative of liver damage caused by fluke migration. These findings are consistent with studies by [9] and [18], which highlight that increased AST and ALT levels result from inflammatory responses, fibrosis, and necrosis induced by the parasites. Elevated ALP levels, as reported by [9, 11], suggest cholestasis due to obstruction by flukes, further corroborating the

hepatobiliary pathology observed in Fasciola infection. Reduced protein and albumin levels, indicating hypoproteinemia and hypoalbuminemia, are likely attributed to hepatocyte damage caused by flukes, impairing normal protein synthesis. Decreased glucose levels, suggesting hypoglycemia due to disrupted hepatic glycogenic pathways by fluke migration, align with findings reported by [17, 19, 41].

In acute bovine fasciolosis, macroscopic changes included hepatomegaly, rounded liver edges with parenchymal inflammation, pinpoint hemorrhages, congestion, pale areas indicating necrosis, and a firm, rough capsule with discoloration within the parenchyma. Juvenile flukes were observed, consistent with previous studies [4, 8, 23, 28, 29, 31, 35] in various livestock species. An engorged gall bladder with bile and the presence of a blackish-brown exudate upon dissection align with previous studies [2, 20]. The absence of fluke eggs in both the gall bladder and fecal samples indicates an early stage of infection. These observations are consistent with reports that suggest variations in the manifestation of gall bladder and bile duct abnormalities could be influenced by breed differences [38].

In chronic fasciolosis, the liver appeared small and firm with a corrugated capsule. The bile duct was engorged; containing immature and adult twisted flukes with a blackish-brown exudate, giving the liver a pipe stem appearance. These findings align with previous studies in various livestock species [8, 20, 23, 29, 34]. The gall bladder showed thickened, edematous mucosa with flukes and eggs, contrasting with findings from [2] who noted thickened mucosa without mentioning size reduction. Reduced gall bladder size may stem from bile duct blockage by flukes, leading to impaired bile synthesis. Fluke eggs were confirmed in wet smear and fecal samples, aligning with their lifecycle, maturing within 10-12 weeks to lay eggs in the bile duct and feces [38].Identification of *F. gigantica* and *F. hepatica* in the gall bladder and bile duct was based on morphology [38].

Microscopic changes observed in acute fasciolosis include eosinophil infiltration, sinusoidal dilation with red blood cell (RBC) engorgement, hepatocyte necrosis, and hemosiderin deposition. In chronic fasciolosis, histopathological examinations consistently reveal several features across different species infected with Fasciola. These features include neutrophil infiltration in sinusoidal capillaries, necrotic hepatocytes, and fibrous tissue proliferation in bile ducts, fibrosis, fatty changes, and epithelial cell metaplasia. These observations are supported by other studies [5, 6, 8, 29, 34].

5. Conculution

This study assessed hematobiochemical, gross and microscopic lesion alterations caused by bovine fasciolosis. Significant differences were observed between fasciola-infected and non-infected cattle. Gross examination revealed juvenile flukes in acute cases and cholangitis with atrophic bladder in

chronic cases, likely due to secondary infection. Microscopically, acute cases showed eosinophil infiltration and congestion, while chronic cases exhibited fibrous tissue and bile duct proliferation, and epithelial metaplasia. Fecal and bile samples were also examined to support the findings. The results of hematobiochemical alterations were consistent with gross and microscopic findings, indicating a significant impact on liver physiology and histology, leading to substantial losses in meat and milk production.

Data sharing statement

All data generated or analyzed during this study are available upon request from the corresponding author.

Ethics statement

Ethics approval was granted by the University of Gondar's College of Veterinary Medicine and Animal Sciences Ethics Research Review Committee (Ref. No. CVMASc/UoG/RERC/20/12/2022) on December 20, 2022. Animal procedures followed ethical guidelines, and verbal informed consent was obtained from the Gondar ELFORA slaughterhouse manager, approved by the ethics committee before sample collection.

Acknowledgments

The author thanks the staff of the College of Veterinary Medicine and Animal Science, University of Gondar, for their unwavering support, and the staff of Gondar ELFORA slaughterhouse for their hospitality and invaluable assistance during sample collection.

Author contributions

ABT contributed to writing the original draft, reviewing and editing, funding acquisition, resourcing, methodology, visualization, validation, investigation, conceptualization, formal analysis, and data curation. TM and MB provided supervision, visualization and validation. MCK, MY, MY, and MMA were involved in visualization, validation, methodology and review editing. BA, AMG, AWT, KAS, ZGW, GKB, BAM, MGF, FE, MTY, GEK, and EM contributed to resourcing, review editing, visualization and validation.

Disclosure

The authors report no conflicts of interest in this work.

Funding

This research was funded by the University of Gondar to support the corresponding author's partial fulfillment of a Master of Science degree in Veterinary Pathology. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

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