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STUDY ON CLINICAL PATHOLOGY, GROSS AND HISTOPATHOLOGICAL ALTERATIONS CAUSED BY TREMATODE INFECTIONS AND THEIR ASSOCIATED RISK FACTORS IN RUMINANTS SLAUGHTERED AT THREE MUNICIPAL ABBATOIRS IN CENTRAL ETHIOPIA

MVSc THESIS

BY

ADISU WAKUMA BOKE

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

MVSc IN VETERINARY PATHOLGY

ACADEMIC ADVISOR: Jirata Shiferaw (DVM, MSc, Asst. Professor)

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JUNE, 2023

BISHOFTU, ETHIOPIA

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ADISU WAKUMA BOKE

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 Veterinary Science in Veterinary Pathology

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Thesis title: Study on Clinical Pathology, Gross and Histopathological Alterations Caused by Trematode Infections and Their Associated Risk Factors in Ruminants Slaughtered at Three Municipal Abattoirs in Central Ethiopia

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As members of the examining board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: Adisu Wakuma Boke entitled **"study on clinical pathology, gross and histopathological alterations caused by trematode infections and their associated risk factors in ruminants slaughtered at three municipal Abbatoirs in central Ethiopia**" and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Veterinary 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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DEDICATION

I dedicate this work to the omniscient God who has reared and nurtured me to this day. Until now, he has supported, loved, and assisted me. My lovely wife Wasane Adisu and my son Wabi Adisu, for their love, patience, moral support, and tolerance during my entire thesis work period.

STATEMENT OF AUTHOR

Firstly, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are permissible without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or imitation of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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LIST OF ABBREVIATIONS

AHI	Animal Health Institute
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CDC	Central Disease Control
DPDx	Centers for Disease Control and Prevention
DPX	Dibutyl Phthalate Xylene
CSA	Central Statistical Agency
DLC	Differential Leucocyte Count
FH	Final Host
GDP	Gross Domestic Product
Hb	Hemoglobin
MCV	Mean Corpuscular Volume \
MCH	Mean Corpuscular hemoglobin
MCHC	Mean Corpuscular hemoglobin
Ml	Milliliter
^{O}C	Degree Centigrade
PCV	Packed Cell Volume
RBC	Red Blood Cell
TEC	Total Erythrocyte Count
TLC	Total Leucocyte Count
WBC	White Blood Cell

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ABSTRACT

A cross-sectional study was conducted to study clinical pathology, gross and histopathological alterations Caused by trematode infections and their associated risk factors in ruminants slaughtered from November 2022 to June 2023 at three municipal abattoirs in central Ethiopia. Thorough ante mortem and post mortem inspection was undertaken in the selected abattoirs. For this purpose, a total of 256 ruminants were involved in the study through systemic random sampling techniques and d analyzed for trematode parasite infestations. From the selected animals, 137 bovines, 64 ovines, and 55 caprines, were analyzed for trematodes parasites. The overall prevalence of ruminant fasciolosis and paramphistomosis was 32.42% (83/256) and 43 (16.80%), respectively. There was no statistically significant variation in the prevalence of ruminant fasciolosis between the different species of animals, but there was a significant association between paramphistomosis and animal species. The prevalence of fasciolosis was higher in caprine (41.82% (23/55) and lower in bovine (29.50%) (40/137), followed by ovine (31.25% (20/64) and was higher in young animals (45, 40.54%) than in adult animals (38, 26.21%), but the prevalence of paramphistomosis in young animals was lower (17, 15.32%) than in the adult group (26, 17.93%). The association of Fasciola prevalence and ages showed that there was a significant difference (p 0.05). The infection rates of fasciolosis and paramphistomosis for ruminants with poor body condition were 47.62% and 28.57%, while for medium body condition they were 32.81% and 28.97%, and for good body condition they were 28.97% and 15.89%, respectively. Based on ruminant origin, Jima (52.63%), and Dukem (22.50%) had the greatest rates of fasciolosis and paramphistomosis, respectively; but lowest in Adama (11.76%) for fasciolosis. Fasciola-infected ruminants show histopathological changes including hemorrhages, hepatocytic wall dilatation, necrosis, hypertrophy, and portal fibrosis, while paraphistomes-affected animals show muscular degeneration, loose of villi and microvilli. Hematological assay results show lower Packed cell volume, Hemoglobin, White Blood Cell, and Red Blood Cell counts and higher liver enzymes, while total protein and albumin are lower in infected animals. The study showed a high prevalence of fasciolosis and paramphistomosis in the study areas. Therefore, Further study is needed on trematode infection, its epidemiological distribution, snail intermediate hosts, and strategic measures for effective control options.

Keywords: *Abattoir, Cattle, Clinical Pathology, Ethiopia, Goat, Lesion, Sheep, Trematode*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, with 70 million cattle, 42 million sheep, 52 million goats, 8 million camels, and 56 million chickens (CSA, 2021). Ruminants have made a significant contribution to Ethiopia's national economy and the livelihoods of many Ethiopians, and they continue to hold the promise of truly completing the country's economic development (Tadele and Worku, 2007). This sector contributed up to 40% of agricultural Gross Domestic Product (GDP), nearly 20% of total GDP, and 20% of national foreign exchange earnings of Ethiopia (World Bank, 2017). Recent estimates showed that 97.8%, 1.9%, and 0.3% of cattle are indigenous, hybrid, and exotic breeds, respectively. The estimates for sheep are 99.6% and 0.3% for local breeds and hybrids, respectively. Nearly all goats (99.9%) are indigenous breeds (CSA, 2020a).

Despite a large population of cows, sheep, and goats and ideal climatic conditions, the country's current output is low. This is due to a variety of complex and interconnected factors, including widespread diseases such as helminths, insufficient feed and nutrition, poor genetic potential of local breeds, market issues, and inefficiencies in livestock development services such as credit, extension, marketing, and infrastructure (Benin *et al*.,2003; Negassa *et al.* 2011).

Helminth parasites belong to two phyla especially Platyhelminthes (flatworms) and Nemathelminths (roundworms). The two classes of parasitic level worms, the Trematoda and the Cestoda, are found in the phylum Platyhelminthes. There are two types of Trematodes: digenes and monogenes. Monogenetic trematodes have direct life cycle and are the primary ectoparasite of aquatic vertebrates. Digenetic trematode has indirect life cycle and are endoparasites of a wide verity of vertebrate species (Ballweber, 2001).

Trematodes are a diverse group of parasites affecting both animals and humans' health worldwide (Bogitsh *et al*, 2019). The Digenea is one of the largest groups of Platyhelminthes and parasitizes a wide range of invertebrate and vertebrate hosts which also includes humans. Within the vertebrate, final host, these worms are found in numerous organs, including the intestine, lungs, liver, and vascular system (Walz *et al.*, 2015, Qian *et al.*, 2016).

The families that include parasites of significant veterinary importance are Fasciolidae, Dicrocoelidae, Paramphistomatidae, and Schistosomatidae. Adult trematodes are commonly referred to as "flukes" (Kassaye and Hana, 2019).

One of the most common parasitic diseases in domestic animals is fasciolosis, which primarily affects cattle, sheep, and goats as well as, very rarely, humans. The two species most frequently implicated as the etiological agents of fasciolosis are *Fasciola hepatica* (*F. hepatica*) and *Fasciola gigantica* (*F. gigantica*). In Europe, the Americas, and Oceania, only *F. hepatica* poses a threat, but in many regions of Africa and Asia, both species' populations coexist (Khaled *et al*.,2010). Fasciolosis is a systemic parasitic disease of domestic ruminants, which is economically significant parasitic disease of most mammalian species. It is causing heavy losses in production and productivity, morbidity and mortality, growth retardation, sterility; poor feed utilization, poor quality of meat and milk, condemnation of affected livers and expense due to control measures (Yesuf *et al.,* 2020).

Fascioliasis is more prevalent and frequently chronic in young animals. Inflammation, bile duct blockage, liver tissue damage, and anemia are all brought on by adult flukes in the bile ducts. This is because young animals' growth rates and feed conversion are significantly influenced by both immature and adult flukes (Kanyari *et al*., 2017).

Paramphistomosis is the ruminant forestomach infection caused by Paramphistomum spp. (Trematoda: Paraphistomatidae) in warmer latitudes. Snails are the intermediate hosts of this parasite. The infective stage for ruminants is the metacercaria in water bodies and on aquatic vegetation. Hypoproteinemia, anemia, and death can result from heavy burdens of immature flukes in the proximal intestine, where they reside before migration to the rumen and reticulum, although the presence of heavy infection in the rumen is usually of limited clinical significance. By burrowing deeply into the wall of small intestines, larvae may reach the peritoneal cavity (Gelberg, 2017).

Paramphistomosis is a pathogenic disease that affects domesticated ruminants and causes significant economic losses in the dairy and meat sectors. It is considered a neglected tropical disease, with the highest prevalence throughout tropical and subtropical regions, particularly in

Africa, Asia, Europe, and Australia (Roy and Lynden, 2019). It is widely distributed geographically, with particular attention in Ethiopia (Melaku and Addis, 2012), Nigeria (Dube and Aisien 2010, and Thailand (Sripalwit *et al*., 2007).

Rumen flukes have a conical form that measures 5–12 mm by 2–4 mm; observed that the adults prefer the rumen and reticulum of ruminants, whereas immature parasites prefer the small intestines and abomasum (Rojo-Vázquez *et al.,* 2012) The adult flukes are generally considered nonpathogenic for their hosts, but migration of immature worms in duodenal mucosa causes severe enteritis, possibly necrosis and hemorrhage and is responsible for anorexia, polydipsia, unthriftiness, severe diarrhea and mortality (Khedri *et al.,* 2015).

In the past, paramphistomes (also known as amphistomes) were thought to be of no clinical significance (Iglesias-Piñeiro *et al*., 2016). But a severe infection with immature flukes, which attach to the lining of the upper small intestine, can result in a life-threatening illness. Reduced weight gains, decreased milk production, or other adverse effects may result from mild infections with the immature fluke. However, most livestock only have minor stomach fluke infections with adult fluke or a few immature flukes, and they do not exhibit any symptoms of illness (Lloyd *et al*., 2007). Even though there may be numerous adults, Paramphistomum in proventriculus is essentially non-pathogenic. Rumen papillae may, at most, lose some of their localized coverage. The duodenal mucosa is where the immature helminths attach (Kahn, 2010).

Another parasitic infection of ruminants, dicrocoeliasis, is brought on by various species of dicrocoelium. Dicrocoeliasis is a parasitic infection in ruminants caused by dicrocoelium species. It infects domesticated and wild ruminants by eating intermediate host ants. Symptoms are less severe than fasciolosis, but it is economically and veterinary significant due to liver damage and slaughter-related removal (Alian, 2021).

Several researchers have reported financial losses in cattle production as a result of these infections due to decreased meat production and quality, milk production, organ condemnation (liver), loss of draught power, mortality, and the risk of contracting zoonotic species, among others (Hossain *et al*., 2011; Odigie *et al*., 2013).However, most abattoir studies so far have focused on the prevalence and financial losses due to fasciolosis neglecting the effects of other

trematodes such paramphistomes, dicrocoelium; despite their significance in veterinary practice. In addition, information is insufficient about how they relate to parasite burdens and pathology. This study aims to fill such a gap and it has been carried out on ruminants slaughtered at three municipal abattoirs in central Ethiopia. Therefore, the study was conducted with planned general and specific objectives;

General objective

- The general objective was to study clinical, pathological, and histopathological alterations Caused by trematode infections and their associated risk factors in Ruminants Slaughtered at Three Municipal Abattoirs in Central Ethiopia.

Specific objectives

- To determine the current prevalence of major trematodes in slaughtered sheep,goats, cattle and the associated risk factors (origin, age, sex, and body condition) in selected municipal abattoirs in central Ethiopia.
- To characterize the pathological and histopathological alterations induced by adult trematodes burden.
- To determine the correlation of adult parasite burden with pathological, histopathological lesions, hematological and serum biochemical parameters induced by trematode infections of ruminants in selected municipal abattoirs in central Ethiopia

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1. LITERATURE REVIEW

1.1. Definition and Taxonomy

Helminth parasites fall under two phyla specifically Nemathelminths (roundworms) and Platyhelminthes (flatworms) Phylum platyhelminths contain the two classes of parasitic level worms, the Trematoda and the Cestoda. The class Trematoda falls into two primary subclasses, the Monogenia which have an immediate life cycle, and the Digenia which require a middle host. There are numerous families in the class Trematoda and those which incorporate parasites of the significant veterinary significance are the Fasciolidae, Dicrocoeliidae, Paramphistomatidae and Schistosomatidae (Urquhart *et al*., 1996). Of the lesser significance are the Troglotrematidae and Opisthorchiidae (Urquhart *et al.,* 2003).

Adult trematodes (flukes) are typically simple to perceive as a result of their level leaf-like shape and the conspicuous presence of suckers. There are two trematode groups of veterinary interest: those found as ectoparasites on fish (Monogenean trematodes) and those that are endoparasites in vertebrates (digenean trematodes). Monogenean trematodes have a solitary oral sucker in addition to numerous suckers mounted on an unmistakable back connection organ (the haptor). They have direct lifecycles and, as there is no transitional host, diseases can spread quickly by direct transmission in hydroponics systems (Jacobs, 2015).

The Digenea is probably the largest group of Platyhelminths and parasitizes a wide scope of invertebrate and vertebrate hosts which additionally incorporates people. Inside the vertebrate, last host, these worms are found in various organs, including the digestive tract, lungs, liver, and vascular system. Diseases with these parasites are liable for significant production misfortunes in the domesticated animal's industry and lessening in the personal satisfaction in people (Mas-Coma *et al*., 2005; Piedrafita *et al*., 2010; Fürst *et al*., 2012; Walz *et al*., 2015; Qian *et al*., 2016).

Digenean trematodes have only two suckers; ventral and oral. The mouth leads from the last option to a solid pharynx which siphons food into two visually impaired consummation caeca. In certain genera, like Fasciola, these are extended to build surface region. There is no butt, so they need to disgorge squander materials through the mouth.

There is an ovary, two testicles and vitelline organs which produce the eggshell. In the liver, Fasciola makes production misfortunes worldwide in ruminants with access wet fields, Dicrocoelium is less harming and found in drier conditions, while Fascioloides is innocuous in wild ruminants yet deadly to sheep. Adult amphistomes are for the most part harmless however their immature stages can have extreme and, in some cases, deadly outcomes in hotter, wetter areas (Jacobs, 2015).

2.2. Fasciola (liver fluke)

2.2.1. Etiology

The two most significant species are *F. hepatica* and *F. gigantic* (Mushtaq, 2011). *F. gigantica* is only found in the tropics and measures (27 to 75 mm) x (3 to 12 mm), but *F. hepatica* is found in temperate areas (high altitude regions in east Africa) and measures (20 to 30 mm) x (10 mm) (Ayele and Hiko, 2016).

2.2.2. Morphology

These flatworms form seven different developmental stages: eggs, miracidia, sporocysts, rediae, Cercariae, metacercariae, and adult flukes (*Ashrafi et al., 2006*). Adult flukes are flattened and leaf-like in shape, measuring 30 by 13 mm. These liver flukes are broader in the front and feature an anterior cone-shaped protrusion followed by a pair of massive laterally orientated shoulders (Hendrix and Robinson, 2006). The tegument is well-armed with backwardly directed spines that, along with the suckers, serve as an efficient method for retaining the parasite in the bile duct (Sures, 2004).

Fasciola eggs are made up of a fertilized ovum with vitelline cells encased by a proteinous shell (Garcia *et al*., 2016). *F. hepatica* eggs are operculated and measure 130-150 micrometers in length and 63-90 micrometers in breadth, having a yellow colour. They were difficult to distinguish from *F. gigantica* eggs (MasComa *et al*., 2009). *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. *Fasciola gigantica* eggs are larger than those of *Fasciola hepatica*, measuring 190 x 100 micrometer (µm) as from measured report of Taylor *et al*. (2007).

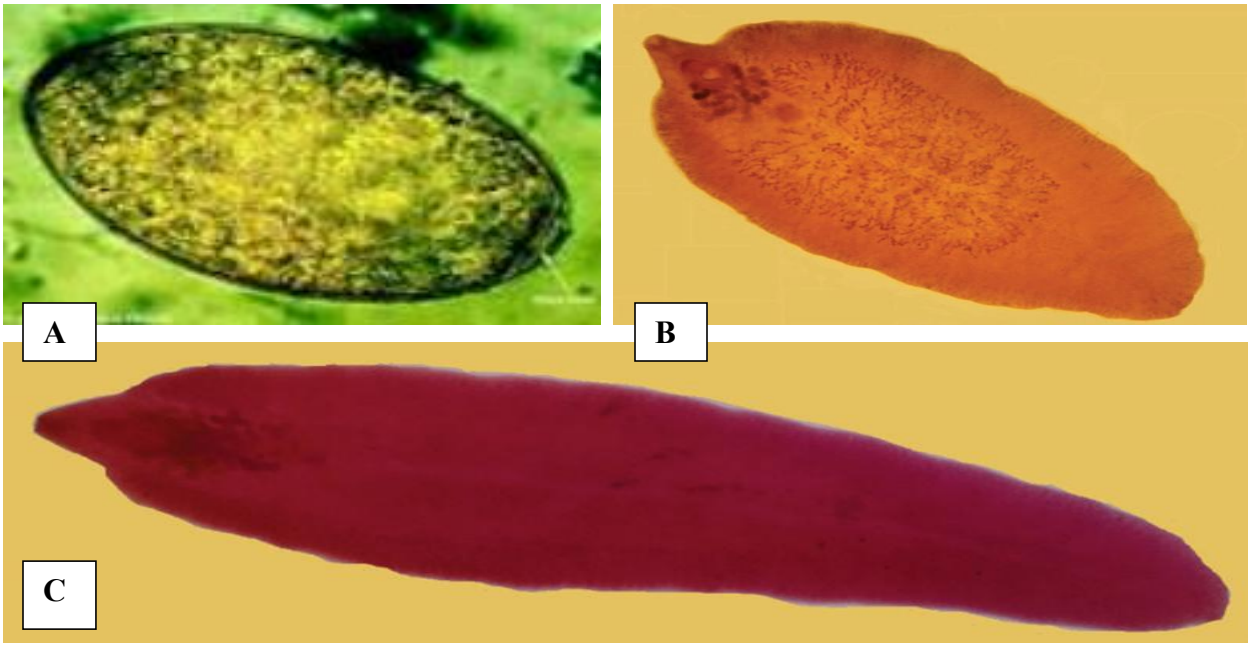

Figure 1: Morphology of Eggs and Fasciola species. (A) Fasciola Eggs (100X) (B) Morphology of *Fasciola hepatica* isolated from sheep and (C) *Fasciola gigantica* isolated from cattle (Source: Reza *et al*., 2014).

2.2.3. Epidemiology

The two most important species are *Fasciola hepatica* found in temperate area and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical area (Biniam *et al*., 2012). Fasciolosis is a serious condition that affects ruminants worldwide in regions with favorable climates for the growth of the snails that serve as their hosts (Neyab *et al*., 2010).

The most prevalent and significant liver fluke is called *F. hepatica*, and it is found worldwide. All domestic species may be infected, but only sheep and cattle have any economic significance (Keyyu *et al*., 2005).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 (Graham-Brown *et al*., 2017).

The ability to find suitable snail habitats, temperature, and moisture levels are also necessary for the development of fluke eggs, for miracidia searching for snails, and for the dispersal of cercaria being shed from the snails (Graber, 2008). This fluke's worldwide distribution occurs in regions where sheep, cattle, and goat are raised. It is widely acknowledged that *F. Hepatica* significantly reduces the value of livestock globally. *F. Gigantica* is more prevalent in Africa and India, where it affects goats and buffalo (Radostitis *et al*., 2010; Keyyu *et al*., 2005).

2.2.4. Life cycle

Infestation with Fasciolosis is usually associated with grazing wet land and drinking from the snail infesting watering places (Radostits *et al*., 2007). F. hepatic has a typical family life history (Bowman, 2003). The parasite matures in the host's bile duct where it infects cattle, goat and sheep (the definitive host), and the eggs of the parasite travel down to the bile ducts before being excreted with the feces. In order for the eggs to exist, hatch, for the miracidia to survive, and for the snails to persist, the necessary climatic and environmental conditions must exist. Warm, moist conditions and the presence of free surface water generally aid the life cycle at this point (Radostits *et al*., 2010).

The miracidium develops in the egg when certain environmental factors, such as humidity, are present (Mandal, 2006).

At 26^0c eggs can be hatched in 10-12 days producing the first larval stage, the miracidium. The miracidium is broad anteriorly with small papillae form protrusion the tegument is ciliated and the organism has a pair of eye spots (Soulsby, 2002) for further development on amphibious snails of the genus lymnae is required (Intermediate hosts). One chemical is seceded by the snails (IH) and the miracidium follow this chemical and reaches the snails and penetrates the soft tissue of the snails (Mandal, 2006) and develops in to the sporocyst, which reaches a length over 1mm (Soulsby, 2002).

Several ages occur in the body of the snail like sporocyst, radia and cercaria. The cercaria comes out the host snails. It has tail appendage and it can move from one plate to another pale and also can swim (Abu, 1999). The cercaria can crawl on the grass blades and aquatic vegetation, soon the cercaria lose their tail appendage and transform in to metacercaria which is the encysted stage formed by the secretion of cytogenous glands present in cercaria and the metacercaria is somewhat resistant to environment (Hammond and Sewell, 2004).

Final host (FH) gets infection by ingestion of the metacercaria along with their food material and drinking water. After ingestion, excystation of metacercaria occurs. Then the immature flukes invade the intestinal wall and migrate through the peritoneal cavity and subsequently penetrate the liver capsule reaching the parenchymatous tissues where are the flukes migrating for a long duration. Then finally these flukes reach the bile duct and get sexually matured (Mandal, 2006).

Adult flukes reproduce by cross and self-fertilization in the bile ducts of the host whereas the immature stages are involved in asexual reproduction. The flukes survive for years in the liver and produce thousands of eggs that are passed out in faeces via the bile duct into intestinal tract and the cycle continues. It may take approximately 3 to 4 months for a fluke to develop into an adult and start producing eggs and lymnaeid snails act as the intermediate hosts (Miliotis and Bier, 2003).

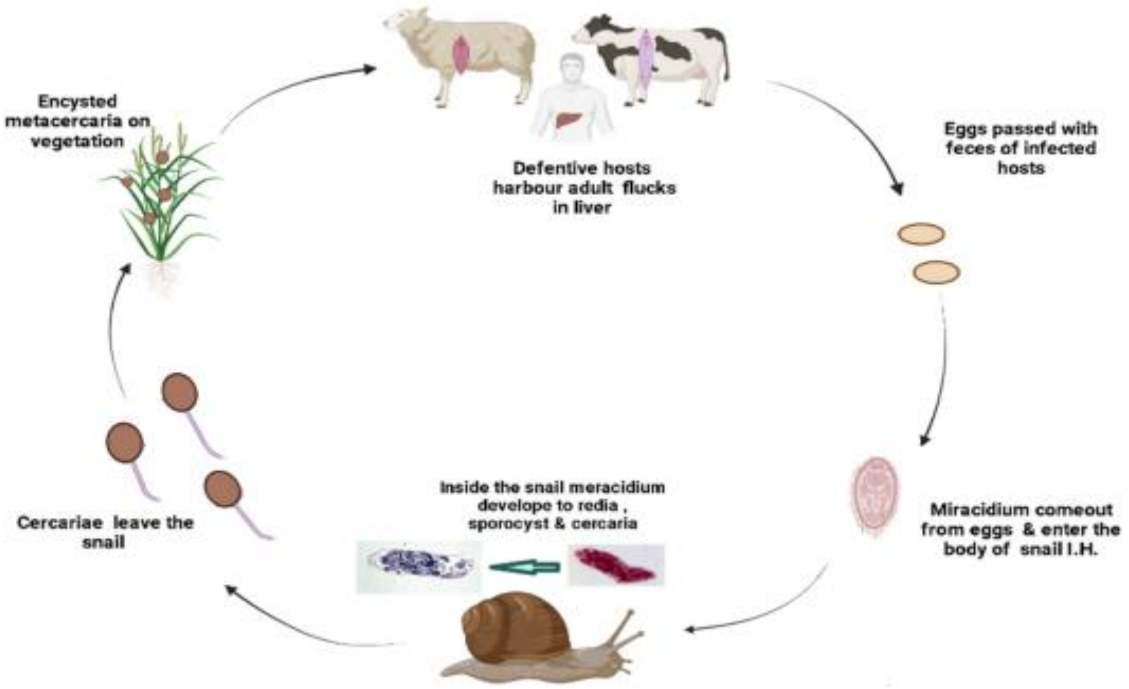

Figure 2: Life Cycle of Fasciola. Source: (Abdullah, 2023)

2.2.4. Transmission

The transmitted vectors for Fasciola spp. are amphibious freshwater lymnaeid snails (Mas-Coma *et al*., 2009a). It was estimated that nearly 30 species of lymnaeid snail are recognized as intermediate hosts for Fasciola spp. globally (Vázquez *et al*., 2018). Galba truncatula is the common lymnaeid act as a transmitter for F. hepatica in endemic temperate and subtropical areas (Artigas *et al*., 2011; Bargues *et al*., 2020).

Different Lymnaea species including: L. cousin, L. columella, L. ollula, L. natalensis, and L. viridis act as an intermediate host for Fasciola spp. (Hussein and Khalifa, 2008). Both Radix (R.) auricularia and R. natalensis lymnaeid snails that live in the subtropical and tropics area, can transmit F. gigantica (Mas-Coma *et al*., 2009b). Biomphalaria alexandrina has also been reported as a transmitter for F. gaigantica (Farag and El Sayad, 1995).

Other cosmopolitan freshwater lymnaeid snails which are responsible for transmission of Fasciola spp. in different areas include: Radix rubiginosa, Austropeplea tomentosa, Pseudosuccinea columella, Stagnicola corvus, and Hinkleyia caperata (Vázquez *et al*., 2018). The high transmission capacity of vectors is connected to the duration and persistence of the life span of the infected snails after infection (Mas-Coma *et al*., 2001).

Humans act as the incidental hosts for liver flukes (Alemneh, 2019). Ingestion of freshwater wild plants including watercress is the main source of infection to humans (Mas-Coma *et al*., 2018). In spite of watercress, various freshwater plant species might be involved in Fasciola transmission and human infection, which depend mainly on geographical distribution of those plants and the dietary traditions of peoples in that region (Mas-Coma *et al.*, 1999). Water had been mentioned as another source for infection in human, either directly by drinking or indirectly by contaminating vegetables, fruits, and kitchen utensils (Chen and Mott, 1990). Humans also become infected with fascioliasis after eating raw dishes prepared freshly from an infected liver with immature flukes (Taira *et al*., 1997).

2.2.5. Host Range

Definitive Host: The final hosts are sheep, goats, cattle, horses, deer, humans, and other mammals whose typical predilection site is the liver (Taylor, 2007). Within the biliary tract of the liver, the immature stage migrates and matures. The agent blocks the duct system as it matures. The adult female lays one egg, which is secreted into the stomach and expelled with the feces (Urquhart *et al.,* 2003).

Transitional Host (intermediate host): In Europe and South America, Galba *truncatula* is the most common intermediate host for *F. hepatica*. Freshwater snails from the Lymnaeidae family are intermediate hosts of *F. hepatica* (Krauth *et al.,* 2015; Mazeri *et al.,* 2016). Planorbidae snails are occasionally used as intermediate hosts for *F. hepatica* (Mas-Coma *et al.,* 2005). The following are significant species involved in the transmission of *F. hepatica* and are responsible for the establishment of miracidium in the cercaria stages of *Fasciola* larvae (Sures, 2004).

2.2.5. Pathogenesis

Essentially, there are two stages in the pathogenesis process: the first stage, which happens during migration in the liver parenchyma and is connected to liver damage and hemorrhages (Swanakar *et al*., 2014). When parasites penetrate the liver's bile ducts, the second phase (the biliary phase) begins. Flukes develop, feed on blood, and lay eggs in biliary channels. Tissue injury causes biliary duct hypertrophy and lumen blockage. Adult flukes are rather innocuous, but liver tissue is generally severely injured, as seen by edema, bleeding, discoloration, necrosis, bile duct hyperplasia, and fibrosis (Urquhart *et al.,* 2003; Taylor, 2007). The inflammation of bile duct, and gall bladder developed at chronic state may causes gallstones and fibrosis (Rahman *et al*., 2017).

Seasons are brought on by adult flukes eating blood and their cuticular spines harming the bile mucosa when the parasite is in the bile ducts (Sileshi and Desalegn, 2007). Different stages of *Fasciola hepatica* in the liver are what cause acute and chronic fasciolosis. The sudden invasion of the liver by numerous young flukes that result in acute fasciolosis may cause enough parenchymal destruction to result in acute hepatic insufficiency (Radostits *et al*., 2010).
The emergence of anemia and hypoalbuminemia, which can result in ascites, submandibular edema, and a pallor of the mucous membranes, are two clinical features of chronic fasciolosis (Graber,2008).

Chronic hepatic fasciolosis takes time to manifest and is brought on by mature flukes in the bile duct. It includes cholangitis, biliary obstruction, fibrosis-causing hepatic tissue destruction, and the release of a hemolytic toxin by the fluke (Radastits *et al.*, 2010).

The severity of the infection may be reduced in animals with previous experience, in which the result is a decrease in helminth size and egg production, a delayed onset of anemia, an earlier increase in the number of eosinophils and lymphocyte infiltration into the liver (Rojo-Vázquez *et al*., 2012).

The eosinophilic response following *F. hepatica* infection is biphasic with an initial peak at 4 weeks post-infection (migratory phase) and a second peak 9 weeks post-infection and an increase of the GGT levels by 10 weeks post-infection (epithelial damage in the bile duct). This response is similar to that observed in sheep infected with *F. gigantica* (Zhang *et al*., 2005).

2.2.6. Clinical signs

Fasciolosis's clinical signs can take on acute, subacute, or chronic forms (Graber, 2008). The animal dies suddenly, blood-stained frothy appear at the nostrils, and blood is discharged from the anus as in the case of anthrax are the acute clinical signs (Soulsby, 2002). Clinical appearance in cattle and sheep, the most common definitive hosts, is classified into four kinds (Urquhart *et al.,* 2003; Radostits *et al.,* 2007; Mushtaq, 2011). Fasciolosis can manifest in one of four clinical forms.

i). Acute Type I Fasciolosis: More than 5000 swallowed metacercariae constitute an infectious dosage. Animals died unexpectedly, with no prior clinical indications. Ascites, abdominal bleeding, icterus, membrane pallor, and weakness may occur (Radostits *et al.,* 2007; Kaya *et al.,* 2007).

ii). Acute Type II Fasciolosis: The infectious dose was 1000–5000 metacercariae swallowed. As previously said, it can cause mortality, but it also causes pallor, loss of condition, and ascites for a short period (Urquhart *et al.,* 2003).

iii). Subacute Fasciolosis: The infectious dose is 800-1000 metacercariae swallowed. Sheep and cattle are sluggish, anemic, and may perish. Weight loss is a prominent aspect (Radostits *et al.,* 2007; Urquhart *et al.,* 2003). Anemia, jaundice, and ill-thrift are symptoms of sub-acute cases, and the migrant flukes cause significant tissue damage, hemorrhage, and in particular liver damage (Radostits *et al*., 2010).

iv). Chronic Fasciolosis: The infectious dosage is 200–800 ingested metacercariae. All varieties of fasciolosis can cause asymptomatic or slow development of bottle jaw and ascites (ventral edema), emaciation, weight loss, anemia, hypoalbuminemia, and eosinophilia (Kaya *et al.,* 2011).

The most prevalent clinical syndrome in sheep and cattle is chronic fasciolosis, which develops in 12 weeks after an infection when the fluke has entered the bile ducts and is sexually maturing (Anne *et al*., 2006). Even though chronic infections typically result in production losses, death can also happen (William *et al*., 2000). Chronic Fasiciolisis is much more protracted and "fluky"; over several weeks, sheep experience weight loss, submandibular edema (bottle jaw), and mucosal pallor. Wool loss and diarrhea are frequent occurrences (Radostits *et al*., 2010).

2.2.7. Diagnosis

This is primarily based on clinical indicators, seasonal occurrence, weather patterns, and historical cases of fasciolosis on farms or the observation of snail habits (Graber, 2008). In endemic regions, a diagnosis is frequently made based on clinical signs like condition loss, anemia, and failure to gain weight (William *et al*., 2000). Several approaches, such as fluke egg count, liver enzyme detection, and post-mortem investigation, are utilized to identify the condition based on this information (Urquhart *et al.,* 2003).

Parasitological examinations: The eggs of fasciolosis are very distinctive, and finding them in the faeces can confirm the diagnosis (Soulsby, 2002). It must be distinguished from other fluke eggs, particularly the big eggs of Paramphistomum. The Fasciola egg had a yellow shell with an unclear operculum, and the embryonic cells are similarly vague. The eggs of the Paraphistomum have clear embryonic cells, transparent shells, and distinct opercula. There is frequently a small Knob at the posterior and pole, and the eggs themselves are frequently bigger than those of the liver fluke. Operacula eggs do not consistently appear in flotation methods, have a barrel-shaped and should be sedimented instead (Mesfin, 2004).

Detecting Liver Enzymes: Typically, two enzymes are tested. When parenchymal cells are injured, glutamate dehydrogenase (GLDH) is produced, and levels rise within the first several weeks of infection. The other gamma-glutamyl transferase (GGT) shows epithelial cell injury lining the bile ducts, and elevated levels are sustained for extended periods (Taylor, 2007).

Subacute or chronic elevations in liver enzyme activity, such as glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH) have been identified in subacute or chronic fasciolosis 12–15 weeks following metacercariae consumption (Kozat and Denizhan, 2010; Sangster *et al.,2021)*

Postmortem Examination: Infection can be also confirmed at necropsy and many farmers use abattoir returns to identify if *F. hepatica* is present in their livestock (Mazeri *et al*, 2016). Liver examination at slaughter house is considered to be the most direct, reliable and cost-effective technique for the diagnosis of liver fluke infection (Borai *et al*., 2013). In acute fasciolosis, there may be peritonitis, particularly on the visceral surface of the hepatic capsule. Due to migration of flukes, there are dark hemorrhagic streaks and foci. The liver is swollen, friable and has capsular perforations marked by hemorrhagic tags. Characteristic lesions seen in chronic fasciolosis include gall bladder enlargement and bile duct calcifications. Progressive biliary cirrhosis in cattle results in a hard, fibrotic liver with noticeable, thick, and fibrous bile ducts (Radostits *et al*., 2007).

2.2.8. Pathological lesions

Gross lesions: Clinically, a fasciola-infected animal exhibits pale visible mucous membrane or anemia due to severe liver damage brought on by immature flukes tunneling through parenchyma (Radostits *et al.,* 2000).Both chronic and acute liver lesions are a feature of the illness. The liver that has been infected with fasciola is pale, firm, and irregularly shaped. The liver's size is reduced, and the bile ducts become thicker, as part of the chronic fasciolosis' gross pathology (Talukder *et al*., 2010). Numerous fibrosis types, including post-necrotic scarring, ischemic fibrosis, and per biliary fibrosis, may also be present . Gallbladder enlargement, bile duct calcification, and abnormal fluke migration are more frequent in cattle (Steyl, 2009).

Immature flukes moving through the liver parenchyma are linked to acute fasciolosis. In this, the liver is visibly enlarged, hemorrhagic, and covered in fibrinous to fibrous exudates on the surface of the capsular layer. Numerous hemorrhagic spots and focal necrosis are found on the cut surface of liver parenchyma. Grossly visible as dark acute hemorrhagic streaks or the typical post necrotic scarring and granulation, the migratory tracts of this parasite are caused by direct trauma.

The gross pathological changes of the liver in chronic Fasciolosis are characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing the adult flukes (Talukder, *et al*., 2010). Chronic fascioliasis causes a chronic inflammation of the liver and bile ducts accompanied by loss of condition, digestive disturbances and a general reduction in productivity (Rana *et al*., 2014).

Figure 3: Gross pathology of sheep liver and bile duct. Hepatomegaly of sheep liver and Bile duct of sheep filled with blackish brown exudates infected with adult flukes (source: Jehangir *et al*., 2018).

Histopathological lesion: The histopathological in chronic Fasciolosis were characterized by the infiltration of fibroblasts mixed with lymphocytes and a few mononuclear cells in the region where young flukes had previously migrated is notable. Huge proliferations of fibrous connective tissue associated with infiltration of lymphocyte and plasma cells in the portal area have also been reported (Okaiyeto *et al.,* 2012; Talukder, *et al.*, 2010). Flukes that are mature cause the epithelium to become necrotic and ulcerated, which results in peribiliary inflammation and severe epithelial hyperplasia (Fairweather, 2011). The liver parenchyma exhibits necrotimigratory tracts produced by immature flukes that are migrating (Steyl, 2009).

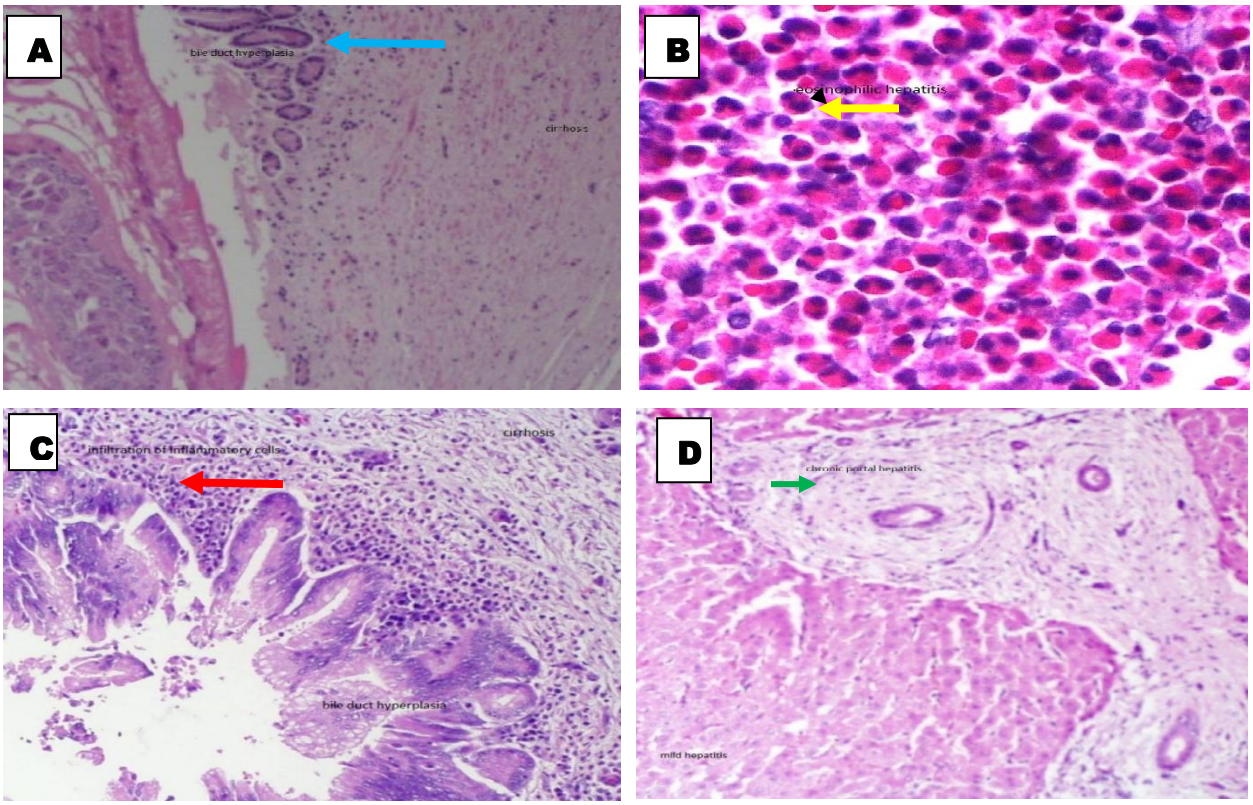

Figure 4: Histopathology of liver (**A).** Bile duct hyperplasia in liver of infected cattle *by Fasciola gigantica* and B). Eosinophils in cattle liver infected by *Fasciola gigantica* (C). Inflammatory cells, bile duct hyperplasia in liver of cattle infected by *Fasciola gigantica* and (D). Chronic portal hepatitis (cirrhotic liver) and mild hepatitis, hepatocyte replaced by fibrotic tissue in liver of cattle infected by *Fasciola gigantica* (Jafar *et al*., 2015).

2.3. Paramphistomes (Rumen/Stomach Fluke)

2.2.1. Etiology

The most notable species include *Paramphistomum Cervi, Paramphistomum cotylophorum, Paramphistomum cracile, Paramphistomum gotoi, Paramphistomum grande, Paramphistomum ichikawai, Paramphistomum leydeni, Paramphistomum liorchis,* and *Paramphistomum microbothrioides* (Lotfy *et al*., 2010).

2.2.2. Morphology

In ruminant fore stomachs (rumen, reticulum), adult Paramphistomes are tiny flukes that are about 1 cm long, conical in shape, and pink or reddish (Toolan *et al.,* 2015). On a microscopic level, their bodies are pear-shaped, with the head at the narrowest end, unlike many other fluke species. The cross-section is nearly cylindrical. They have two suckers, one oral and one ventral, with the latter being larger and located closer to the back. Like other flukes, they don't appear to be segmented on the outside. The mouth closes off in the pharynx, a muscular tube that allows for sucking. The digestive system is branching as opposed to linear, as in other animals, and ends in numerous blind ducts because it is blind (i.e., without anus: the only opening is the mouth) (called coeca). Like other flukes, rumen flukes are hermaphrodites, which means they have both male and female reproductive organs (Roberts *et al*., 2016). As in hermaphrodites, the male and female reproductive systems are both present in the posterior region of the body. The ovary is located anterior to the lobed testes. The eggs have a clear shell, resemble barrels, and have an operculum on one end (Lotfy *et al*., 2010).

2.2.3. Life Cycle

The rumen fluke life cycle requires two hosts: mammals, primarily ruminants, as the final hosts and snails as intermediate hosts. The final host becomes infected after ingesting encysted metacercariae that are floating in the water or attached to plants (González *et al*., 2013). They require intermediate hosts like snails as well as definitive hosts like ruminants for their indirect life cycle.

The hermaphrodite, sexually mature monoecious excretes the egg after self-fertilizing in the mammalian rumen. Miracidia with cilia form from eggs in water. The bodies of snails from the genera Bulinus, Planorbis, and Stagnicola, which serve as intermediate hosts, are then invaded by miracidia (Bowman, 2008).

In the stomach, adult flukes lay eggs that are expelled outside with the faeces. About two weeks later, miracidia hatch from their eggs. They explore the water until they come across a snail that suits them. Once inside the snail, they develop into sporocysts and rediae, which have the ability to reproduce asexually and give rise to daughter rediae. About 15–30 cercariae are present in each medium. It uses its two eyespots and long, thin tail to find aquatic plants or other suitable substrates, to which it adheres and encysts to become a metacercariae (Jones *et al.,* 2017).

Mammalian hosts ingest the pathogenic larvae. By the time they reach the duodenum and jejunum, their cysts have been removed (Yan *et al*., 2013). After aggressively attacking the mucosa, they burrow through the intestinal wall to reach the rumen, where they develop into adult flukes and start to lay eggs. Metacercariae finish developing and start laying eggs 2 to 4 months after being ingested by the last host (prepatent phase) in Sanabria and Romero (2008).

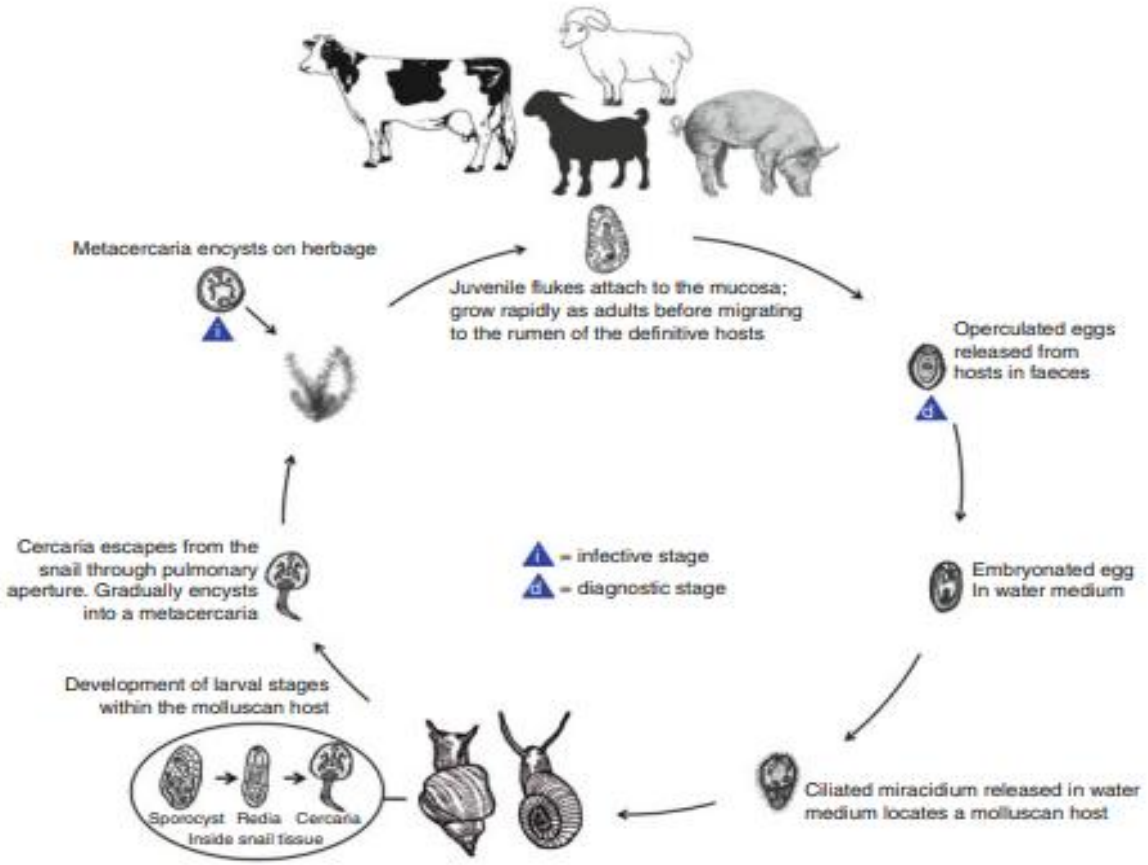

Figure 5: Life-Cycle of the Rumen Fluke (Paramphistomum) (Tandon *et al.*, 2014)

2.2.4. Epidemiology

Although Paramphistomum is found all over the world, it is considered a neglected tropical disease, with the highest prevalence throughout tropical and subtropical regions, particularly in Africa, Asia, Europe, Russia and Australia (Roy and Lynden, 2019; RojoVázquez *et al.,* 2012). Paramphistomosis epidemiology is governed by several parasite-host-environment interactions. The infection rate in pastures is the most important epidemiological variable in determining worm loads in animals. It is also regulated by the climatic requirements for pasture egg hatching, development, and survival (Ozdal *et al.,* 2010).

Paramphistomosis has been reported in several locations in Ethiopia, with approximately 45.83% in western Gojam, 28.6% in Bishoftu, and 6.7% in Hawassa (Yeneneh *et al*., 2012; Melaku and Addis, 2012 and Tagesse *et al*., 2014), respectively.

The major epidemiological variable influencing worm burdens of animals is the infection rate from pastures. It is also influenced by the climatic requirement for egg hatching, development and survival of the larvae in pasture (Ozdal, 2010)

2.2.5 Host Range

Definitive Host: Paramphistomes: Cattle, sheep, goats, and other animals, as well as numerous wild ruminants, are all susceptible to Paramphistomum. Ruminants are the most reliable hosts (González *et al.,* 2013).

Intermediate Host (Transitional Host): Paramphistomes: Snails of the genera Bulinus, Planorbis, and Stagnicola serve as intermediate hosts (Kifleyohannes *et al.,* 2015).

2.2.6. Pathogenesis

The pathogenic impact of rumen fluke is connected with the intestinal phase of infection. Because the immature fluke feeds on plugs, the duodenal mucosa is severely eroded. In severe infections, this results in enteritis, which is marked by edema, bleeding, and ulceration (Dube

and Aisien, 2010). This paramphistomosis is considered a very pathogenic illness in tropical climates. In livestock animals, it causes enteritis and anemia, resulting in significant output and economic losses (Dorny *et al.,* 2011).

Pathological signs are caused by immature flukes. When the immature flukes begin to congregate in the bowel, there is watery and fetid diarrhea, which is frequently linked with high mortality (even up to 80-90%). In ruminants, up to 30,000 flukes can accumulate at one time and assault the duodenal mucosa, causing acute enteritis. The immature helminths adhere to the duodenal mucosa via their strong complete ventral sucker and are firmly embedded in the mucosa, producing severe enteritis, duodenitis, hypoproteinemia, edema, bleeding, and perhaps necrosis in previously uninfected young animals. Pathological lesions caused by immature paramphistomum cause anorexia, polydipsia, severe diarrhea, and mortality in domestic ruminants (Juyal *et al.,* 2003).

Ruminal lesions have also been linked to heavy infection with the adult worms *Paramphistomum ichikawai* (Rojo-Vázquez *et al.,* 2012) and *P. microbothrium*, which may have hampered digestion and absorption, leading to diarrhea, anorexia, anemia, and weakness (Dorny *et al.,* 2011). *P. cerviare* are plug feeders that cause significant disease by burrowing into the duodenum's submucosa and feeding on epithelial cells of the burner gland, causing anorexia, perfused fetid diarrhea, and a reduction in plasma protein concentration, and anemia that weakens the animal (Dube and Aisien, 2010).

2.2.7. Clinical Signs

Paramphistomosis, also known as amphistomosis, is a condition that mostly affects cattle and sheep. Its symptoms include copious diarrhea, anemia, and lethargy, and if left untreated, it can lead to death. The most common clinical indications of stomach fluke infection are enteritis (small intestine inflammation) and severe diarrhea (watery scour) with blood traces as a result of dehydration, dullness, weight loss, and so on. Anemia and bottle jaw are also possibilities. Mature paramphistomum is also responsible for irregular rumination, rumenitis, decreased nutritional conversion and body condition, anorexia, polydipsia, and severe diarrhea (Williams, 2012).

The immature rumen fluke is a plug feeder that causes serious disease by burying itself in the submucosa of the duodenum and feeding on the epithelial cells of Brunner's gland, causing anorexia, perfusing fetid diarrhea, a drop in plasma protein concentration, and anemia, all of which weaken the host (Melaku and Addis, 2012).

2.2.8. Diagnosis

Paramphistomosis is considered to be one of the most important emerging diseases affecting livestock worldwide (Taylor et al.,2007). The clinical indications of rumen fluke are frequently associated with young animals in the herd, as well as the history of grazing areas near the snail habitat. Because the disease arises during the prepatent period, a fecal sample investigation is of limited use. A postmortem examination and recovery of the small fluke from the rumen can be used to confirm the diagnosis (Urquhart *et al.,* 2003).

Fecal Sampling and Examination: Fecal samples (Approximately 10 gram) from bovine and ovine can be collected directly from the rectum of the animal. The sample then put in a plastic container with detailed water history about age, group, sex and the district of individual animals (Urquhart *et al*.,1996). Ten percent formalin could be added and the sedimentation technique is applied for detecting trematode eggs in the feces (Tariq, *et al*.,2008).

Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in sediment three grams of feces weighed or measured using a sensitive balance and transferred into container 1. Then 42 ml of tap water poured into Container 1. It could be mixed thoroughly with a stirring device. The Faecal suspension filtered through the area strainer into container 2. The filtered material poured into test tube and centrifuged at 1500 rpm for 5 minutes. After centrifugation, the supernatant removed and a few drops of 5% methylene blue added. Then the sediment transferred to a micro slide, covered with a coverslip and examined fewer than 10x objective microscope (Sintayehu *et al*., 2012).

Ante Mortem Examination: Antemortem inspection can be carried out on the animals before slaughter to assess their general health status. To identify the effect of snail burden, most of scientific studies focus on the origin and management of the animal. Some *Paramphistomatic* animals may be anemic up on the examination before slaughter (Sintayehu *et al*.,2012).

Post Mortem Examination: Affected Animals showed that there is muscular atrophy, subcutaneous edema and accumulations of fluid in the body cavities and the fat deposits are gelatinous. In the upper part of the duodenum, the mucosa is thickened and covered with blood-stained mucus and there are patches of hemorrhage under the serosa. Large numbers of small, lesh-colored flukes (3-4 mm long and 1-2 mm wide) are present in this area but decrease in number toward the ileum. There may be none in the abomasum and fore stomachs. There may be a few in the peritoneal cavity and on histopathological examination, the young flukes are present not only on the mucosal surface but are also embedded in the mucosa and deeper layers (Jones *et al*.,2017)

2.2.9. Pathological lesions

Gross lesion: The rumen and duodenum of cattle and sheep submitted for post mortem examination were assessed macroscopically (gross examination of ruminal papillae). All samples were stapled to a numbered cardboard during fixation in a 10% buffered formalin solution for a minimum of 48 hours. The sample was collected and stored for a minimum of 24 hours in 10% buffered formalin, embedded in paraffin and sectioned at 2 μm, and stained with haematoxylin and eosin for histopathological analysis. The stained slides were then examined at 100× and 400× magnification using an Olympus 40x microscope (Kern *et al*.,2016).

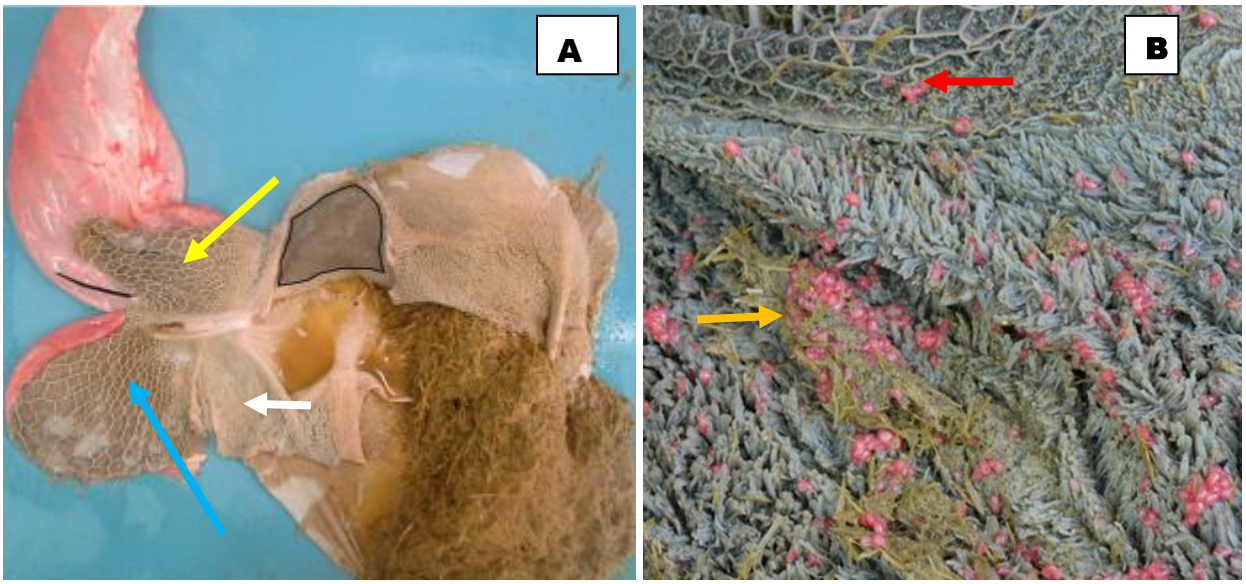

Figure 6: Gross pathology of rumen fluke in the reticulo-rumen of a bovine. (A). Opened reticulum and rumen (B). Adult Rumen Flukes in the reticulo-rumen of a bovine (Busin *et al.,* 2023).

Histopathological lesion: histopathological, inflammation of the mucosa and submucosa, and evidence of chronic changes such as fibrosis or granulomas are present (Day *et al*., 2008). Variable mucosal oedema and villous atrophy are seen on histopathological examination of the affected intestine, including concomitant hyperplasia of mucosal crypts and submucosal Brunner's glands and infiltration of the mucosa and submucosa by lymphocyte, eosinophils, cells, plasma cells, and globule leucocytes (Fuertes *et al*., 2015).

2.4. Dicroceilum (Lancet Fluke)

2.4.1. Etiology

Digenean lancet liver flukes of the family Dicrocoeliidae (Trematoda: Digenea) can infect the bile ducts of a variety of wild and domesticated mammals and humans around the globe (Otranto and Traversa, 2003). There are five species of parasites, namely, *D. chinensis, D. dendriticum, D. hospes, D. orientalis,* and *D. suppereri*, with *D. dendriticum* the most prevalent (Beck *et al*, 2015).

There are two intermediate hosts and a final host in the life cycle of the parasite. The first intermediate host is terrestrial snail of the genus *Helicella* and *Zebrina* and the second intermediate host *Formica* ant, with the final host being mammals including herbivores (cattle, buffalo, sheep, goat, horse, deer, camel, rabbit, and wild ruminants), pig, rodents, polar bear, primates, and humans (Cabeza-Barrera *et al*, 2011). Dicrocoelium dendriticum, which causes liver fluke disease in ruminants and is of zoonotic and economic importance. It is prevalent in many regions of the world (Shinggu *et al*., 2019; Khanjari *et al*., 2014).

Dicrocoelium dendriticum lives in the adult stage, in the bile ducts, canaliculus, and gallbladder of its hosts (cow, sheep, goat, and pig). The main economic impact of dicrocoeliasis in livestock is due to the rejection of livers from slaughtered animals at meat inspection (Rojo-Vazquez *et al*., 2012). However, in severe infections, affected animals may show clinical signs including poor food intake, ill thrift, poor milk production, and alteration in fecal consistency, photosensitization and anemia (Manga-Gonzalez *et al*., 2004; Sargison *et al*., 2012).

2.3.2. Morphology

Dicrocoelium species are characterized by a lancet shaped body, with an oral and a ventral sucker. *Dicrocoelium* sp (Lancet liver fluke) is 6-10 mm in length and 0.2-0.4mm in width and its size is directly proportional to the size of the final host (Bec*k et al*, 2014); semitransparent and pied, with a black uterus and white vitellaria visible to the naked eye. The eggs are oval, dark brown, typically operculate, small (38– 45 µm x 22–30 µm), with two characteristic dark points (so called “eye spots”), and contain a miracidium (Euzéby, 1971). The species differ in the position of testis, the length of the vitellaria and the position of the ventral sucker in relation to the cirrus sack (Schuster, 2002).

Dicrocoelium spp. are helminthes of lancet shape, with weakly developed suckers of similar size in the anterior part of body. The mouth opens in the center of oral sucker into the pharynx, which enters into a short thin esophagus with two straight branches of intestinal caeca, located alongside the body and lacking other openings. The trematodes are hermaphroditic. The uterus fills the trematode’s hind body and consists of descending and ascending branches (Shelyakin and Stepanov, 2013).

2.3.2. Epidemiology

The epidemiology of Dicrocoelium depends upon the environment and on the presence of its intermediate and definitive hosts. The occurrence of *D. dendriticum* is related to dry and calcareous or alkaline soils, which represent favorable biotopes for their intermediate hosts (Manga-Gonzalez, 2001). Dicrocoeliosis is present worldwide in lowland or mountain pastures, which provide adequate conditions for the survival and development of terrestrial snails and ants (Gideon, 2009); it has been described in sheep and goats rather than in cattle (Ducommun and Pfister, 1991). The epidemiology of Dicrocoelium depends upon the environment and on the presence of its intermediate and definitive hosts (Manga-Gonzalez, 2001) and is distributed throughout Europe, Asia, North and South America, Australia, and North Africa (Arias *et al*., 2011). In this environment, *D. dendriticum* eggs are highly resistant because they can over-winter and remain infectious for up to 20 months on pastures.

2.3.3. Life cycle of dicrocoelium

The life history of Dicrocoelium spp. is indirect and may take at least six months to complete. Monoecious and both sexually reproducing and self-fertilizing adults are found in the bile ducts. Eggs containing fully-developed miracidia are shed in faeces and must be eaten by land snails before hatching. Miracidia penetrate the gut wall of the snails and undergo asexual replication and development into cercariae, which then escape from the snails in their slime trails, and are eaten by ants. One cercaria migrates to the head of the ant and associates with the sub oesophageal ganglion; while up to about 50 cercariae encyst in the gaster as metacercariae (Martín-Vega *et al*., 2018).

The larval stage that develops in the ant's head alters its behaviour, making it cling to herbage and increasing the probability of its being eaten by an herbivorous definitive host. Unlike Fasciola spp., larval flukes migrate to the liver via the biliary tree and develop to adults in the bile ducts (Manga Gonzalez *et al.,* 2001). Several species of land snails and ants are known to be intermediate hosts within the same geographical location (Mitchell *et al*., 2017).

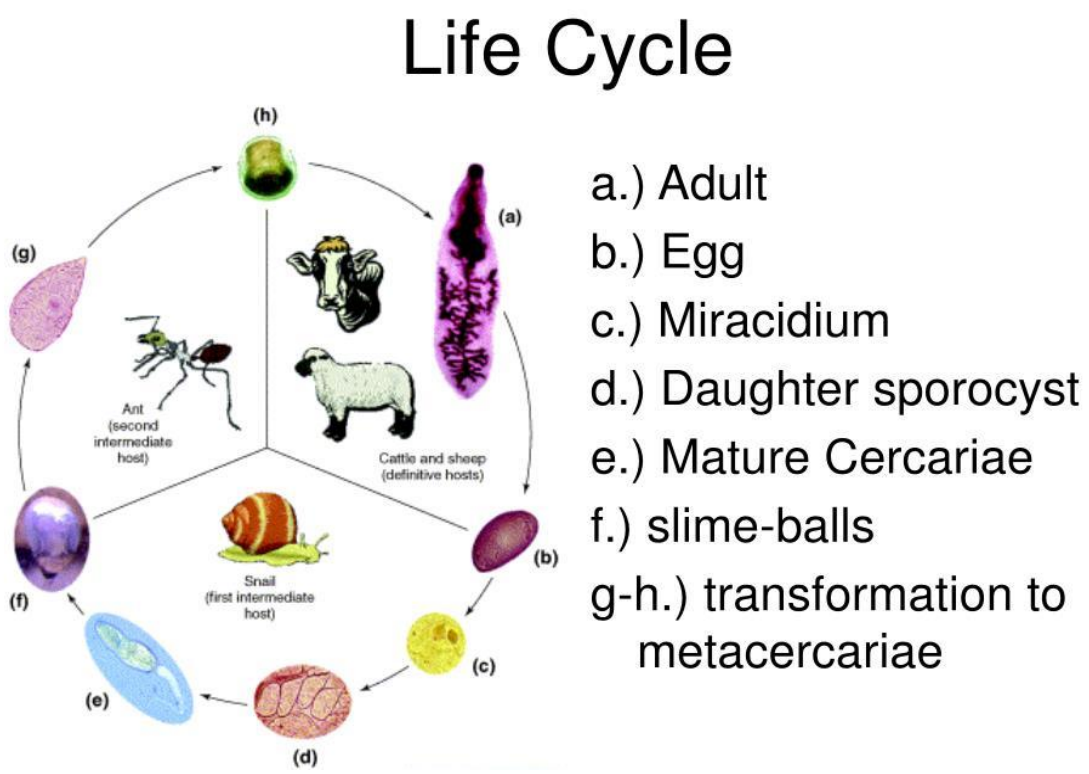

Figure 7: The life cycle of Dicroceilum.(Source:Schmidt & Roberts, 2005))

2.3.4. Pathogenesis and lesions

The young flukes migrate directly up the biliary duct system of the liver without penetrating the gut wall, liver capsule, or liver parenchyma as in fasciolosis. Clinical symptoms are not usually manifested, even in heavy infections, and therefore, major lesions, due to liver impairment, are detectable only at necrotic examination of the liver (Theodoridis *et al*., 1991). Dicrocoeliasis causes severe pathological changes of the liver and bile system such as abscesses, granulomas, and fibrosis. Cholangitis with the thickened bile ducts appearing as white spots on cut surfaces of the liver was diagnosed. Chronic disease can develop into cirrhosis which can lead to death (Abdi *et al*, 2013).

Pathological changes associated with dicrocoeliasis include pallor, hardened liver, distension, and inflammation of bile ducts. The presence of parasites in the gallbladder and bile ducts may also result in whitish foci on the liver, scarring, fibrosis, and cirrhosis, depending on the severity of the infection (Yener *et al*., 2016).

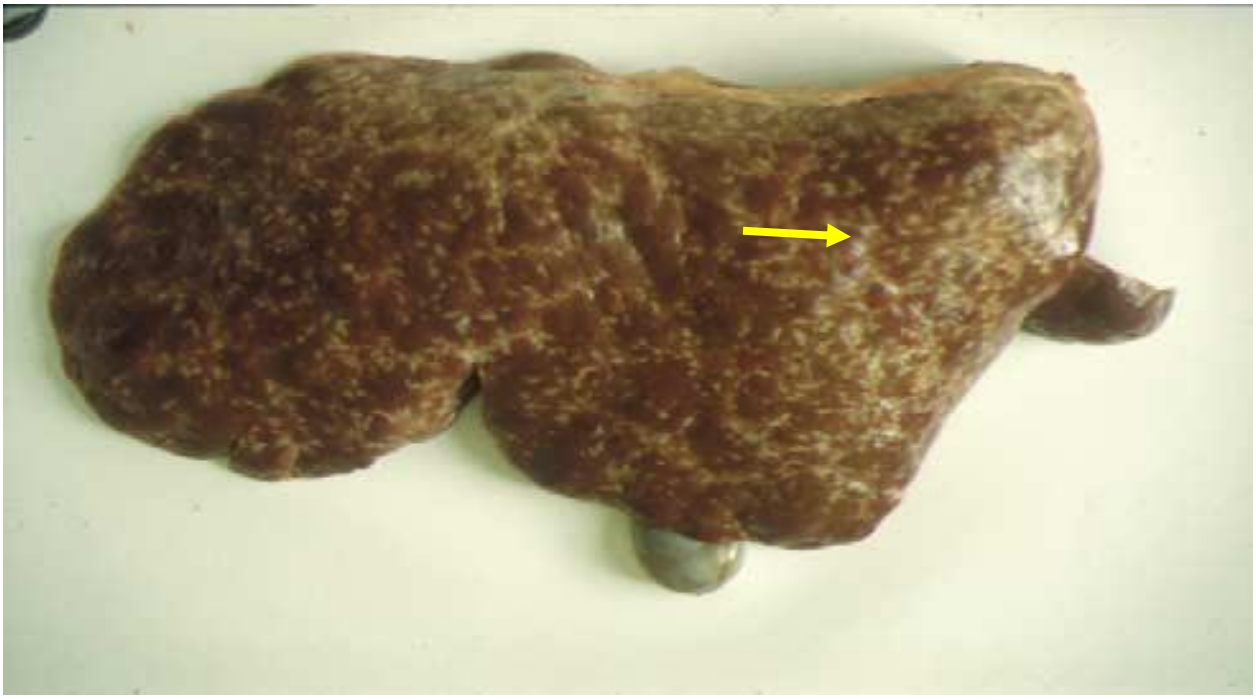

Figure 8: *D. dendriticum* with severe fibrosis with enlarged liver (Otranto and Traversa, 2002).

2.3.5. Diagnosis

The diagnosis of dicrocoeliosis in live animals is usually based on the identification of characteristic eggs in faecal samples, for example by floatation in saturated zinc sulphate solution (Sargison *et al*., 2012). However, coprological methods only detect patent infections, and their sensitivity may be low.

2. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Bishoftu, Dukem and Gelan Municipal Abattoirs from November, 2022 to June, 2023 in the Oromia Region, central Ethiopia. Bishoftu has an altitude of 1950 meters above sea level and experiences a bimodal rainfall pattern with a long rainy season from June to October (84%) and a short rainy season from March to May. The average annual rainfall and averages maximum and minimum temperature of the area are 800mm, 26°C and14°C, respectively. The geographical (astronomical) location of Bishoftu town is approximately located at 8° 45' N latitude and 38° 59’ E longitudes, 47.9 km south east of Addis Ababa at an altitude of 1950 meters above sea level (CSA, 2021).

Gelan and Dukem town have an altitude between 1,800 and 2,300 meters above sea level. They lie directly adjacent to each other along the highway between Addis Ababa and Djibouti. Geographically, the towns lie between 8° 53’N and 8°45’N latitudes and 38° 46’E and 38° 56’E longitudes, 25 km and 37 km respectively south of Addis Ababa. This region has an average temperature of 190 °C and an annual precipitation of 861 mm (NMS, 2013).

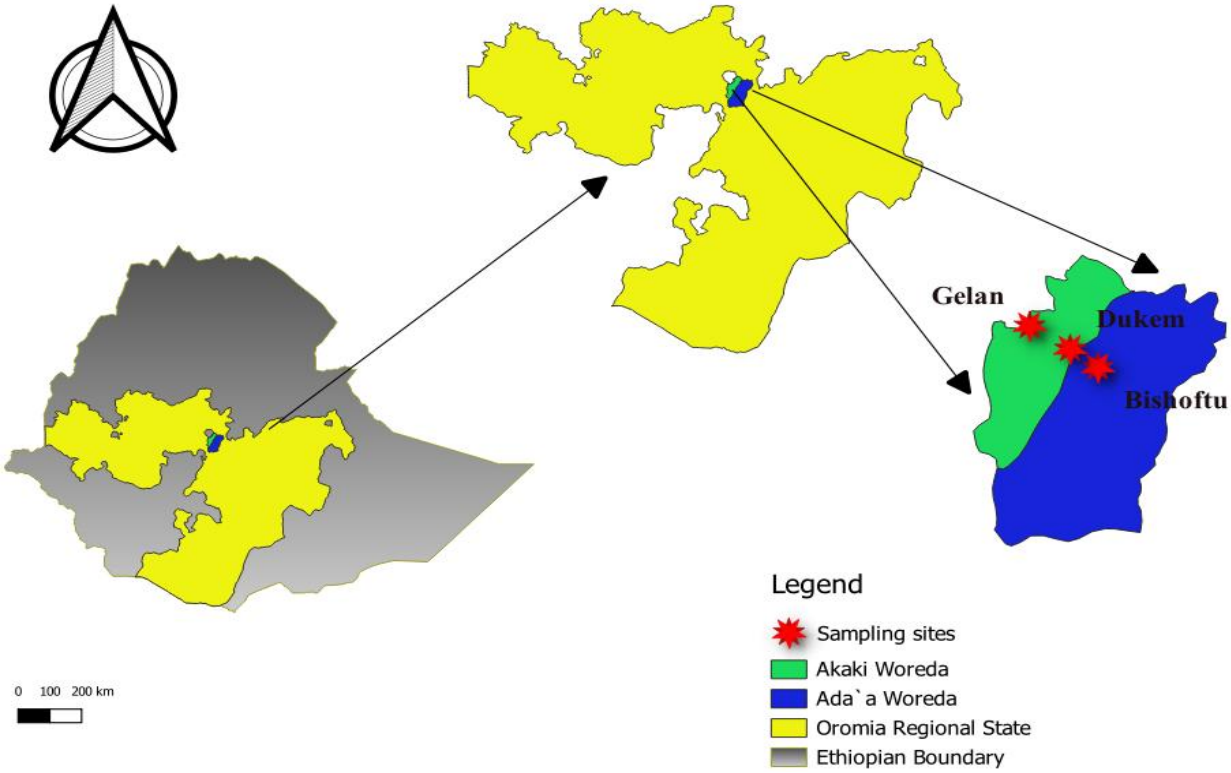

Figure 9: Map of Stud Area

3.2. Study Animals

The study animals were cattle, sheep, and goats of different sexes, ages and body conditions brought to three municipal abattoirs in central Ethiopia. The study animals were different ages and body conditions brought from different parts of the country to the abattoir for the purpose of meat production. In this study, ruminants were categorized into two age groups; young (<5years) and adult (> 5years) for cattle; for sheep and goat <2 years as young and >2 years as adult based on dentations (Meberhatu and Beka, 2011). Similarly, the sample units were grouped into three categories of body condition namely poor, medium and good with equal distribution of the sample among cattle, sheep and goats among body condition scores. The study animals comprised male and female indigenous Zebu cattle that were presented for slaughter from Gimbicu, Wolayita, Jima, Akaki, Arsi Negele, Gelan and Hararghe (Gelan, Dukem) and Adama, Bishoftu, Dukam, and Modjo (Bishoftu) Municipal abattoirs. A total of 137 cattle, 64 sheep and 55 goats were examined following ante-mortem and post-mortem inspection procedures.

3.3. Study Design

The study was conducted from November, 2022 to June, 2023 using a cross-sectional study design to study clinical, pathological and histopathological alterations caused by trematode infections, and their associated risk factors in ruminants slaughtered at Bishoftu, Dukem and Gelan municipal abattoirs in central Ethiopia, using ante-mortem (blood, fecal sample) and post-mortem examination (liver, bile duct, rumen, and gall bladder samples) of each selected animal. Animals were included in the study using systematic random sampling method (used to conduct abattoir survey) where only the first animal was chosen randomly. The prevalence of abattoirs was estimated with respect to a number of risk factors such as host factors (origin, sex, age, species and body condition of the animals).

3.4. Sample Collection and Sample Processing

Blood and feces samples were collected at ante-mortem and liver, bile duct, gallbladder, and stomach samples were collected at post-mortem inspection from the previously identified animals. Post-mortem inspection of infected organs was followed by visualization, palpation, and systematic incision to get well adult flukes based on routine meat inspection guideline by Souls by, 1982.

Gross pathological lesions characterizations were assessed, and a serum sample was analyzed biochemically following routine procedures. The specimens for histopathology were collected from affected livers, bile duct, gall bladders, rumens and reticulum preserved with 10% neutral buffered formalin and transported to laboratory.

3.4.1. Fecal Sample Collection and Examination

Fecal samples were collected randomly between November, 2022 to June, 2023 from cattle, sheep, and goat. Fecal samples were collected directly from the rectum of these ruminants placed in universal bottle and closed tightly. Bottles with samples were labeled with the corresponding animals ID and transported to the parasitology laboratory of the College of Veterinary Medicine and Agriculture. The fecal samples were examined immediately upon arrival in the laboratory, or

stored in a refrigerator at 4°C until the examination. Fecal sedimentation technique described by Antonia *et al*. (2002) and Bowman (2014) was used to detect *Fasciola* species and rumen fluke eggs. The diagnosis of dicrocoeliasis in live animals is usually based on the identification of characteristic eggs in faecal samples, for example by floatation in saturated zinc sulphate solution (Sargison *et al*., 2012)

3.4.2. Ante-mortem Inspection and Post mortem examination of animals

The identification mark at ante mortem were the marking tagged on animals and ear tags 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 were presented for slaughter were male and female local breeds. During the post mortem examination, the infected organ of ruminants was collected and ordered according to the animal code.

Accordingly, the primary examination involves visualization and palpation of the organs; the secondary examination involves more incision of liver; opening of bile duct and hepatic lymph nodes. For generalized liver fluke infection (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 distribution of the lesion to hepatic lobes will be also thoroughly examined.

The livers and gallbladders will be appropriately examined for the presence of *Fasciola* species and all gross pathology, during which the fasciola spp. and all gross pathological changes was noted and recorded. At first liver and bile duct were systematically inspected for the presence of *Fasciola* spp. by applying the routine internal organ inspection procedures. If evidence of fascioliasis is found, they were classified as mature or immature and gross lesions was characterized (Sohair and Eman, 2009).

After evisceration, the liver particularly the bile ducts and the gall bladder, rumen, reticulum and duodenum of ruminants was thoroughly examined (visual, palpation and incision if necessary were made depending on the inspected organs) for trematode identification as these organs are known to be predilection site for liver and rumen flukes (Soulsby 1986).

3.5.5. Tissue processing and examination

After recording the gross changes, 4mm thickness of organ from the infected cattle, sheep and goats were collected and fixed in 10% buffered formalin. Histopathology was done at the Animal Health Institute (AHI), Sebeta, Ethiopia. Tissues were processed by an automated tissue processor machine with its increasing alcohol concentration (70, 80, 95 and 100%, 100%, 100%, 100%), cleared by xylene and embedding was done accordingly. Tand tissue blocks were sectioned at 5µm. The sections were dewaxed, rehydrated, and stained using hematoxylin and eosin (H & E) stain. The slides were mounted with Dibutyl phthalate xylene (DPX) and allowed to dry before examination under a light microscope following the procedures of *Bancroft* and Gamble, 2002.

3.6. Hematological studies and serum biochemical analysis

3.6.1. Blood sample collection

Blood samples were drawn from the jugular vein and kept at +4°C in evacuated EDTA tubes. Within 6 hours, the samples were analyzed. A manual method was used to count erythrocytes and leukocytes. A technical haemometer was used to measure hemoglobin. Hematocrit was determined using capillary tubes (Alexander & Griffiths, 1993). Differential leukocyte counts and cell changes were noticed on blood smears using immersion microscopic observation (Brown, 1976). The hematological parameters were examined at Addis Ababa University, College of Veterinary Medicine and Agriculture in clinical pathology laboratory.

3.6.2. Blood Sample Processing

Hemoglobin (Hb) Determination: The hemoglobin concentration was determined by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobin meter according to the methods described by Dein (1984). Distilled water was poured into a graduated cylinder containing diluted (0.1N) hydrochloric acid and 20 ml of blood sample until the color of the diluted blood sample matched the glass standard. The dilution was measured by the blood sample's hemoglobin level, as described by Philippe (2009).

The Total Erythrocyte Count (TEC) : TEC was carried out using blood dilutions of 1 :200 in Haym's solution. In an RBC dilution pipette, blood was taken up to 0.5 marks, and solutions were taken up to 101 marks. Mechanically, the pipette was completely shaken by holding it between the index finger and thumb, and the first drop was discarded while the second drop was placed on the counting chamber. The cells were stabilized for 1-2 minutes before counting total red blood cells in each mm area at high magnification (40x); and total red blood cells were determined manually using a hemocytometer according to Dein (1984)

Total Leucocyte Count (TLC) : TLC was evaluated by sucking fresh blood up to 0.5 levels in a WBC dilution pipette and then sucking 0.1N HCl up to 11 markings. The pipette was gently rotated mechanically by holding it between the index finger and thumb. Total WBC were counted at low magnification (10x) and determined manually using a hemocytometer following Dein's (1984) methodology.

Packed Cell Volume (PCV) and Blood indices : PCV was calculated using a microhematocrit reader (75x16 mm). According to Ibrahim (2012), capillary tubes were filled with blood and centrifuged at 12,000 rpm for 5 minutes before the percentage of RBC was recorded by a hematocrit reader by comparing the figure to the normal value of the bovine

Differential Leucocyte Count (DLC) : After preparing the blood smear, it was air-dried. The smear was fixed in methanol for 5 minutes before being stained with a working Giemsa solution for 35 minutes. It was then washed with tap water, blotted, and inspected under 100x microscopy for differential leukocyte counts. Each cell (neutrophil, basophil, eosinophil, monocyte, and

lymphocyte) was counted until 100 white cells were enumerated and the percentages of each WBC were determined.

Biochemical Analysis : Blood samples were taken in plain vacutainers and stored at room temperature in an inclined position for 20 minutes. They were then refrigerated to prevent glycolysis and complete clot retraction. The samples were then centrifuged at 3000 rpm for 10 minutes to separate the clear serum, which was carefully collected and stored in serum kit tubes at -20°C until biochemical assessments (Nasreldin and Zaki, 2020), and the blood with anticoagulant was used for Giemsa staining according to Hodzic et al. [38] procedures. Through biochemical examination of serum parameters from infected animals, laboratory tests can detect a variety of clinical-pathological abnormalities. These tests often include measuring serum alanine transaminase (ALT) and aspartate transaminase (AST), which are the most sensitive indicators of hepatocellular damage. Alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum proteins, and bilirubin are also utilised used to assess cholestasis and the liver's synthetic capability (Boone *et al*., 2005).

3.7. Data management and statistical Analysis

The data collected from the study areas was entered in to Microsoft Excel 2016 and the data was coded appropriately and analyzed using STATA version 17 statistical software's. The association of age, origin and body condition with trematode infection in the organ was assessed by Chi square (X^2) test). The gross and microscopic lesions and trematode infections data are also analyzed using Microsoft excel and was presented by descriptive results (frequency and percentages). The statistical analysis system (SAS, 2000) was used to determine the mean, range and standard deviation of hematological data. Logistic regression analyses were used to assess correlation of selected factors and abaittor prevalence of trematoda infection. 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.

3.8. Ethical Clearance

Ethical clearance for this research was obtained from the Addis Ababa University College of Veterinary Medicine and Agriculture Animal Research Ethical Review Committee, and all animal work was conducted according to animal research ethics with Ref No:VM/ERC/18/03/15/2023 (appendix VIII).

4. RESULTS

4.1. Overall prevalence of Fasciolosis and Paramphistomosis

Among the 256 herds (sheep, goat and cattle) at Bishoftu, Dukem and Gelan municipal abattoir, 83(32.42%) and 43(16.80%) animals were positive for fasciolosis and paramphistomosis respectively. The highest prevalence was for goat 23(41.82%), sheep 20(31.25%) and followed by cattle 40(29.20%). Statistically analysis showed that there was no significant difference among species ($p>0.05$) (Table 1).

Among the risk factors considered the overall prevalence was highest for animals in young age group (40.52%), followed by adults 38 (26.21%). Accordingly, fasciolosis prevalence was highest in animals from Jima (52.63%), while the lowest in animals from Adama (11.76%). There was no significant difference in the prevalence of fasciolosis among ruminants based on origin (Table 1). Also, a high prevalence of fasciolosis infections was found in poor body condition animals (47.62%), followed by 32.81% and 28.97% prevalence in medium and good body condition animals. No significant differences were found in fasciolosis prevalence with the body condition of animals (Table 1).

Paramphistomosis was more common in ovine species (34.38%) than in cattle (7.30%), with a significant correlation between animal species but no significant variation based on origin, sex, age, or physical condition (Table 2).

Table 1: The overall Prevalence of fasciolosis based variables.

Risk factors	Categories	Number of examined	Number positive	Prevalence (%)	χ^2	P-value
Species	Bovine	137	40	29.20		
	Ovine	64	20	31.25	2.9067	0.234
	Caprine	55	23	41.82		
Origin	Adama	17	2	11.76		
	Modjo	34	11	32.35		
	Bishofu	49	16	32.65		
	Dukem	40	13	32.50		
	Gelan	17	5	29.41	10.5294	0.395
	Wolayita	12	5	41.67		
	Hararghe	5	2	40.00		
	Arsi Negele	8	1	12.50		
	Gimbichu	27	11	40.74		
	Jima	19	10	52.63		
	Akaki	28	7	28.00		
Age	Young	111	45	40.54		
	Adult	145	38	26.21	5.8955	0.015
Sex	Male	214	67	31.31		
	Female	42	16	38.10	0.7381 P	0.390
BCS	Poor	21	10	47.62		
	Medium	128	42	32.81	2.8038	0.246
	Good	107	31	28.97		
	Total	**256**	**83**	**32.42**		

BCS: Body Condition, x^2: Chi Square

Table 2: The overall Prevalence of paramphistomosis based on variables

Risk factors	Categories	Number of examined	Number of positive	Prevalence (%)	χ^2	P-value
Species	Bovine	137	10	7.30		
	Ovine	64	22	34.38	23.40	0.000
	Caprine	55	11	20.00		
Origin	Adama	17	3	17.65		
	Modjo	34	7	20.59		
	Bishoftu	49	11	22.45		
	Dukem	40	9	22.50		
	Gelan	17	3	17.65		
	Wolayita	12	0	0.00	7.84	0.644
	Hararghe	5	1	20.00		
	Arsi Negele	8	0	0.00		
	Gimbichu	27	4	14.81		
	Jima	19	2	10.53		
	Akaki	28	3	10.71		
Age	Young	111	17	15.32		
	Adult	145	26	17.93	0.31	0.579
Sex	Male	21	33	15.42		
	Female	42	10	23.81	15.42	0.184
BCS	Poor	21	6	28.57		
	Medium	128	20	15.63	2.27	0.321
	Good	107	17	15.89		
	Total	**256**	**43**	**16.80**		

4.2. Abattoir Survey

From a total ruminants examined, 137 cattle, 64 sheep, and 55 goats slaughtered and examined at Bishoftu, Gelan, and Dukem municipal abattoirs and 83 (32.82%) and 43 (16.80%) were found positive for *Fasciola* spp. and *Paramphistomum* spp., respectively. However, the prevalence in

caprine (41.82%) was significantly higher (p>0.05) than that of ovine (31.25%) and cattle (29.20%). Analysis of the frequency of fasciolosis by species in ruminants was considered in the current study and no statistically significant differences (p > 0.05) (Table 1). The highest frequency rate was recorded in cattle (Table 2).

In percentage composition *F. hepatica* was the highest with 38 (45.78%) while *F. gigantica* was the lowest 27 (32.53%). Mixed infection with the two species was recorded in (21.68%) of animals. The prevalence was shown in table 4. The specific prevalence of *Fasciola*, *Paramphistomum and dicroceilum* species was 32.82%, 16.80% and 0% respectively (table 4).

Table 3: Abattoir Percentage of *Fasciola* species in ruminants in selected abaittors.

***Fasciola* species**	**No. of infected liver**	**Percentage (%)**
F. hepatica	38	45.78%
F. gigantica	27	32.53%
Mixed	18	21.68%

Table 4: Specific prevalence of Trematodes in ruminants in selected abaittors

Genus of Trematode Identified	**No positive**	**Prevalence (%)**
Fasciola	83	32.82%
Paraphistome	43	16.80%
Dicroceilum	0	ND
Total	**126**	**49.62%**

NB: % = Prevalence, ND = Non defined

4.2. Hematological profile

Hematological analysis showed fasciolosis and paramphistomosis in infected and non-infected livers, with lower hematocrit, Hb, RBC, and WBC counts. A significant association was observed between hematocrit and hemoglobin in ruminants. Differential leucocyte count (eosinophils were higher; neutrophils, monocytes, and lymphocytes were lower). A statistically

significant association was observed in Overall hematological result fasciolosis and paramphistomosis between eosinophils of the ruminants ($p < 0.05$) (Table 5 And 6)

Table 5: Overall hematological result of mean comparison and Trematoda infection. (By Two-sample t test with equal variances).

Parameters	**Infected**	**Uninfected**	***P-value***
	Mean ± Std. dev.	**Mean ± Std. dev**	
Hematocrit (%)	30.55172±8.08525	38.47143±6.25989	0.0000
Hb (g /dl)	10.30172±3.33127	12.72143±2.40073	0.0000
TEC $x10^6$	8.793319±40.0247	11.67058±62.2627	0.3340
TLC$\times 10^3$	13.33729±3.65653	70.95104±723.215	0.1959
MCV	60.68707±16.2477	60.1135±9.7376	0.6363
MCH	3.307929±0.33795	3.354483±.654985	0.7675
MCHC	33.54526±6.55064	33.08043±3.38238	0.7671

MCV: Volume, MCH: Mean Corpuscular hemoglobin, MCHC: Mean Corpuscular hemoglobin Concentration, Hb: Hemoglobin, TEC: Total Erythrocyte Count, TLC: Total Leucocyte Count

Table 6: overall differential leucocyte count result of mean comparison and Trematoda infection by Two-sample t test with equal variances.

Parameters	**Infected**	**Uninfected**	***P-value***
	Mean ± Std. dev.	**Mean ± Std. dev**	
Neutrophils	34.08621±10.4703	37.33571±10.17658	0.0063
Lymphocytes	48.46552±9.731554	52.34286±9.290493	0.0006
Monocytes	4.439655±2.090512	5.257143±2.926858	0.0061
Eosinophils	8.47±6.758	7.1444±3.021	0.001
Basophils	0.1206897±0.3271796	0.2642857, 4425354	0.0020

4.3. Biochemical Analysis Result

The mean values with the serum biochemical parameters, including alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin in affected ruminant was presented in table 13. From the result, the fasciolosis infected ruminants had statistically reduced ($P<0.05$) total mean of total protein (4.265±0.49) and albumin (3.97±0.65) and AST, ALT and ALP (88.895±5.40, 62.95±11.30, 127.21±12.8) had statistically increased, respectively.

Table 7: Comparisons of serum biochemical analysis in infected and non-infected liver by ruminant Fasciolosis.

No	Liver condition	AST (u/l)	ALT (u/l)	ALP (u/l)	Total protein (g/dl)	Albumin**(g/dl)**
		Mean ±SD				
1	Uninfected	19.53±3.96	22.33±4.10	75 ±5.21	7.393±0.92	6.463±0.31
2	Infected	88.895±5.40	62.95±11.30	127.21±12.8	4.265±0.49	3.97±0.65
	p-value	0.0000	0.0000	0.0000	0.0000	0.0000

4.4. Gross pathological lesions

Gross pathological lesions often identified at post-mortem in this study include firm, pale, swollen and irregularly outlined liver with tough texture. Adult liver fluke and calcification were observed in bile duct and liver parenchyma. In chronic cases, livers were smaller in size, had focal and multifocal nodules, as well as small hemorrhages on the parietal surface. When a piece of the bile duct was cut through, there was abnormal fluke migration and, in some cases, signs of calcification. Postmortem examination revealed thickened and swollen bile ducts with adult flukes, degraded debris, and cholangitis.

In cases of acute fluke infection, the liver was greatly enlarged (swollen), with rounded edges, a pale color, and numerous small and large hemorrhagic spots dispersed around the parietal surface. Flukes were also seen in the migratory tubes of the parenchyma, and there were enlarged hepatic

lymph nodes and abnormally cloudy thick fluid that flowed up on cutting. A viscous yellow substance poured from the cut ends of hard, dark, and brown-colored bovine livers that had many soft abscesses and were surrounded by hyperemic zones on the surface. In chronic cases reduced size of livers, focal and multifocal nodules, and pin-point hemorrhages on the parietal surface of the liver were also examined. Distension of the gall bladder with bile from pressure exerted by the nodules.

The gross pathological changes of the liver in chronic Fascioliasis characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. In acute form, the livers were slightly swollen or enlarged with rounded edges and the color became paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface of the left, right and caudate lobe (Figure 10-14). Following a gross examination of the rumen viscera, ruminal papillae atrophy and ulceration at the site of fluke attachment was seen (Figure 15-16).

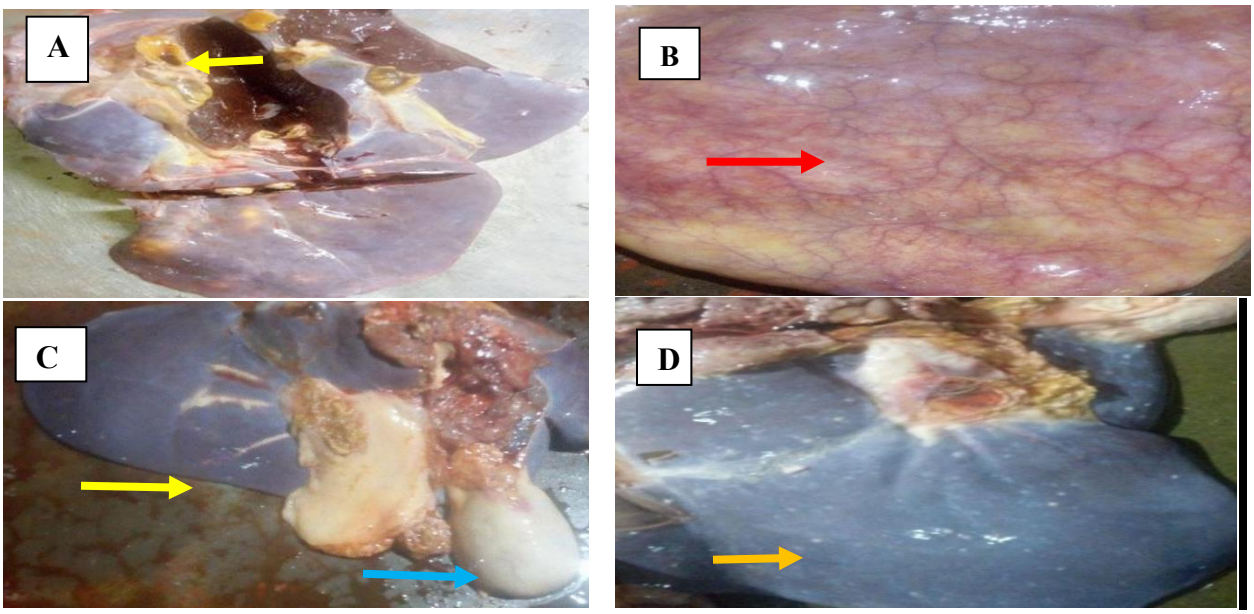

Figure 10: Gross pathology of liver and gall bladder in bovine. (A) Engorgement, hyperplasia of bile duct and paleness in some areas which was due to the necrosis (yellow arrow). (B) Cholecystitis (red arrow) (C) Chronic liver fluke infection in cattle, with bile duct (yellow arrow) and gall bladder distension (blue) (D) Calcification (orange arrow).

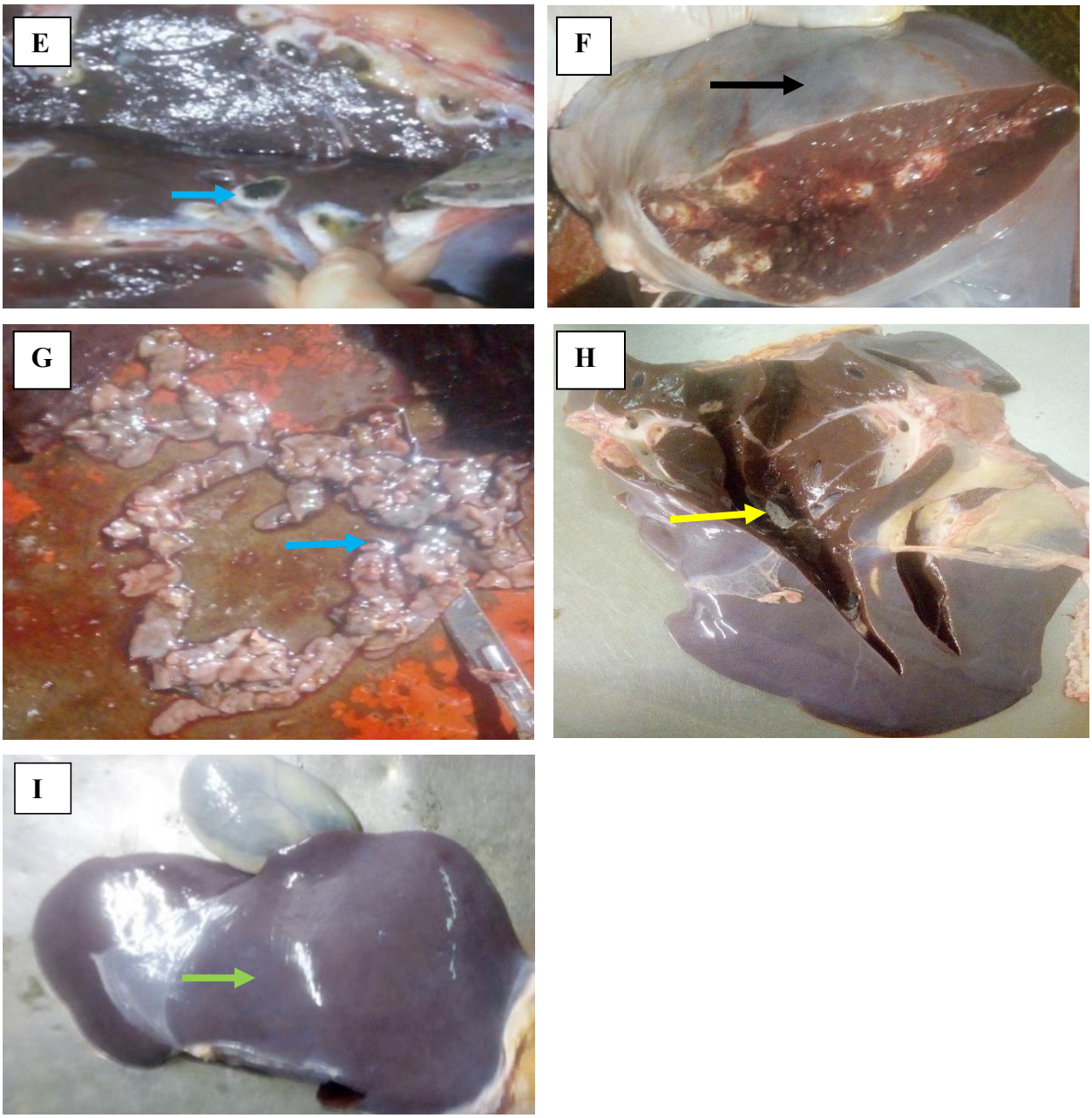

Figure 11: Gross pathology of adult Fasciola and normal liver. (E) Heavily Infected liver (pipe stem appearance) and Bile duct of Bovine filled with blackish brown exudates infected with *Fasciola gigantica.* (F) Fibrosis and Necrosis of Liver (G) Adult liver fluke infestation from bile duct in chronically infected cattle. (H) Fibrosis of bile duct (I) normal bovine liver.

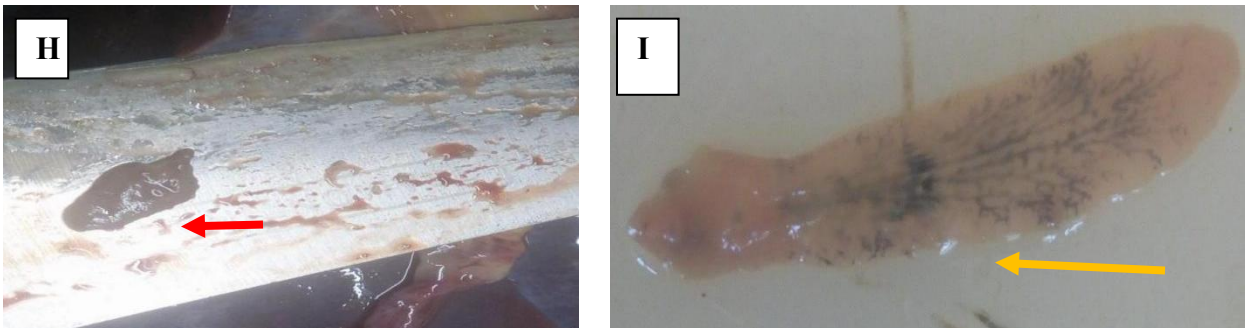

Figure 12: Adult *Fasciola Hepatica* and *Gigantica*

The gross pathological changes of the liver in chronic Fascioliasis characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. In acute form, the livers were slightly swollen or enlarged with rounded edges and the color became paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface of the left, right and caudate lobe.

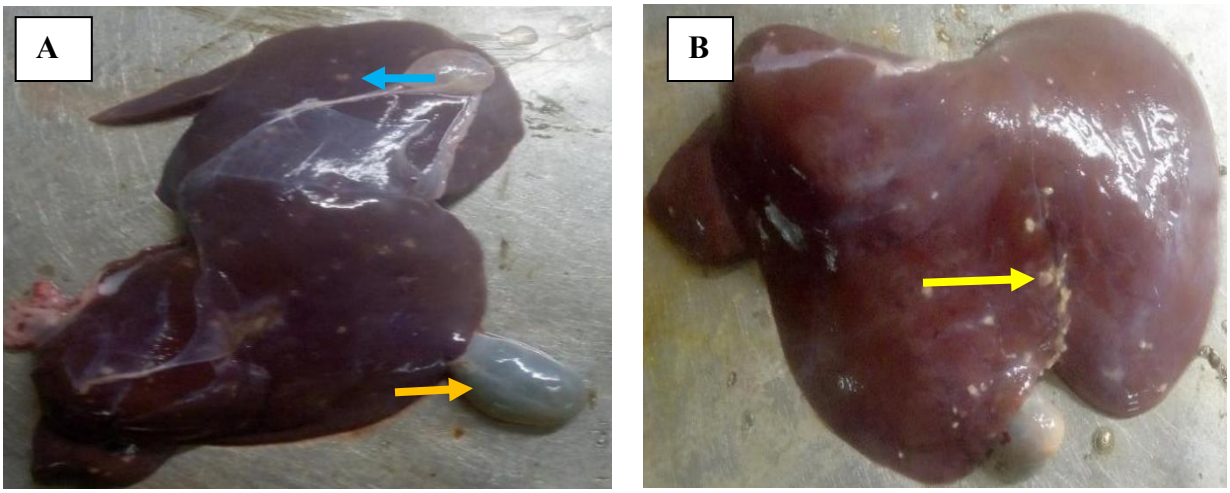

Figure 13: Gross pathology of liver in caprine. (A) fibrosis of liver and distended of gall bladder (B) Calcification of liver

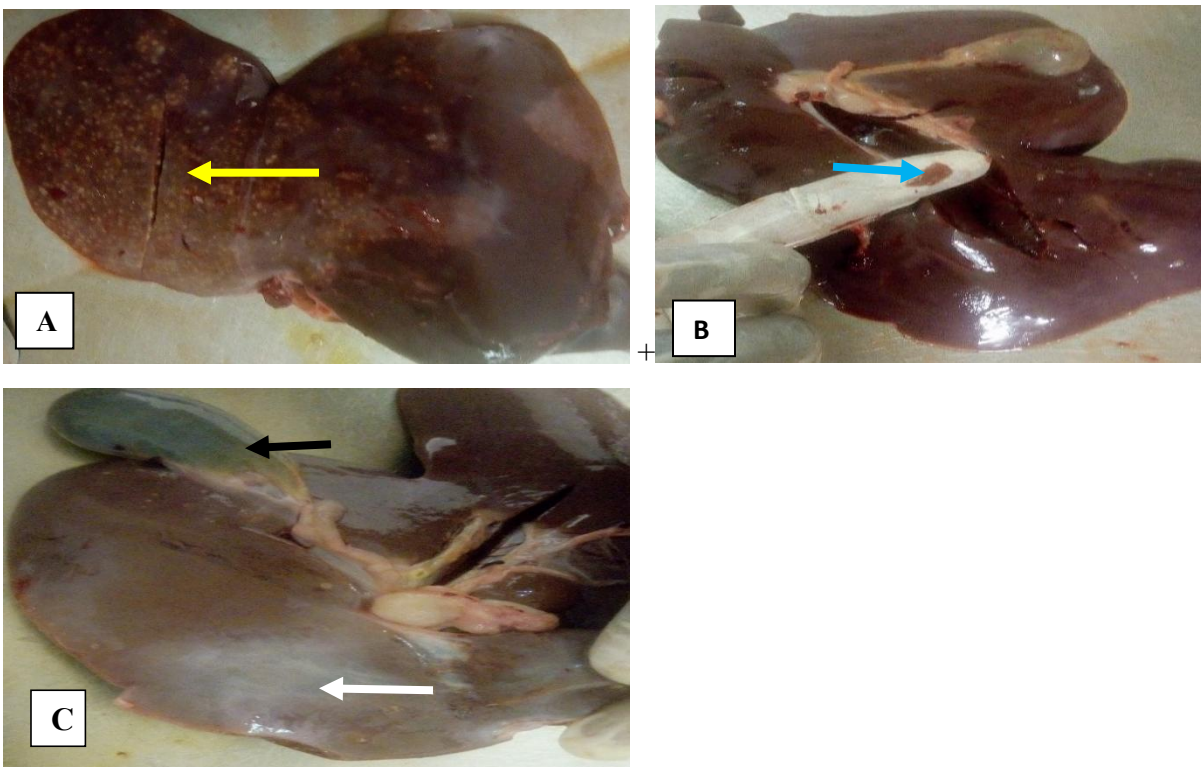

Figure 14: Gross pathology of liver in ovine. A) second stage of cirrhosis B) Adult Fasciola hepatica in ovine bile duct (C)Fibrosis, Distended Gall Bladder

As gross pathology examination, ruminal papillae atrophy and ulceration at the site of fluke Attachment.

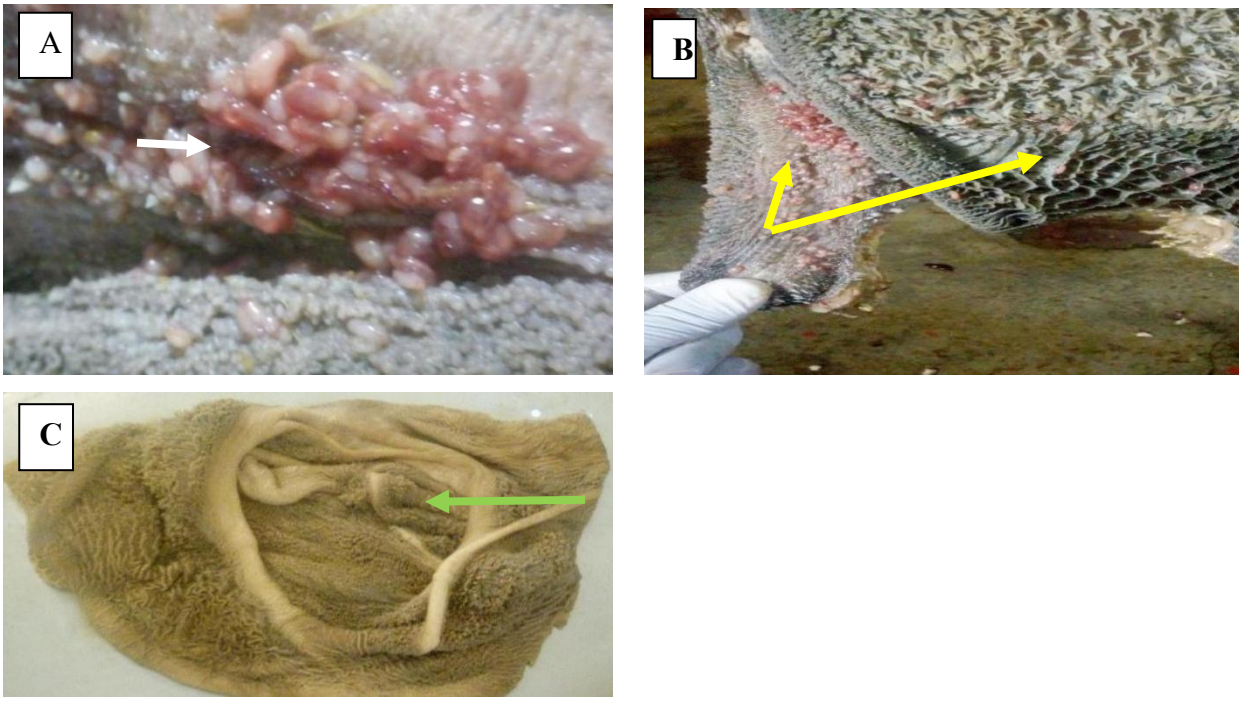

Figure 15: Growth pathology of rumen. (A) and (B) Gross pathology of adult rumen fluke attached to rumen papillae and reticulum in bovine liver (C). Normal bovine rumen.

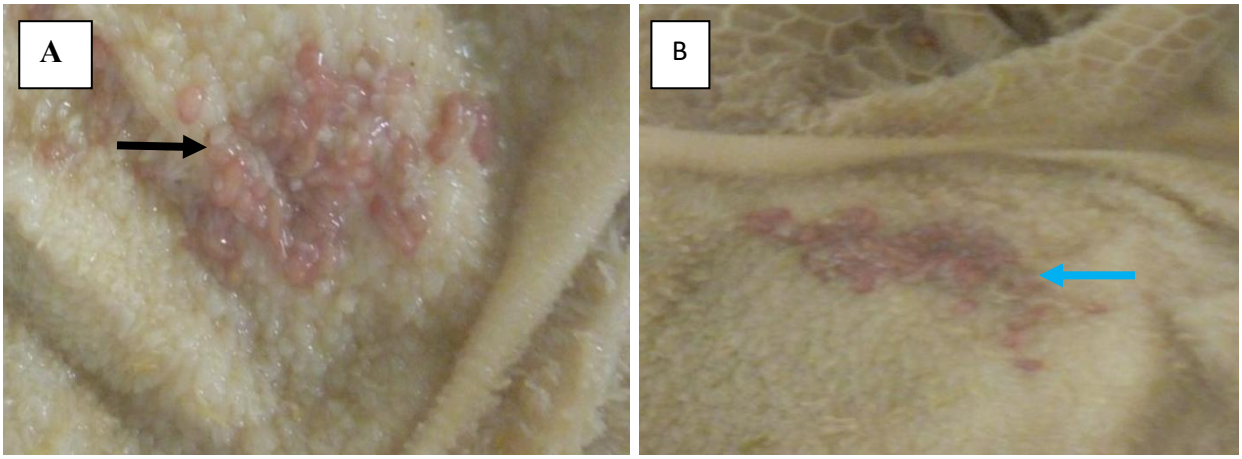

Figure 16: Adult rumen fluke attached to rumen papillae in small ruminants

4.5. Histopathological Results

Microscopically, various histopathological changes were observed in the liver and bile duct sections of Fasciola-infected ruminants. The characteristic histopathological lesions of fasciolosis showed hemorrhages in the portal and central veins, purulent exudates in the liver parenchyma, hepatocytic wall dilatation with infiltration of mainly eosinophils and lymphocytes, and calcification. A liver section also showed necrosis of hepatocytes due to chronic fasciolosis. Some sections also revealed the hypertrophy of the central wall, hemorrhages in the interstitial space, infiltration of eosinophil cells, and thickening of the hepatic wall. Inflammatory cells released in response to fasciolosis, eosinophilic cells, had shown a greater number than other leukocytes. Portal fibrosis, characterized by extensive fibrous connective tissue proliferation in the portal area with infiltration of mononuclear inflammatory cells and compression atrophy of hepatocytes adjacent to the fibrosis zone, was recorded. Congestion and edema of the portal vein, granulomatous, fibrosis enclosing inflammatory cells, thinly contained exudates in the central vein, cystic granulation, disarrangement of the portal triads, thickening and narrowing of the portal triads Some sections showed cellular infiltration, fluid-filled areas, degeneration, and fragmentation of the wall in the bile duct (Figure 17-31).

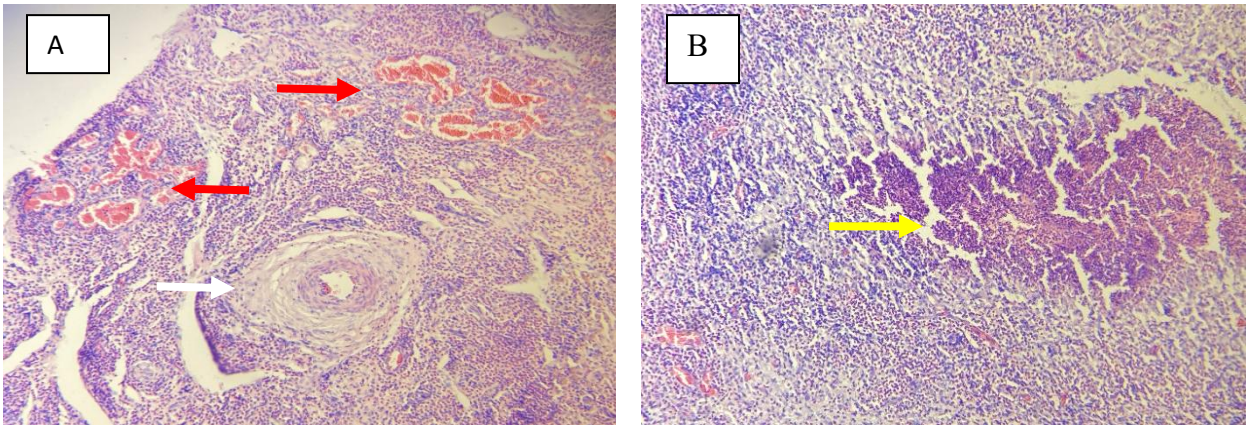

Figure 17: Histopathology of Liver with Fibrosis and granulomas. (A) hemorrhages in the portal vein, fibrosis encapsulating in the central vein (white arrow), and hemorrhage surrounded by infiltration of inflammatory cells especially lymphocytes; and (B) Multiple, multifocal, eosinophil-dominated inflammatory cells aggregation.

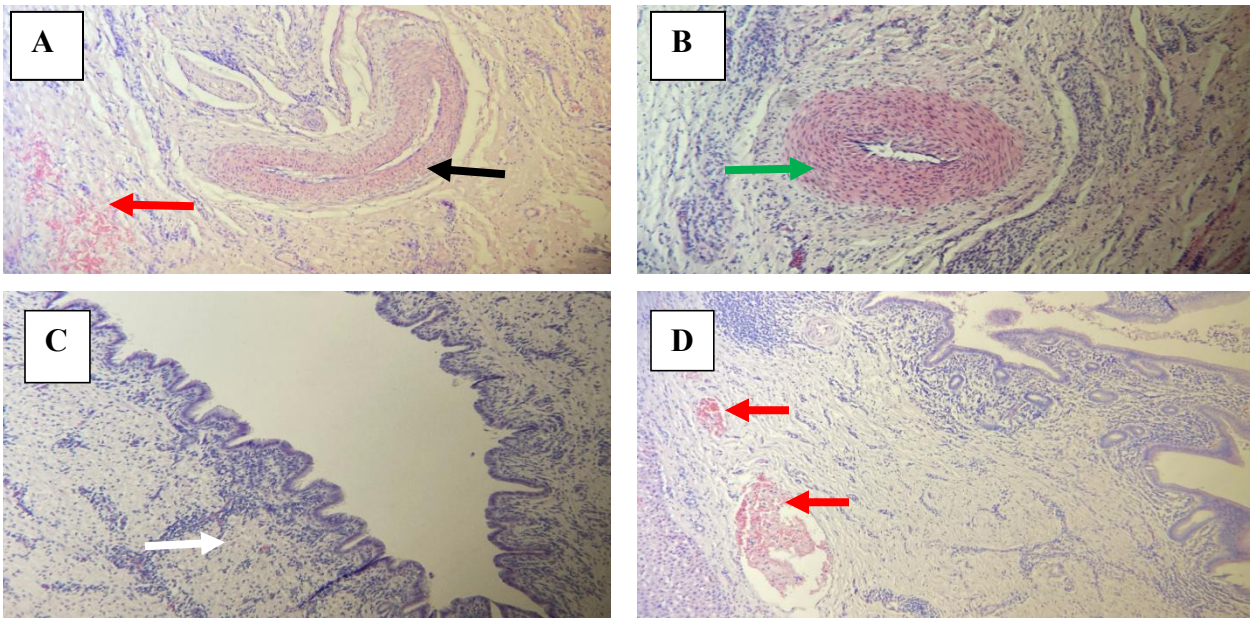

Figure 18: Histopathology of liver with different lesion in bovine. (A) hypertrophy of central vein with their wall (black arrow), hemorrhages in interstitial spaces and (B) infiltration of eosinophilic cells with thickening of hepatic artery, lymphocytes and few plasmas cells (C) extra hepatic biliary duct hyperplasia, hepatocytes necrotized, infiltration of inflammatory cells (D) hemorrhage, congestion in central vein, infiltration of inflammatory cells, connective tissue multiplication (fibrosis).

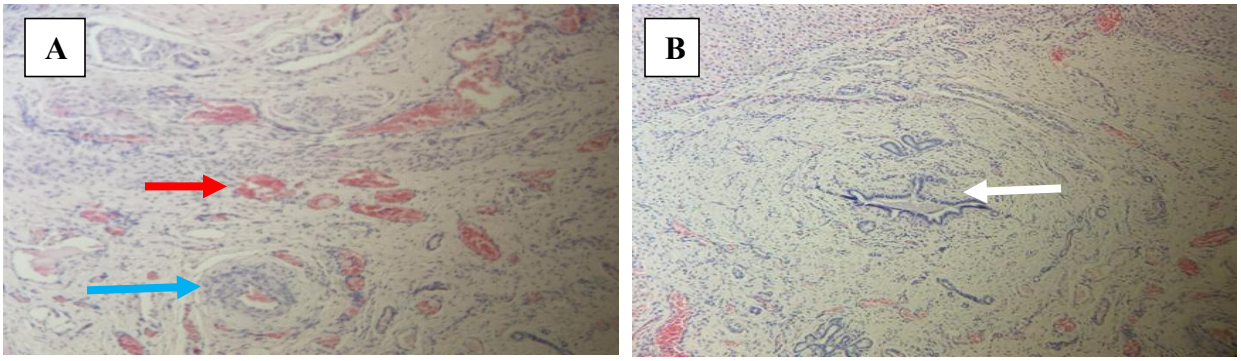

Figure 19: histopathology of liver with hemorrhages, fibrosis and dilatation of bile duct. (A) hemorrhages (red arrow), fibrosis enclosing inflammatory cells (blue arrow) and B) thinly, dilatation and proliferation of bile duct.

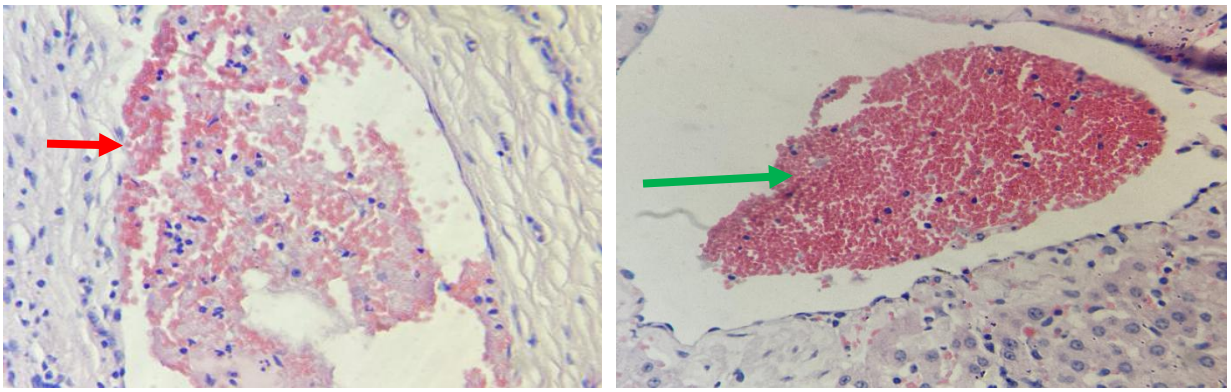

Figure 20: histopathology of liver and bile duct with different lesion (A) the RBCs are inside the central vein (B) congestion with Infiltration of Inflammatory Cells in central vein.

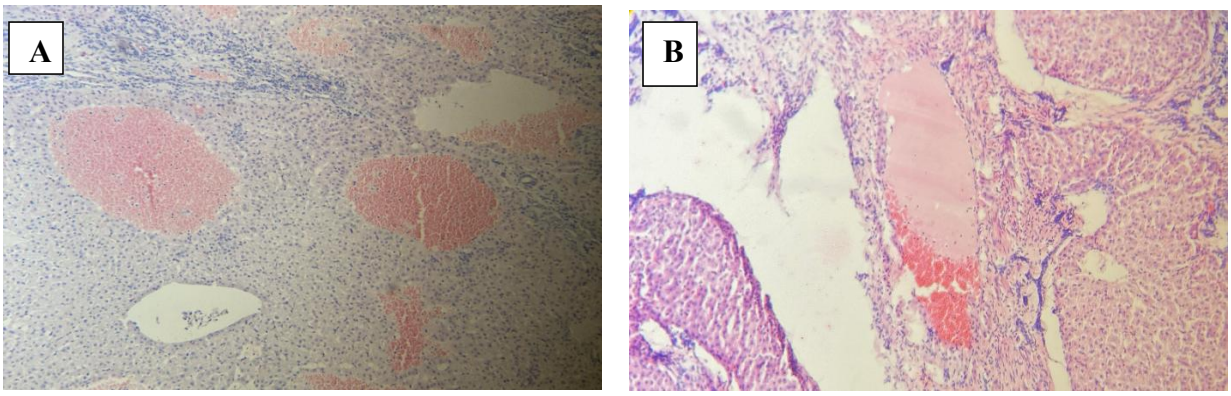

Figure 21: Histopathology of Liver with Different Lesion. A) periportal hepatitis, infiltration of inflammatory cells in periportal area mainly lymphocyte, hepatocyte is necrotized with nuclear rhexis, some and multiple hemorrhages with free RBC out of the vessels and multiple hepato necrosis. (B) cystic granulation and hemorrhage.

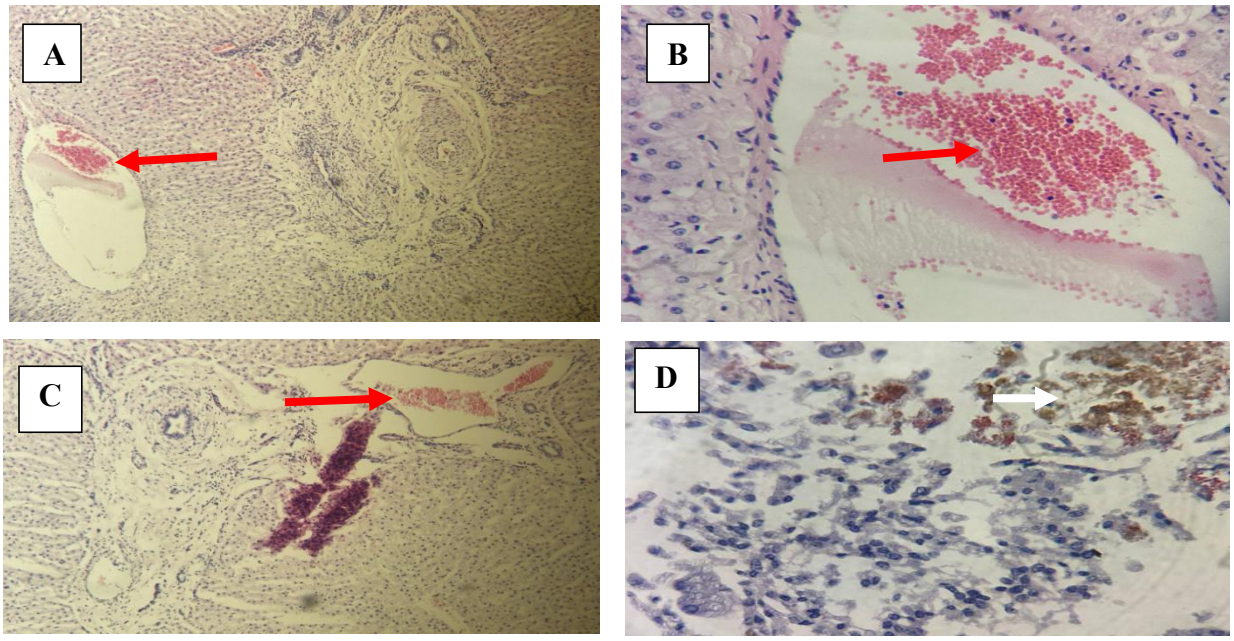

Figure 22: Histopathology of bovine liver with congestion and infiltration of cells. (A) congestion, thinner vein walls and a few infiltrations of inflammatory cells (B) Magnified (A) with congestion and some infiltration of lymphocytes (C) congestion, infiltration of inflammatory cells (red arrow) (D) histopathological section of bovine liver infected with *Fasciola spp*, showed hemosiderin pigmentation (white arrow)

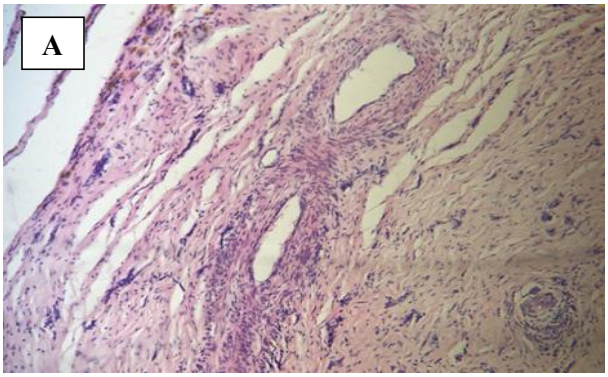

Figure 23: Histopathology of portal triads derangement and another lesion. (A) derangement of portal triads, thickening and narrowing of portal triads.

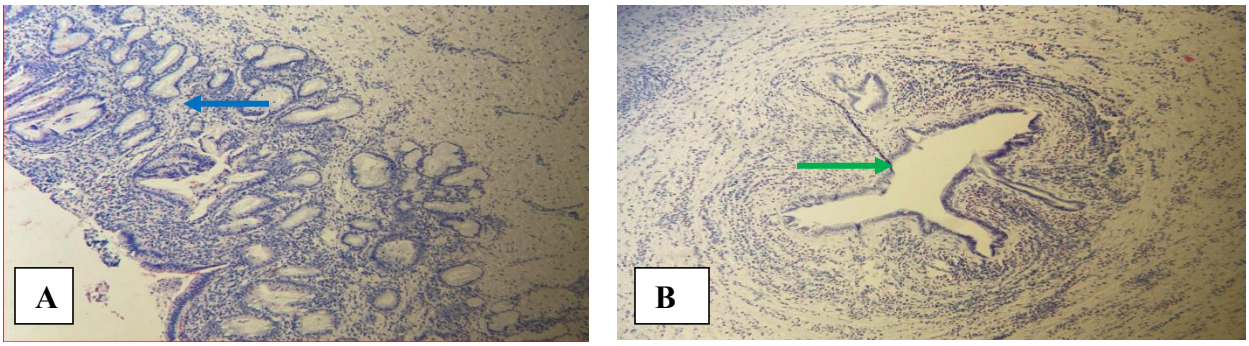

Figure 24: Histopathology of bile duct (A) infiltration inflammatory cells, multiple small ducts are formed and fluid filled area of bile ducts (B) distension of bile duct enclosed by inflammatory cells (green arrow).

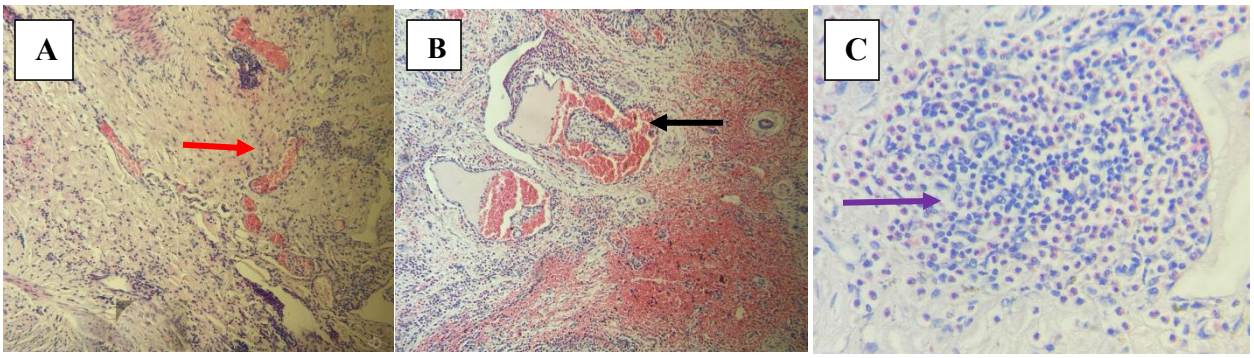

Figure 25: Histopathology of acute hemorrhages hepatitis. (A) Hemorrhages and infiltration of inflammatory cells (B)Hemorrhagic area of hepatic ducts and (C) infiltration of inflammatory cells

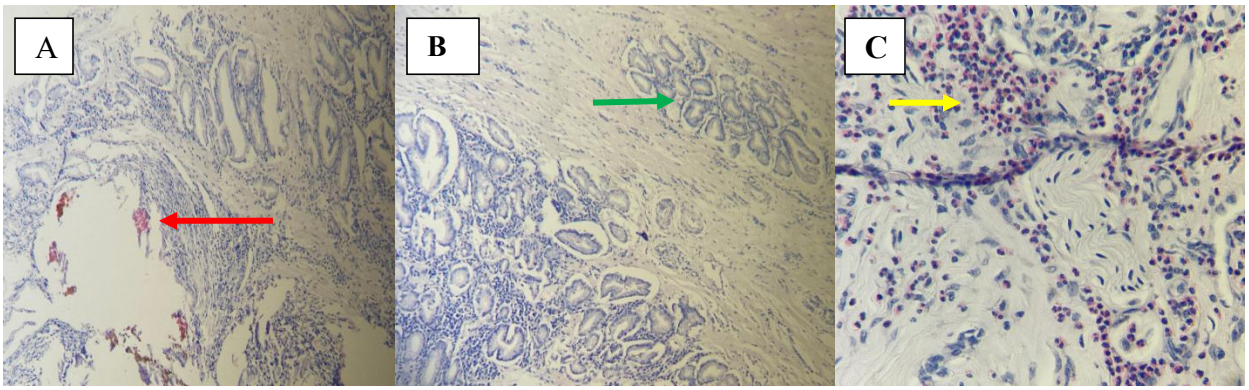

Figure:26: Histopathology of bile duct. (A) Hemorrhages in bile duct (B) free and multiple peribiliary glands and (C) infiltration of eosinophils.

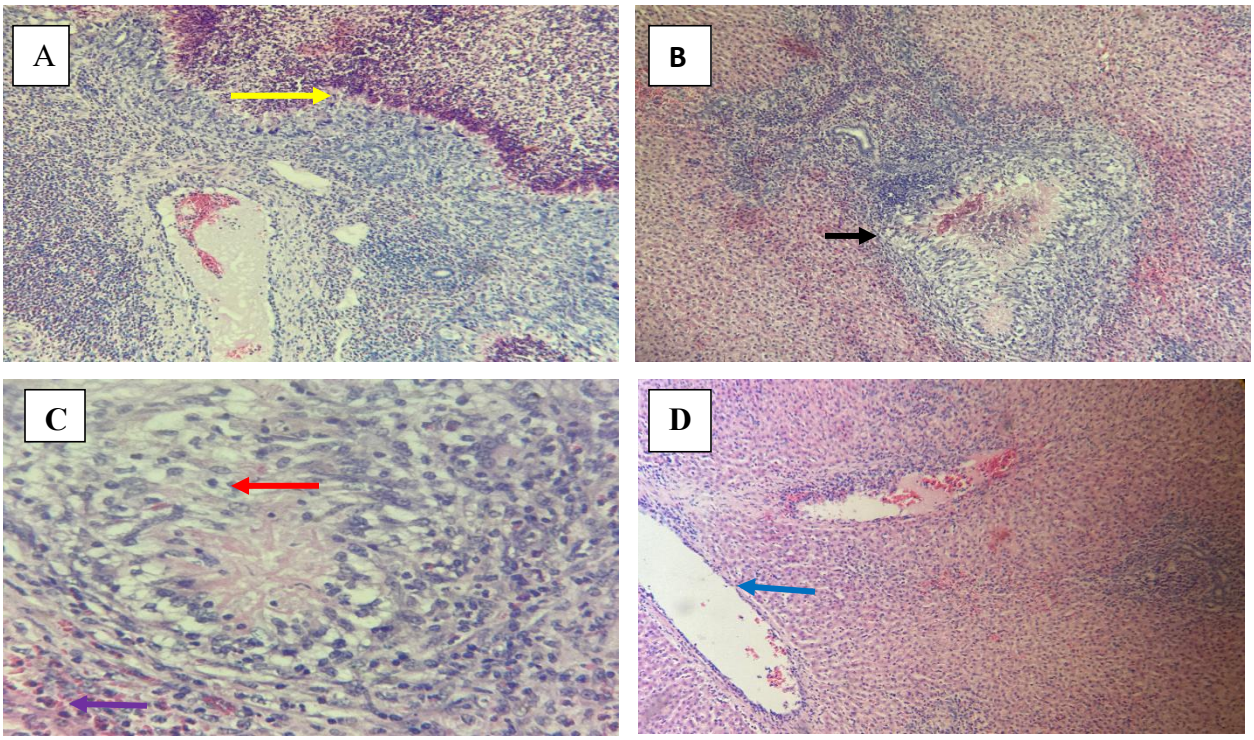

Figure 27: Histopathology of liver. (A) necrosis, (B) Granulomatous, (C) infiltration of eosinophilic and lymphocytes and (D) Thickening of central veins.

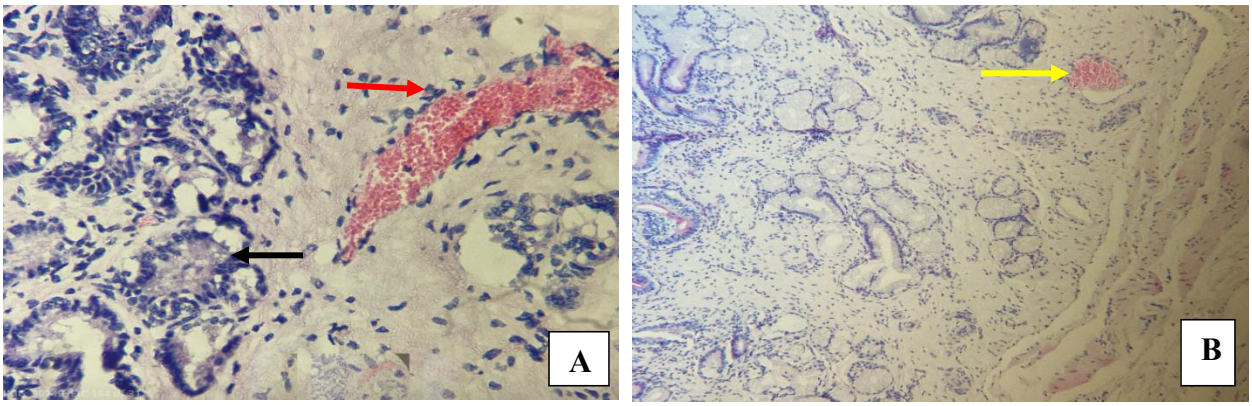

Figure 28: Histopathology of bile ducts. narrowing of hemorrhagic region in bile ducts and infiltration of inflammatory cells

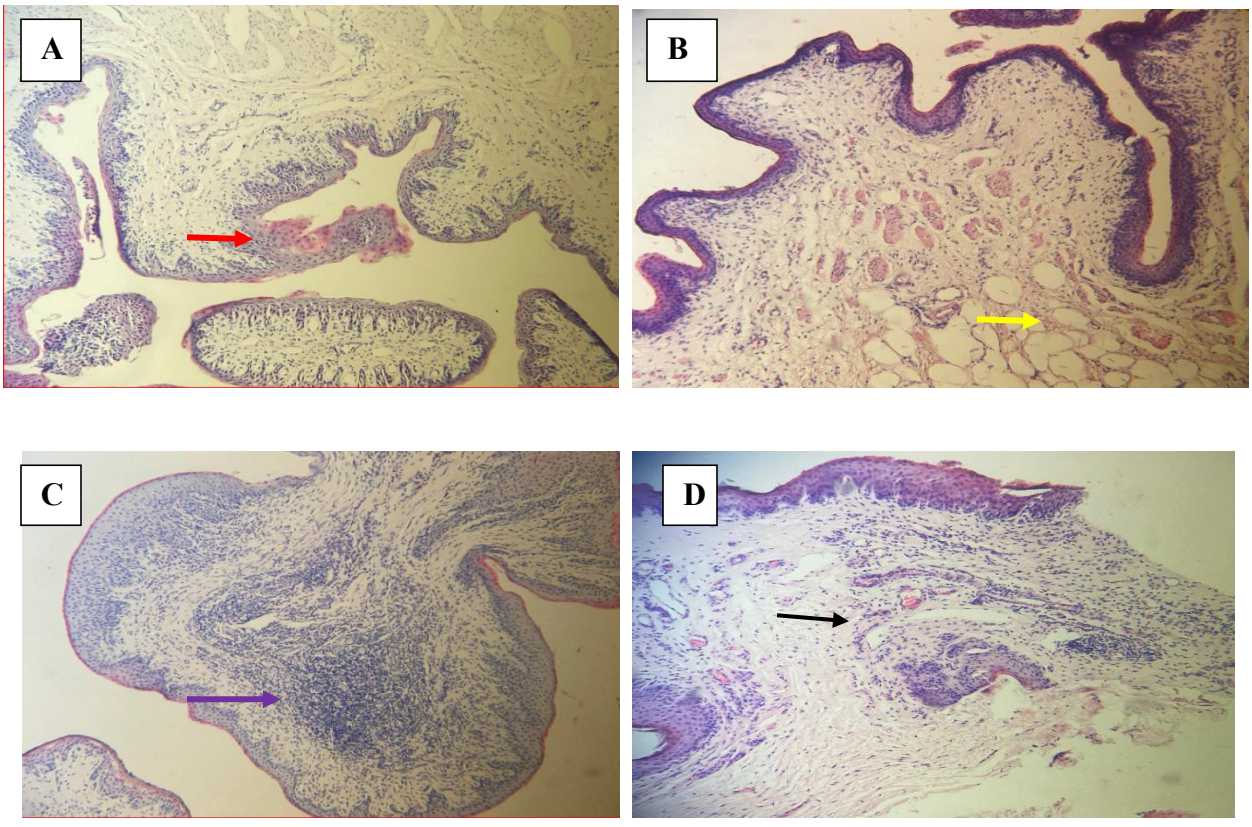

Figure 29: histopathology of rumen. (A)hemorrhage in muscular (B)glandular degeneration, loose of villi and microvilli and disarrangement of the muscular and submucosa layer (C) Infiltration of muscular cells (D) Disarrangement of muscular and submucosa layer.

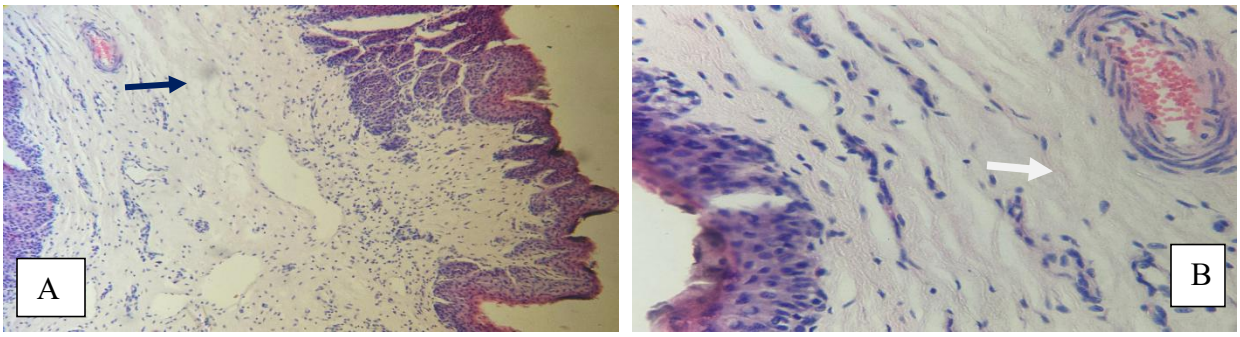

Figure 30: Histopathology of rumen with different lesions. (A) Degeneration of muscular layer, hypertrophy of papillae (B) devoid of villi and edematous region in the glands

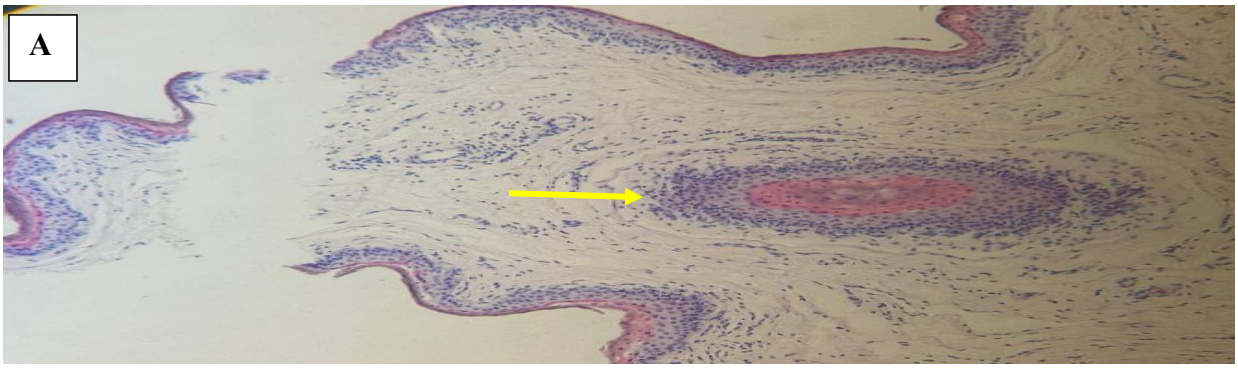

Figure 31: Histopathology rumen with granulated regions encapsulated with eosinophils infiltration and external surrounded by leucocytes

5. DISCUSSION

From the total of 137 cattle, 64 sheep and 23 goat that were brought to the Bishoftu, Dukem and Gelan municipal abattoir and slaughtered, the overall prevalence of fasciolosis was 32.42%.

This study's finding is aligned with the result of Fikirtemariam e*t al*., 2013 who indicated 36.72% for the overall prevalence of fasciolosis in Bahir Dar. The present finding was found to be lower than the results of previous study conducted by Tolossa and Tigre (2007) who reported a prevalence of 46.58% on postmortem examination of livers from Jima and Agaro. Although, the result of this study on the overall prevalence of bovine fasciolosis is notably lower than the finding by (Yilma and Mesfin, 2000) who reported 90.65%, and the study by (Tadele and Worku, 2007) that reported 46.58%, and the study by (Dejene, 2008) that reported 50.98% at Gondar abattoir, Ethiopia, Jima municipal abattoir, and Arsi, Ethiopia respectively.

However, the results of this study on the Investigation of clinical pathology, characterize of the gross and histopathological lesions in different tissues of ruminants at three municipal abattoirs were remarkably higher than the results of studies by Edilawit *et al*., (2012), Mulat *et al*., (2012), Fetene and Addis, (2014), and Birhan *et al*., (2019) at Wolaita Sodo abattoir, Gondar ELFORA abattoir, Dangila municipal abattoir, Debre tabor municipal abattoir who reported 25.33%, 29.75%, 30.21%, and 28.6% respectively on overall prevalence of bovine fasciolosis. The variation observed in these studies could be due to the increasing climate change, the availability of a suitable habitat for the vectors, the method employed for the diagnosis and the increasing trend of animal deworming by farmers.

The overall prevalence of ruminants paramphistomosis recorded in the current study is 16.80%. This study's finding is aligned with the result of Buzuwork *et al*., 2022 who indicated 20.8% for the overall prevalence of fasciolosis in Bishoftu municipal abattoir. The overall Prevalence of paramphistomosis observed in this study is lower than the result of Abebe *et al.*, (2011) which was 57.52% in and around Jima; 40.1% by Melaku and Addis, (2012) at Bishoftu and 51.82% reported by Ayalew *et al.,* (2016) at Gondar Elfora Abattoir. These variations seen in prevalence between this and other similar studies elsewhere probably may be attributed to mainly to the

differences in the geographical locations, climatic and ecological conditions such as altitude, rainfall, and temperature.

The prevalence fasciolosis noticed in the current study which is 32.42% and specifically, 29.20% in bovine,31.25% in ovine and 41.82% in caprine, respectively showed no statistically significant association between the occurrence of fasciolosis and specific species which means, those animals are equally susceptible to fasciolosis.

The result of the current study showed that age had a significant effect on the prevalence of fasciolosis in ruminants with a prevalence of 26.21% and 40.54% in adult and young respectively. This study result was in agreement with (Yusuf *et al*., 2016) at Haramaya municipal abattoir with a prevalence of 16.3% and 73.5% in adult and young animals respectively; being higher in young animals than the adult. There was a decrease in infection rate as age increased. This may be because of increased acquired immunity with age which is manifested by a humoral immune response and tissue reaction in the ruminant's liver due to previous challenges. These are in agreement with experimental study conducted by (Radostits *et al*. 2007) which confirmed the occurrence of higher infection rate in younger animals. But difference prevalence between the two studies may be due to variation of the sample size. The current result study is in disagreement with the findings of Mariam *et al*., (2014) which showed that, age had no effect on the prevalence of fasciolosis.

This is in agreement with the findings of (Keyyu *et al.,* 2006); who reported a significant difference in prevalence between the age groups. The results of the present study indicated that body condition of the animal had no significant association with the occurrence of fasciolosis and paramphistomosis with the prevalence of 47.62%, 32.81% and 28.97% for fasciolosis and 28.57%, 15.63% and 15.84% for paramphistomosis with poor, medium, and good body condition respectively. This finding is in agreement with the finding of (Aragaw *et al.*, 2012) at Addis Ababa abattoir and (Turuna, 2019) at the Nekemte municipal abattoir who reported a high prevalence of fasciolosis and paramphistomosis in poor body conditioned animals than medium and good body conditioned animals. The prevalence of fasciolosis and paramphistomosis was higher in the animals with poor body condition because this body condition in cattle is manifested when fasciolosis and paramphistomosis reaches at their chronic stage. However, this

finding is in agreement with the finding of Phiri *et al*., (2005) and Gojam and Tulu, (2018) who reported that the prevalence of bovine fasciolosis does not show a statistically significant association with the body condition of animals.

According to the findings of this study, the origins of the cattle had no significant effect on the prevalence of ruminants fasciolosis, (P>0.05); being higher in Jima (52.63%) than Bishoftu (32.65%). The highest prevalence occurred at Jima due to favorable environment for fasciolosis and intermediate host. But it is not significant effect on the prevalence of paramphistomosis. The difference of result may be due to the variation in sample and management system.

The hematological examination resulted, PCV, Hb, WBC, RBC, neutrophil, monocytes and lymphocytes were found minimum in the infected liver. however, eosinophil, were found maximum in the affected liver. Total protein and albumin in this study is lower in infected liver of ruminants. The damage and/or necrosis, degeneration of hepatocytes in this study were due to increased plasma concentration and highly increased concentration of ALT level in cytoplasm. The present report is in agreement with the result of 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 liver and its increment is associated with high deaths of hepatocytes due to liver fluke which in agreement with report of Kilad *et al*. (2000) supposed increase in ALT is due to hepatocyte death from liver fluke infection

Histopathology of infected liver was carried out. Microscopically, the infected liver tissue appeared pale in color, greatly swollen indicating fibrosis, large patches scattered over the parietal surface and the pipe stem appearance of the liver were noticed. There was a dilation in the central vein (Talukder *et al*., 2010).

Gross fibrosis of bile duct was as a result of migrating fluke in the liver tissue. Damage to the hepatic cells is as a result of the method of feeding by the premature parasites and it is most common in bovine cirrhosis (Njoku-Tony and Okoli, 2011). Haroun *et al.,* (1986) also reported the degenerative and necrotic changes in hepatocytes, associated with hemorrhage, fibrosis, increased lobulation of the liver, mononuclear cell infiltration with hemosiderin deposition in fluke tracks and portal areas and the formation of granulomata around fluke eggs and fluke

remnants in the sheep naturally infected with F. gigantica. Also, Odigie *et al.,* (2011) reported gross examination of infected livers and revealed that the capsules were enlarged; this enlargement is due to the presence of fluke (both adult and young fluke) and was seen in the bile duct.

6. CONCLUSION AND RECOMMENDATIONS

The results of this study showed the clinical pathology, gross and histopathological changes that accompany the infection of trematodes in ruminants. In addition, it gives an insight a better understanding into the transimission and pathogenesis of the disease. The study confirmed that fasciolosis and paraphistomosis *were* the main diseases of ruminants affecting their liver, rumen as well as reducing weight gain. This may be due to the fact that the origins of animals have suitable ecological condition for the existence and multiplication of the intermediate host. Therefore,the study concluded that the fasciolosis and paraphistomosis causes severe economic losses from reduced carcass weight and condemned organ and causes pathological lesions in liver and rumen of ruminants. Based on aforementioned, the following recommendations are forwarded:

- Detailed ruminant municipal abattoir inspection should be implemented.
- Implement Management practices such as hygienic conditions and strict biosecurity from the prevalence of the intermediate host prevalent.
- Strict supervision is essential for the diagnosis and control of flukes' implementation.
- Every animal owner should be able to regularly treat their animals with proper anthelmintic.
- Solid epidemiological research of the current state of trematode infection in the country should be conducted.
- Serious care and attention are necessary of both veterinary personnel and public health planners to ensure that severely damaged organ is not passed on for human consumption due to their distorted nutritional values and health risks.
- Formulating functional meat inspection policies for organs and carcass approval/ rejection.

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8. APPENDICES

Appendix I: Sample Collection Sheet

No	Bovine code	Species of animal	Age	Sex	Body condition	origin	Blood sample	Fecal sample	Postmortem finding
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

Appendix II: The hematological parameters and fecal test of ruminants recording sheet

					Differential Leucocyte Count (%)					Blood Indices		
bovine code	**PCV (%)**	**Hb(g/dl)**	**TEC (10^6)**	**TLE (10^3)**	**Monocyte**	**Lymphocyte**	**Neutrophil**	**Eosinophils**	**Basophils**	**MCV**	**MCH**	**MCHC**
1												
2												
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10												

Appendix III: Ethical clearance sheet

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/18/03/15/2023

Name and affiliation of applicant: **Addisu Wakuma Boke (BSc, MSc student)**
Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Investigation of clinical pathology, gross and histopathological alterations caused by Trematode infections in ruminants slaughtered at three municipal abattoirs, Central Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Abattoir Survey**
Target animal species:	**Ruminants**
Number of animals involved:	**No live animal use**
Study area:	**Central Ethiopia**

Minutes No. and date of review: **VM/ERC/03/15/022, 25/01/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Adisu Wakuma.

Professor Getachew Terefe (DVM, PhD)
Chairman

The Federal Democratic Republic of Ethiopia * Addis Ababa University College of Veterinary Medicine and Agriculture *

Signature

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Appendix IV: The procedures of blood hemoglobin determination (Ibrahim, 2013).

1. Take 0.1N HCl (1%) into central graduated tube up to mark
2. Suck the blood exactly up to mark 20 (20µl) with the help of sahlis 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 minutes.
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

Appendix V: The procedures of blood PCV determination (Bancroft and Gamble, 2002; Ibrahim, 2013).

1. The blood is filled in to a micro hematocrit tube to (3/4th) and seals it with sealer.
2. Centrifuge the filled hematocrit tube in a hematocrit centrifuge at 12000 rpm for 4-5 Minutes.
3. Read the value (the tube) with hematocrit reader and record the result.

Appendix VI: Differential white blood cell count

Materials and reagents:

- ✓ Un-coagulated whole blood
- ✓ Microscope
- ✓ Glass slides
- ✓ Immersion oil,
- ✓ Giemsa stain
- ✓ Xylene
- ✓ Methanol alcohol,
- ✓ a piece of gauze,
- ✓ Distilled water and Staining rack

Procedure:

- A drop of blood is placed near to the one end of a clean glass slide.
- A second slid is hold at 45^0, attached to the drop of blood, then pushed along the surface of the first in forward direction to form a thin smear. The smear is dried in the air.
- The smear is fixed in methanol for 3-5 minutes.
- The smear is stained with giemsa staining dye and allowed for 30 minutes.
- The slide is rinsed with water and dried in air.
- The stained smear is put on the microscope and one drop of oil is added on the smear. Using oil-immersion objective (100x), 100 WBCs are counted by Battleship method.
- Each type of leukocyte is identified, and results are recorded as % of the total leukocyte count then changed to absolute for more accuracy.

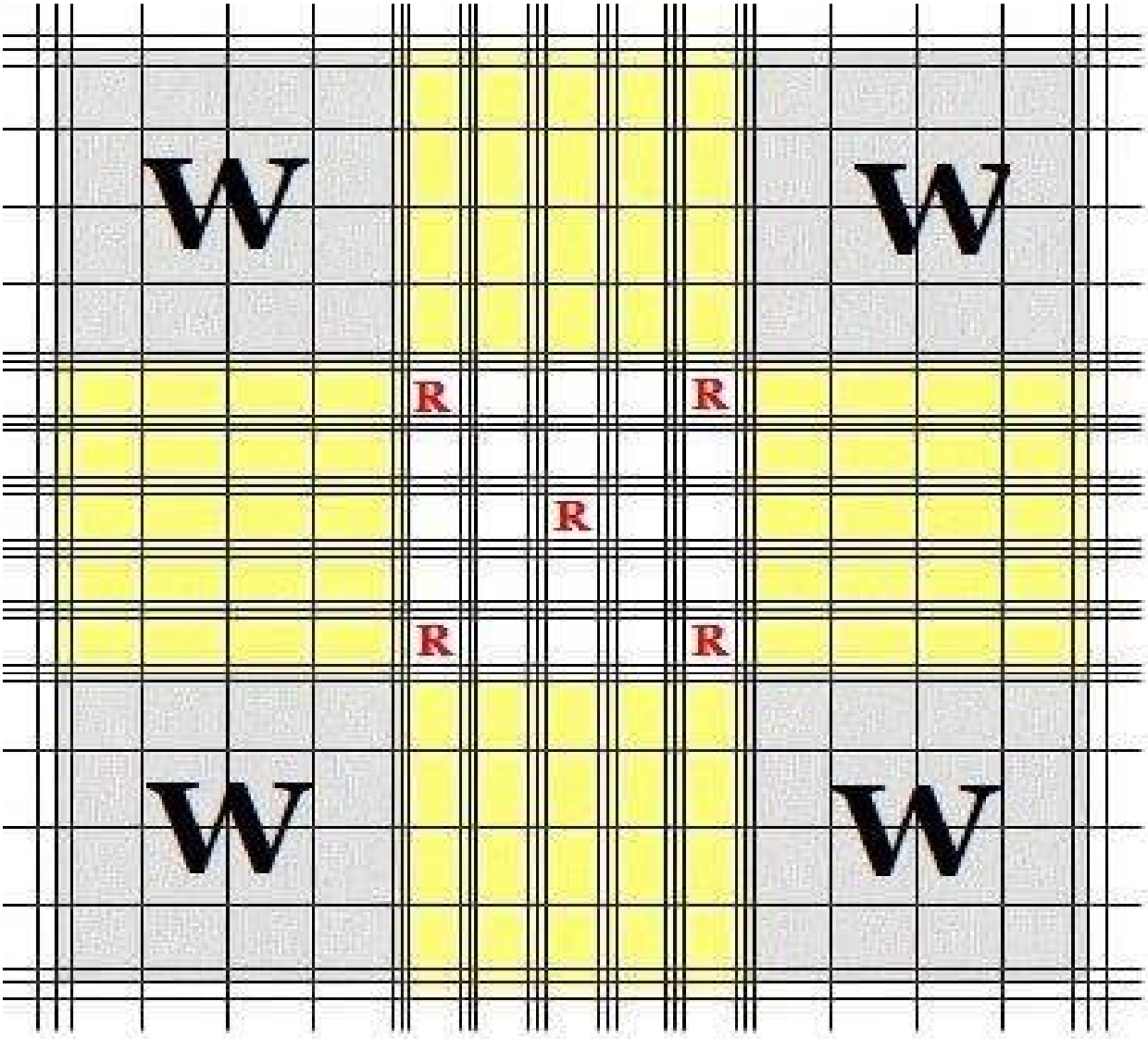
Appendix VII: The procedures of the total RBC count (Ibrahim, 2013).

1. Take the blood in to RBC pipette up to 0.5 marks
2. Immediately draw the RBC diluting fluid up to mark 101
3. Rotate the pipette between thumb and other fingers with finger. This gives a dilution of 1:200.
4. Clean the counting chamber of hemocytometer and 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 objective under microscope
• Don't counting the cell touching the bottom and right lines
• Count first from left to right directions and then vice versa
• Counting the cell touching the left and tope lines

Appendix VIII: Ruled area on improved Neubauer hemocytometer



NB: R – RBC areas W – WBC areas

- Each square of the Central Square (divided into 25 squares) contains 16 small squares
- Total no. of the area to be counted for RBC Count :16 × 5 = 80 small squares

Appendix IX: Laboratory techniques for sedimentation methods (Hansen and perry, 1994)

- Weigh or measure approximately 3gm of faeces into container and mix with 40-50 ml of tap water
- Mix(stir) thoroughly with a stirring device and filter the suspension through the tea strainer or double layer of cheesecloth into another container
- Pour the filtered material into a test tube and leave it for 5 minutes to sediment
- Remove the supernatant very carefully and resuspend the sediment in 5 ml of water and leave it for 5 minutes to sediment
- Again, discard the supernatant very carefully and take a small amount of sediment by using pipette and drop on the microscope slide
- Then cover with the cover slip and examine it under a microscope at a magnification 100x for trematodes egg.

Appendix X: Histopathological technique (Talukder, 2007)

1.Fixation of tissue 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 to fit 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.

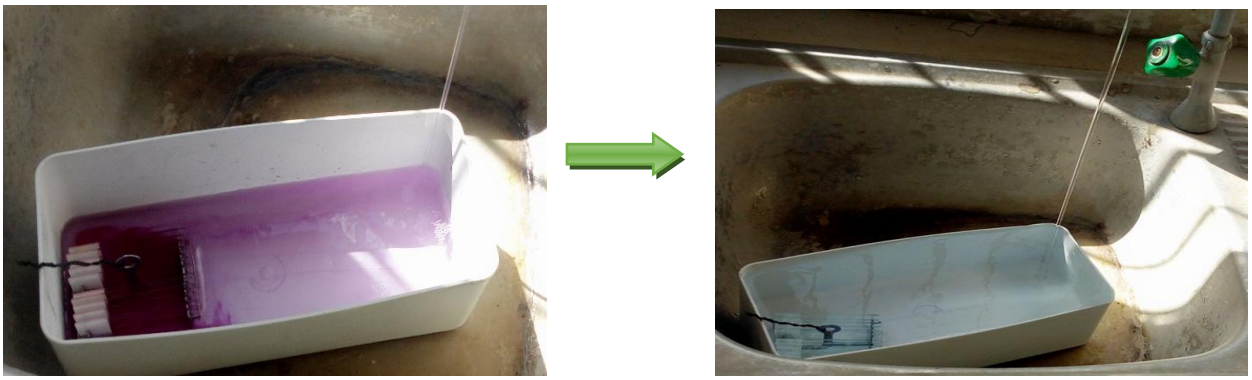
4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and

then fresh melted wax (54-60o 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 overnight.

6.Staining: Hematoxylin Eosin stain procedure.

A. Deparaffinize slides in 3 changes of xylene for 3 minutes each.
B. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
C. Place in Hematoxylin for 8 - 15 minutes.
D. Rinse in tap water until water runs clear.



E. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.

F. Rinse in tap water until ripples disappear from slides.

G. Dip in Bluing Agent, 3 - 5 long dips.

H. Wash in lake-warm tap water for 5 minutes (37-40°C.)

I. Stain in Eosin for 30 seconds - 2 minutes.

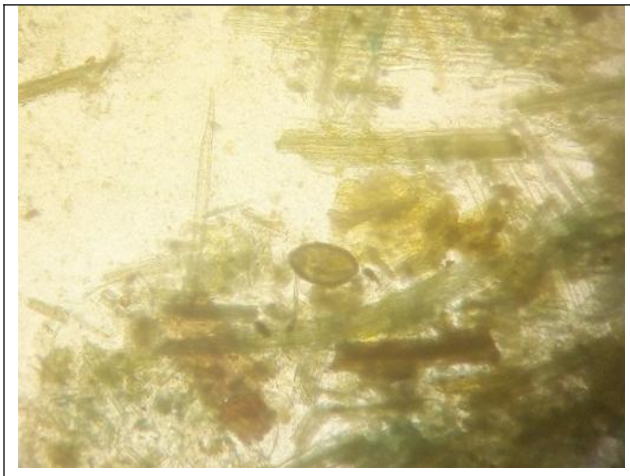
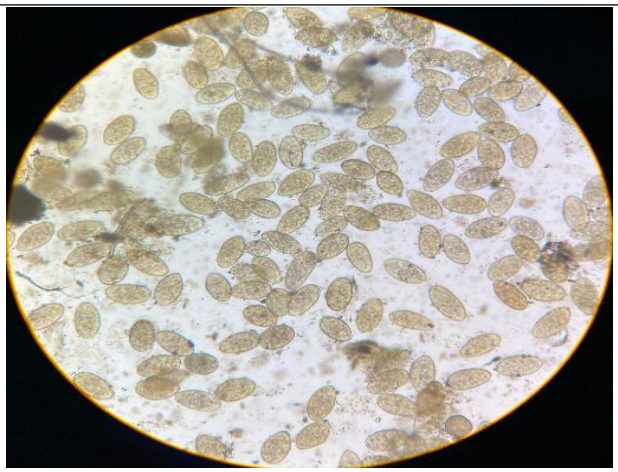
J. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.

K. Clear in 3 changes of xylene for 2 minutes each.

L. Mount cover glass using Canada Balsum or D.P.X (Deapistix)

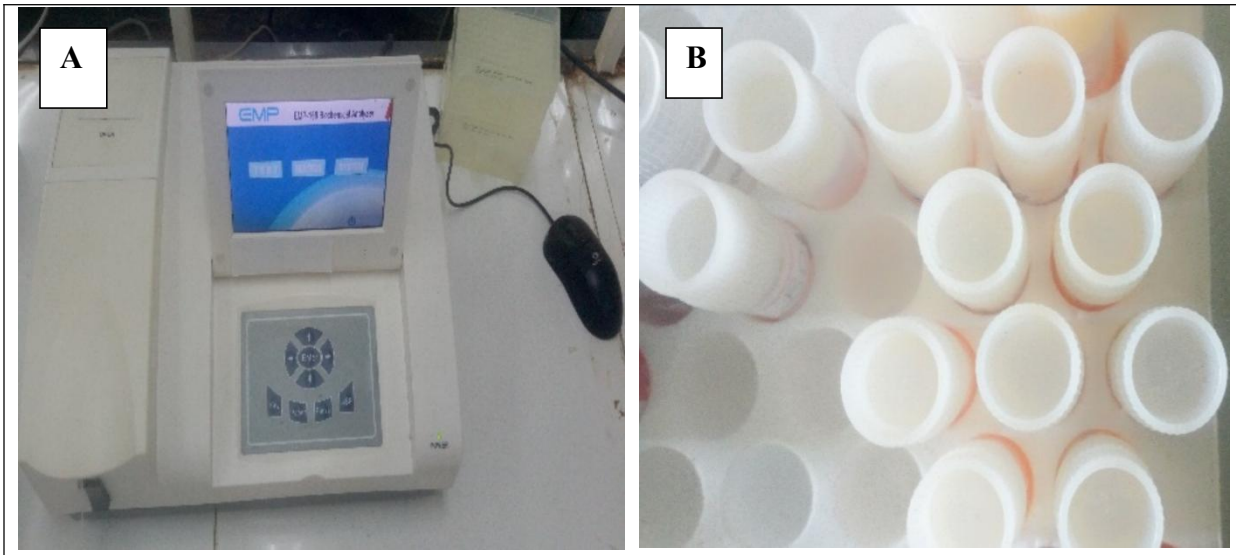
Appendix XI: Bishoftu municipal abattoir slaughter house with Photographs of cattle in the fence

Appendix XII: Different pictures captured during the study period

Egg of fasciola in fecal sample and gall bladder

(A). Fecal sample collection from goats (B). Sample transportation material (C). Data recording at laboratory (D). PCV reading (E). Hemoglobin determination (F). RBCs picture captured from light microscope in 40x magnification power from hem-cytometer chamber



(A).Spectrophotometer (B). Serum Kits

APPROVAL SHEET

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Pathology and Parasitology

Thesis title: study on clinical pathology, gross and histopathological alterations caused by trematode infections and their associated risk factors in ruminants slaughtered at three municipal abbatoirs in central Ethiopia

Submitted by: Adisu Wakuma Boke_____ _____

Name of Student. Signature Date

Approved for submittal to MSc thesis assessment committee.

	Signature	Date
1. Dr. Jirata shifera (DVM, MSc, Asst. Prof.) Principal Advisor	_____	_____
2. Professor Yacob Hailu (PhD) Co- Advisor	_____	_____
3. Professor Getachew Terefe (DVM, PhD) Department head	_____	_____