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Pathological and Serum Biochemical Study of Liver Fluke Infection in Ruminants Slaughtered at ELFORA Export Abattoir, Bishoftu, Ethiopia

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Abstract

A cross sectional study was conducted from September 2013 to July 2014 on a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) to assess pathological and biochemical changes on liver infected with fluke at ELFORA export abattoir in Bishoftu, Ethiopia. Liver and blood samples of the same animals were collected using systematic random sampling. Gross and histopathological lesions and serum biochemical alterations were assessed. On the basis of gross pathological examination study animals were grouped into three: group-A (78.87

Index terms— ELFORA-abattoir, serum biochemicals, liver, liver fluke, pathological changes, ruminant, bishoftu, ethiopia.

1 Pathological and Serum Biochemical Study of Liver Fluke Infection in Ruminants Slaughtered at ELFORA Export Abattoir, Bishoftu, Ethiopia

Abstract-A cross sectional study was conducted from September 2013 to July 2014 on a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) to assess pathological and biochemical changes on liver infected with fluke at ELFORA export abattoir in Bishoftu, Ethiopia. Liver and blood samples of the same animals were collected using systematic random sampling. Gross and histopathological lesions and serum biochemical alterations were assessed. On the basis of gross pathological examination study animals were grouped into three: group-A (78.87%) showed no fasciola spp. and no visible gross lesion (taken as control groups), group-B (12.32%) confirmed with fasciola and fasciola indicative lesion and, group-C (8.80%) were co-infected (fasciola presence, fasciola indicative lesion and other lesions). The gross lesions in fasciola positive livers include firm, enlarged livers with tense capsule, haemorrhagic spots, multi focal nodules and enlarged hepatic lymph nodes. The bile ducts were thickened and distended with adult fluke especially in chronic cases. Frequently observed histologic lesions were, hepatic portal fibrosis with large amount of fibrin in the portal area, hepatocytes degeneration, fatty changes and periportal necrosis. Fibrous connective tissue of various amount with, fibroblasts and infiltration of mononuclear cells, in particular lymphocytes were common lesions in tracts migrated by parasites; biliary cirrhosis with fibrous connective tissue and with epithelial hyperplasia were also observed. Eosinophilic hepatitis, talengechtasis and hepatocytes degeneration and necrosis were lesions in the parenchyma. Activity of serum ALT and AST were higher in cases of acute parenchymal lesions like eosinophilic hepatitis and necrosis, while ALP was significantly elevated with fibrosis and cirrhosis. The findings of the present study indicated that serum biochemical changes were consistent with pathological lesions; hence serum biochemical analysis could be used with other tests in diagnosis of ruminant fasciolosis. However, additional studies would be needed to establish association between serum biochemical and pathological changes with ruminants' liver fluke infection.

2 Introduction

iver fluke infection caused Fasciola hepatica and F.gigantica remains economically significant parasite of livestock and is emerging zoonotic infection. It causes morbidity and mortality in most mammalian species and by far important in sheep and cattle (Hodzic et al., 2013). A study conducted by Keyyu et al. (2006), reported up to 100% liver condemnation rates in slaughter slabs in Iringa region in Tanzania in cattle. In Ethiopia the prevalence of fasciolosis is as high as 83.08% (Mulualem, 1998) in cattle, 62.7% (Zeleke et al., 2013) in sheep and 17.2 % (Sirajudin et al., 2012) in goats. The variation in climato-ecological conditions such as altitude, rainfall and temperature, and livestock management system influences the prevalence of fasciolosis together with survival and distribution of the parasites as well as their intermediate host (snails).

Clinical examination of the Fasciola infected animal showed pale visible mucous membrane or anemia (Radostits et al., 2000). On post mortem fasciola infected liver is an irregular outline, and pale and firm.

According to Talukder et al. (2010), gross pathology of chronic fasciolosis is characterized by reduced in size of the organ and thickened bile ducts. Several types of fibrosis such as post necrotic scarring, ischemic fibrosis and peribiliary fibrosis may also present (Steyl, 2009). In cattle calcification of bile ducts, enlargement of the gallbladder and aberrant migration of flukes are more common. Fluke eggs may sometimes stimulate a granuloma-like reaction with obliteration of the affected bile ducts. Histopathologically, infiltration of fibroblasts admixed with lymphocytes and few mononuclear cells in the area previously migrated by young flukes are appreciated. Mature flukes cause necrosis and ulceration of the epithelium and severe hyperplasia of the epithelial layer.

Acute fasciolosis is associated with immature flukes migrating through the liver parenchyma and create migratory tracts. In this, grossly the liver is enlarged and haemorrhagic with fibrinous to fibrous exudates on the capsular surface. Numerous haemorrhagic spots and focal necrosis are found on the cut surface of liver parenchyma. The migratory tracts from direct trauma of this parasite is grossly seen as dark acute haemorrhagic streaks of typical post necrotic Dinaol Belina Kitila ? & Yoseph Cherinet Megersa ? scarring and granulation (Affroze et al., 2013).

Histopathologically migratory tracts created by immature flukes migrating through the liver parenchyma are seen as necrotic tracts (Steyl, 2009).

The lesions in the liver are only partially a result of mechanical action of liver fluke, because the injury of the liver can be induced by parasites excretory products, decomposed products of parasites, bile and hepatic tissue. Hence serum biochemical tests including serum liver enzymes are also helpful to assess the severity of hepatocellular injury and to monitor the progress of the disease in ruminants **??**Lee et al., 2005).

Serum biochemical analysis also used to evaluate the degree of cholestasis and synthesizing capacity of the liver (Hodzic et al., 2013). Therefore, the objectives of this study were to assess pathological changes of the liver, gall bladder and hepatic lymph nodes; to evaluate serum biochemical changes associated with liver fluke infection, and to establish association between serum biochemical parameters and pathological changes of the ruminants liver.

3 II.

4 Materials and Methods

5 a) Study Area

The study was conducted from September 2013 to July 2014 at ELFORA export abattoir. Currently the abattoir is one of the most facilitated modern export abattoirs in Ethiopia and is exporting meat of small ruminants though cattle are slaughtered for local market. During the study on average 400 and 500 sheep and goats respectively, were slaughtered at this abattoir per day. On the other hand, on average 70-85 cattle were slaughtered per week based on local market needs. The ruminant animals slaughtered at the abattoir were purchased from different zones of the country particularly Borana, Arsi, Bale, Gondar, Jimma and some parts of SNNP region. Therefore, animals were encountered different ecological areas and management conditions at their origin.

6 b) Study Design and Study animals

A cross-sectional study was conducted on systematically selected local breeds of apparently healthy cattle, sheep and goats destined for slaughter. The study animals were only males of different body condition and age groups, and they were transported to the abattoir by vehicles.

7 i. Sample size determination

The sample size was determined by the formula described by Thrusfield (2005), at 95% confidence level and 5% precision, and considering the previous combined prevalence of 12.37% ruminant fasciolosis at ELFORA export abattoir (Meskerem, 2006); accordingly, the total sample size would be 167. However, to maximize the precision the sample size was increased by 1.7 folds and a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) were included.

ii. Sampling Method and procedures Animals were included in the study using systematic random sampling method where only the first animal was chosen randomly. After such selection animals were grouped in to young and adult according to Gatenby (1991), Steele (1996) and Johnson (1998) and moderate and excellent body condition scores (Belina et al., 2012). Then blood sample was taken during ante mortem and livers of the same animals were appropriately inspected for presence of fasciola spp. and gross liver pathology after slaughter. After slaughter animals were clustered in to three groups based on presence of liver fluke and gross liver lesions: group-A/control (no liver fluke and no visible gross lesion), group-B/only affected by fasciola (fasciola presence and fasciola indicative lesion) and, group-C/ co-infection (fasciola presence and fasciola indicative lesions together with other lesions). Livers showing absence of fasciola spp. and fasciola indicative lesion, but with evidence of non fasciola lesions were totally excluded from the study.

Liver with irregular outline, pale, firm and with bile duct distention and thickening, and calcified bile ducts were grouped as chronically infected. When migrating flukes were observed in biliary tract and gallbladder, and adhesions of the gallbladder to adjacent organs were present the lesion also taken as chronic fasciolosis (Meskerem, 2006). When the liver is enlarged with blunt edge and the capsule is tense it was grouped as acute fasciolosis. Presence of haemorrhagic spots with fibrinous exudates on the capsular surface and the dark acute haemorrhagic streaks and juvenile flukes in the migratory tunnels were also considered as acute fasciolosis (Steyl, 2009;Affroze et al., 2013). c) Study Methodology i. Blood samples and serum biochemical analysis 8ml of blood were collected from jugular vein using sterile plain vacutainer tubes, labeled according to the neck tag of animals and taken to laboratory. At the laboratory, blood samples were rendered to stand at room temperature for 3 hours to allow serum separation (Hodzic et al., 2013). Then sera were transferred in to 2ml eppendrof tubes and stored at -70c o until the time of analysis. Analysis of samples were then took place after bringing the samples to room temperature. The serum sample was analyzed with ALT, AST and ALP commercially available respective enzyme working reagent of the test kits, using humastar 80 chemistry analyzer. The instrument humastar 80 chemistry analyzer was calibrated using calibrator (Autocal), and quality control samples normal (Humatrtol N) and pathological (Humatrol P) each day before running the samples. The livers and gallbladders were appropriately examined for the presence of fasciola and its gross pathology, during which the fasciola spp. and all gross pathological changes were 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 were characterized (Sohair and Eman, 2009). Accordingly the primary examination involves visualization and palpation of the organs; secondary examination involves more incision of liver; opening of bile duct and hepatic lymph nodes. For generalized liver fluke infection (fascioliosis) incision were 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 were also thoroughly examined. Then parts of the affected organ were sampled into 10% neutral buffered formalin.

For histological lesion characterization, the fixed tissue samples were trimmed to 5mm and processed (dehydrated through a series of ascending grades of alcohols, cleared in three changes of xylene and impregnated with paraffin wax) and finally, embedded in melted paraffin (60c o). The tissues were then sectioned at 5µm and stained routinely with haematoxylin and eosin (Okaiyeto et al., 2012), and examined.

8 d) Data Management and Analysis

Data was entered into Microsoft excel 2010 and analyzed by SPSS statistical software version 20. Prevalence differences of study variables were analyzed by chi-square and descriptive statistics. P ? 0.05 was considered as statistically significant at 95% CI. The serum biochemical parameters infected groups were compared with those of the controls. The data was expressed as mean ± standard error and range; one way ANOVA was used for multiple comparisons and to see their correlation.

9 III.

10 Results

11 a) Prevalences

The results of the gross pathology showed that 21.13% (n=284) of ruminants were found to be infected with fasciolosis. The result also depicted the infection prevalence was significantly higher (p=0.00) in cattle (50.65%) than sheep (16.16%) and goats' liver (4.63%) (Table **??** 1).

12 b) Pathological Lesions

In this study, the gross pathological changes observed in cattle and sheep livers were almost similar except repeated severe calcification in chronically infected cattle livers. The commonly observed gross lesions include firm, pale and irregularly outlined liver with tough consistency. In chronic cases variably sized livers, focal and multifocal nodules, Pin-point hemorrhages on the parietal surface of the liver were also examined. When a section of the bile duct was cut through there was aberrant migration of flukes and in some cases evidence of calcification was noted. Thickened and distended bile ducts containing adult flukes, decomposed materials and cholangitis were also observed at post mortem (figure.1). The ventral lobe was mostly affected and reduced in size. In goats' liver however, no multifocal nodules and less extensive ductular thickness was found in the chronic form of the infection.

In acute fluke infection, on the other hand the liver was highly enlarged (swollen) with rounded edges and the color was paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface. The capsule was tense with fibrous exudates on the capsular surface of the liver. Hepatic lymph nodes were enlarged and an abnormally cloudy thick fluid oozed up on cutting, and flukes were also observed in the migratory tunnels of the parenchyma. In cattle hard, dark and brown color liver with multiple soft abscesses surrounded by hyperemic zone on the surface were noted and up on cutting section, a viscous yellow material oozed from the cut ends. Grossly in coinfected livers abscesses of different sizes and consistency, and cysts were observed in both acute and chronically infected livers in addition to fasciola and fasciola indicative lesions examined. Microscopic lesion patterns were more or less similar in acutely affected cattle and sheep but less pronounced in goats liver. The biliary tract was less affected than liver parenchyma in acute form of the fluke infection. Migratory tracts traveled by fasciola spp. were infiltrated with macrophages and eosinophils (figure .2A). Multifocal hepatitis and the necrotic lesions with deep eosinophilic cytoplasm, karyorhexis and karyolysis were examined.

Focal hepatitis with neutrophils and eosinophils infiltration in parenchyma was another lesion types frequently observed. Numerous eosinophils admixed with few lymphocytes surrounded by hemorrhagic and edematous area in which hepatic blood vessels were dilated, engorged with blood, occluded by thrombi and foci with extensive hemorrhagic streaks were significantly observed. There were also mild fibrosis and bile duct proliferation and distortion of the hepatic cords in some areas. Moreover, congestion with focally extensive parenchymal necrosis and the degenerative changes manifested by vacuolation of the hepatocytes particularly around central vein were noted. In acute infection edema and neutrophilia were more severe in co-infected livers than in livers only fasciola and fasciola indicative lesion positives. : Rows with superscripts x , y and z are significantly different (Fcalcul.>Ftabul.; p?0.05; 0<r<1) from(j , k , m and n), (b , c and d) and (e , f , g , h and i), respectively; and similar superscripts in different rows indicate equal significance from respective healthy mean values In current study age and status of fluke infection wise multiple comparisons were also made and the results showed statistically significant differences (p<0.05) of serum ALT, AST and ALP values between asciola positives (group-B and C), and control (group-A) animals. In age wise comparisons between infected young and adult cattle, sheep and goats, transaminases showed statistically no differences except significantly higher serum activity of ALT found in adult infected sheep (Tables : 7, 8 and 9). IV.

13 Discussions

The overall combined prevalence of fasciolosis in cattle, sheep and goats was 21.13% and this is higher when compared to reports of Meskerem (2006), who reported 12.37% from the same abattoir. However, the present prevalence was by far lower than (31.70%) Ozung et al. (2011), reported from Ikom abattoir of Nigeria. The variation in the prevalence may be due to geographical origin of studied animals. According to Yohannes and Abebaw (2012), the difference in incidence and severity of the fluke infection are evident in various geographical regions depending on the local climatic condition, availability of permanent water and system of management.

Statistically, in agreement with the report of Meskerem (2006), in cattle, sheep and goats and Ayana, et al. (2009), only from sheep and goat; our current study showed significant differences in the prevalence of liver fluke infection among cattle, sheep and goats. Accordingly, cattle were found highly infected and goats were the least infected ones. These prevalence differences among species might have some connection with their feeding habits as goats are commonly browsers. Similar to the findings of Talukder et al. (2010) and El-Hallawany and Abdel-Aziz (2012), both chronic and acute forms of liver fluke infection were detected in the present study. Adult flukes mainly localized in the bile ducts and gallbladder and they cause chronic fasciolosis while the immature flukes wander in the parenchyma and are responsible for the acute fluke infection.

The gross pathological changes observed in the chronically infected livers were reduction in size and pin-point hemorrhages on the parietal surface which is partly due to the inflammatory changes and later fibrosis that took place in the parenchyma. These findings are partially in agreement with the report of Okaiyeto et al.

(2012), as they reported hemorrhages on the surface of the liver in chronic fasciolosis from dairy farm. The pinpoint hemorrhagic foci on the surface represented the points of entrance of the immature parasites into the liver structure (Borai et al., 2013). In agreement with report of Jones et al. (1997), Molina et al. (2005), and Sayed et al. (2008), chronic cases of the current study also revealed that the livers were firm, tough in consistency and the cut section showed evidence of calcification while the affected ducts were enlarged/distended, thickened and cholangitis with adult fluke in the ducts. These might be because of the immunological reaction of macrophages and lymphocytes infiltration that merges with fibrotic healing of the necrotic areas during the later stage of fasciolosis. Progressive irritation by the adult flukes wandering in the biliary tract cause biliary inflammation like hyperplastic cholangitis, ductular wall thickening, and intra-and extrahepatic biliary dilatation (Catalano et al., 2009). Evidence of calcification showed the brilliant cell in the adenomatous proliferation of the biliary epithelium particularly in cattle liver. However, bile ducts in goats (Talukder et al., 2010) relationship to host resistance. It is also probable that the calcification seen in cattle interferes with feeding habits of fluke and these effects are noted in its size.

In this study no multifocal nodules were noted and even less extensive ductular thickness was recorded in chronic form of goats' fasciolosis. However, El-Hallawany and Abdel-Aziz (2012), reported focal and or multi focal liver nodules, from small ruminants which might be due to abnormal host immune response that resulted against fasciola eggs, dead larvae or the fluke byproducts trapped into the liver parenchyma.

Attributing to our finding Talukder et al. (2010), reported extensive enlargement and huge ductular fibrosis resulting the thickening of the bile ducts which also confirms the findings of Sohair and Eman (2009). Because the inflammatory changes in the parenchyma leads enlargement of the liver in size, and presence of adult flukes in the bile duct cause continuous irritation resulting hyperplastic proliferations and extensive ductular fibrosis.

Enlarged hepatic lymph nodes and multiple soft abscesses on the liver surfaces surrounded by hyperemic zone was also seen during the study, such findings were also reported by Molina et al. (2005) and Sohair and Eman (2009). According to Adrien et al.

(2013), the liver is enlarged in response to acute inflammation as the wandering juvenile fluke mechanically damage the parenchyma. Molina et al. (2005), also stated at the cut surface, the liver is irregular, firm and edematous with hemorrhagic channels and adhered fibrosis in the hepatic parenchyma and also fibrinous tags on the capsule and exudates between organs. These authors justified fibrinous tags on the capsule are due to capsular reaction to the flukes penetrating it. Supporting the current study Adrien et al. (2013), reported the hepatic and mesenteric lymph nodes are reactive and enlarged and they ooze edematous material up on opening. Hepatic abscesses are usually resulted from bacterial infections and subsequent lysis of neutrophils and are later surrounded by a fibrous capsule. Therefore, multiple soft hepatic abscesses in our study might be resulted from secondary bacterial complication. On the other hand, this may be due to host response against parasitic infestation and continuous mechanical irritation along with parenchymal destruction accompanied by intensive haemorrhagic lesions and immunological reactions (El-Hallawany and Abdel-Aziz, 2012).

Microscopically fibroblasts admixed with lymphocytes, plasma cells and few macrophages in the area previously migrated by the young fluke was observed in chronic fluke infection of this study. This might be indicative of granulomatous hepatitis which is resulted from aggregations of activated macrophages with an epitheloid appearance, mostly accompanied by lymphocytes and plasma cells (Sohair and Eman, 2009). Fatty degeneration which is manifested by lipid accumulation inside hepatocytes may be due to tissue hypoxia resulted from anemia and vascular damage (Abd El-Baky and Salem, 2011), as more than 0.5ml blood per fluke can be lost per day when adults become established in the bile ducts (Molina et al., 2005) and consequently the tissue is atrophied as in this study. The ductular epithelium inflammation and focal and multi focal hepato-necrosis of the current study were in agreement with the reports of Mahmoud et al. (1989) and Sayed et al. (2008), which are due to the effect of toxic products liberated by fasciola worms. Hyperplastic bile duct is produced as an attempt to regenerate hepatic tissue when the liver cells have lost their capacity to regenerate themselves (Kelly, 1985). Also, Sohair and Eman (2009), mentioned that the hyperplasia of the ductular epithelium occurs as a result of fluke toxic products which cause changes in the structural integrity of the ductular cells in non specific and potentially destructive manner. The presence of mature worms within the lumen of intrahepatic bile ducts also brings about continuous irritations that lead to hyperplastic proliferations.

Portal and multilobular biliary cirrhosis in cattle and sheep, and coagulative hepatocyte necrosis in goat were the microscopic findings of the chronic fasciolosis in present study. Sayed et al. (2008) and Darwish (1996), also found similar pathological changes from infected livers of buffalo and camel, respectively. A progressive mechanical irritation of hepatocyte by the liver fluke in chronic fasciolosis enhances hepatocyte destruction with persistent healing which results fibrous connective tissue proliferation leading to hepatic cirrhosis as well as mononuclear cell infiltrations, and if the cirrhosis is developed in the portal triad, it is called portal cirrhosis (Heidelbaugh and Bruderly, 2006). On the other hand, the parasite itself chemically digest and destruct the liver parenchyma and biliary epithelium by enzymes like proteases and toxins that produced by the fluke, and such condition later come up with hepatic fibrosis and cirrhosis (Sohair and Eman 2009). In addition to this, the obstruction to intrahepatic bile flow leads to upstream bile ductular proliferation, inflammation and necrosis of adjacent periportal hepatic parenchyma, generalized choleostasis, portal tract scarring and bridging fibrosis. Moreover, chronic extrahepatic and intrahepatic biliary obstruction, and choleostasis together with chronic hepatitis develop to cirrhosis (Salem and Hassan, 2011). Mild irritation to the hepatocyte by fasciola also causes a decrease in blood flow to the irritated area followed by protein denaturation which forms coagulative hepatocyte necrosis (Molina et al., 2005). Granulomatous lesion of the current study indicates circumscribed focal aggregation of epitheloid cells and mononuclear cells, and encapsulated by delicate fibrous connective tissue capsule. Such lesion is created due to host immune response that resulted against fasciola eggs or dead larvae trapped in the liver C parenchyma that causes granulomatous reaction (El-Hallawany and Abdel-Aziz, 2012). Zongping (2003), stated the hepatic necrosis may be related to the enteric infection which can invade the liver by way of septicemia or ascending cholangio-billary infection, and adult flukes moving in the biliary tree also cause biliary colic or cholangitis. Furthermore, invasion of the liver by migrating immature liver fluke damages the tissue and results in reduction of the oxygen tension (anaerobic condition), that allowed the germination and proliferation of closteridial spores with concomitant release of its toxins and induce hepatocellular necrosis (Jones et al., 1997;Sayed et al., 2008).

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In our current study, the histopathological examination also revealed the presence of numerous eosinophils admixed with few lymphocytes and accompanied by hemorrhage and edema in acute fascioliosis. These lesions partially correlated with the findings of Talukder et al. (2010), from goats' liver fluke infection, and completely agreed with report of Borai et al. (2013). Eosinophilia occurs due to sensitivity to the foreign protein of the parasite, which may be a part of immune phenomena. Thus, eosinophilia is likely to be seen when parasites are migrating through the tissue in large animals (Kerr, 2002; Borai et al., 2013).

Edema/excessive fluid in hepatocyte interstitial space may be due to decrease in plasma colloidal osmotic pressure as a result of hypoalbuminemia. The acute cases in this study also revealed dilated blood vessels with disorganized and distortion of the hepatic cords while the hepatic cells showed variable degrees of necrosis and degeneration, which are in agreement with Whenever the liver is injured or damaged, the liver enzymes spill into the blood, causing elevations of serum liver enzyme (Mert et al., 2006). Therefore, in combinations with the physical examination and history, the evaluation of serum liver enzymes should aid in differentiating the source of increased enzymes like AST, ALT and ALP levels (Yasuda, 1988). Different mean values of AST, ALT and ALP were also measured among animal spp., which may arise from physiological adaptations, maturation of metabolic pathways, growth effects, body composition, and or nutrition (Sharon, 2013). The variation among animal species might also due to variability in parasite load that indicate innate differences in the host immune systems (Semrad and Gay, 2013). Furthermore, in "Overview of hepatic disease in large animals" these authors stated the serum levels of hepatic enzymes vary even with ages, breeds and sexes.

In present study, the mean value of serum liver AST and ALT were sufficiently higher ($P<0.05$) with acute lesions like hepatocyte degeneration, telangiectasis and eosinophilic hepatitis unlike with chronic lesion like biliary cirrhosis and fibrosis, which are in agreement with statements of (Yasuda, 1988), in large animals, serum concentrations of liver specific enzymes like transaminases are generally higher in acute liver disease than in chronic liver disease. They may be within normal limits in the later stages of chronic hepatic disease. Consequently AST markedly increased with intrahepatic cholestasis and mildly increased with extrahepatic cholestasis. This increment of serum transaminases at the early stage of the infection could be related to the hepatocellular necrosis and degenerative changes produced by migrating juvenile flukes through the liver parenchyma (Dias, 1996). In previous findings of experimental study on F. hepatica in goats, activity of AST highly elevated first and returns to normal values 11 weeks post infection (Hodzic et al., 2013). The elevation of serum AST and ALT activity in affection with liver parenchyma than in biliary tract damages in our study also partially agreed with the findings of Al-Quraishy and Al-Moussawi (2001) in cows, (Adama et al., 2011) in goats, that the elevation of AST indicates injury to liver parenchyma and or acute liver fluke infection. The cytosolic location of transaminases allows their immediate release with even minor changes in hepatocellular membranes as a result raise their concentration in serum (Sharon, 2013). AST is present in both the cytoplasm and mitochondria of hepatocytes and will elevate together with ALT in states of altered membrane permeability (Yasuda 1988), though the mitochondrial AST isoenzyme is less likely released with most of the conditions which result in increased membrane permeability (Kerr and Steiner, 2012).

ALT is present in high concentration in the cytoplasm of hepatocytes and is considered to be liver specific in small animals and ruminants like camel. Its plasma concentration increases with hepatocellular damage/necrosis or degeneration and hepatocyte proliferation (Hodzic et al., 2013). The previous findings of Mbuh and Mbwaye (2005), also detected the raise of ALT in fasciolosis may be due to hepatocyte destruction since ALT is primarily found in the liver parenchyma. On the other hand, the raise in mean value of ALT in this study may be due to hepatocyte death from liver fluke infection causing complete or partial bile ducts obstruction and then returning of bilirubin to hepatocyte (Dias, 1996;Kilad et al., 2000). Accoding to Adama et al. (2011), serum ALT remain raised for some days after acute liver fluke infection indicating epithelial damage in the bile ducts which agrees with elevated serum ALT in hyperplastic cholangitis of cattle and goats but not in sheep, in our study. Thus, in hyperplastic cholangitis AST and ALT elevation may be due to parenchymal damage as a secondary effect of cholestasis (Salem and Hassan, 2011) Apart from serum transaminases, ALP serum activity showed insignificant decrease with parenchymal lesions like hepatocyte degeneration in sheep and goats, with eosinophilic hepatitis in sheep, and significantly elevated with chronic liver lesions in all animals. Mbuh and Mbwaye (2005), also reported significantly elevated ALP in chronic goats fasciolosis. Serum ALP is known to be excreted via the bile duct and its elevation is usually significant as fascioliasis progresses which may be synchronized with the arrival of flukes to the bile ducts constraining biliary clearance (Adama et al., 2011). Attributing to our current study, Hacariz et al. (2009), also justified that ALP is significantly higher in chronic cases such as cirrhosis and fibrosis in sheep, because ALP is derived from the liver or biliary tract as the adult flukes reside in the bile ducts. In line with this, ALP was evidently raised in cases of hyperplastic cholangitis of the present study. The elevation of ALP is associated with irritation or destruction of biliary epithelium and biliary obstruction (Semrad and Gay, 2013). Attributing to the report of Kocatepe (2012), elevated serum ALP was however, also recorded with hepatocytes necrosis in this study; thus, hepatic ALP can rise following hepatocytes necrosis due to secondary intrahepatic biliary obstructions and as part of the nodular regeneration process.

Age was another variable used for comparisons of mean values of serum liver enzymes of cattle, sheep and goats in this study. Accordingly transaminases were statistically revealed no differences between infected youngs and adults, except significantly higher serum activity of ALT found in infected adult sheep (Table.9). These findings were partially in agreement with reports of Rumosa et al. (2012), who found insignificant differences in serum activity of both AST and ALT between young and mature goats suffering with liver infection. However, Kocatepe (2012), evaluated significantly elevated AST in adult goats with liver infection than in infected youngs.

Semrad and Gay (2013), also reported that AST levels in foals is elevated compared to its values in adults for many months.

In this study, highly significant differences were detected in ALP mean values between infected young and adult groups of each animal spps.; which is in agreement with the study of Kocatepe (2012). Accordingly higher values of ALP were found in young cattle, sheep and goats than in their adults, which could be due to leakage of the enzyme from the growing bones and intestines into the blood, beside ALP released from the affected liver in high concentration.

On the other hand, Rumosa et al. (2012), reported lower values of ALP from infected mature goats, which correspond with the lower values of phosphorus in adult animals serum compared to their equivalent youngs as ALP activity is associated with the process of calcification that accompanies growth.

15 V. Conclusion and Recommendations

This study attempted to assess liver lesions and serum biochemical alteration that accompany liver fluke infection in ruminants. Grossly livers were: irregular in outline, firm, pale and tough in consistency. Multifocal nodules, thick and distended bile ducts were also examined in chronic cases whereas enlarged liver with tense capsule, numerous hepatocyte haemorrhagic spots, enlarged hepatic lymph nodes and multiple soft abscesses were found in acute fasciolosis. Parenchymal and biliary fibrosis, atrophy, fatty changes, hyperplasia, cirrhosis and necrosis were histopathologic changes observed in chronic forms of the fluke infection though goats' liver was not registered as cirrhotic. On the other hand, multifocal eosinophilic hepatitis, talengechtasis and different hepatocyte necrosis and degeneration were histopathologic changes observed in acute cases. Concentration of serum liver enzyme analysis was also used to evaluate liver fluke infection and indicated significant elevation of serum AST and ALT with acute In these study results of serum biochemical changes were also consistent with pathological findings and therefore, serum biochemical analysis could be used as complementary in diagnosis of ruminant liver fluke infection. In the study we used ruminants with no liver fluke and no gross liver lesions at all, as control groups for serum biochemical comparisons but further experimental studies with case-control should be done to establish better association between liver pathological changes and serum biochemical alteration.

Figure 1:

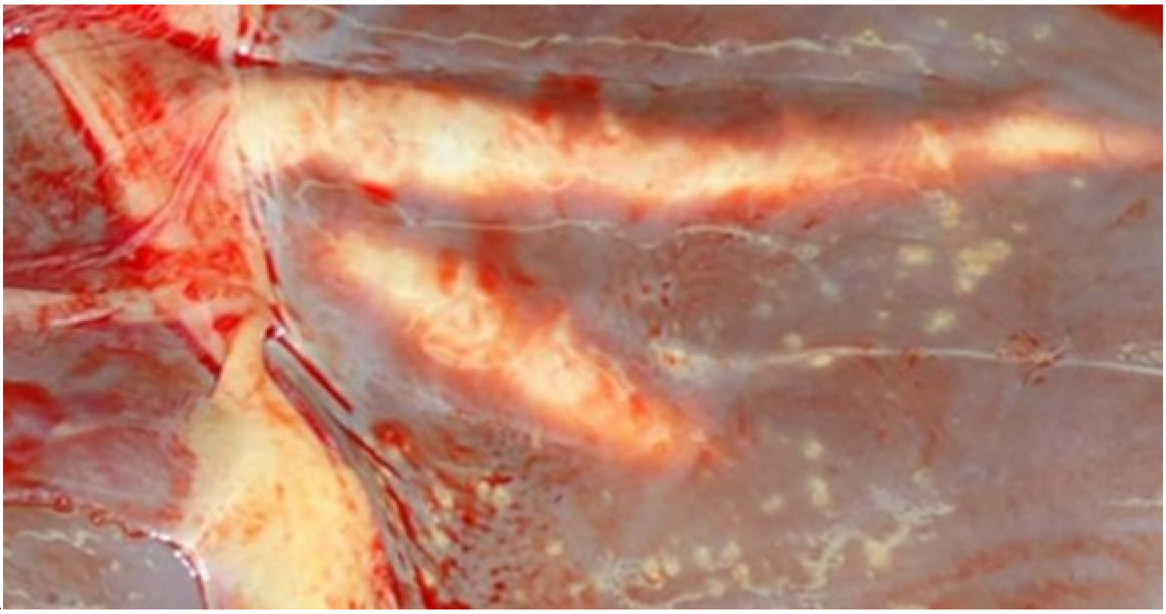
1 

Figure 2: Figure 1 :C

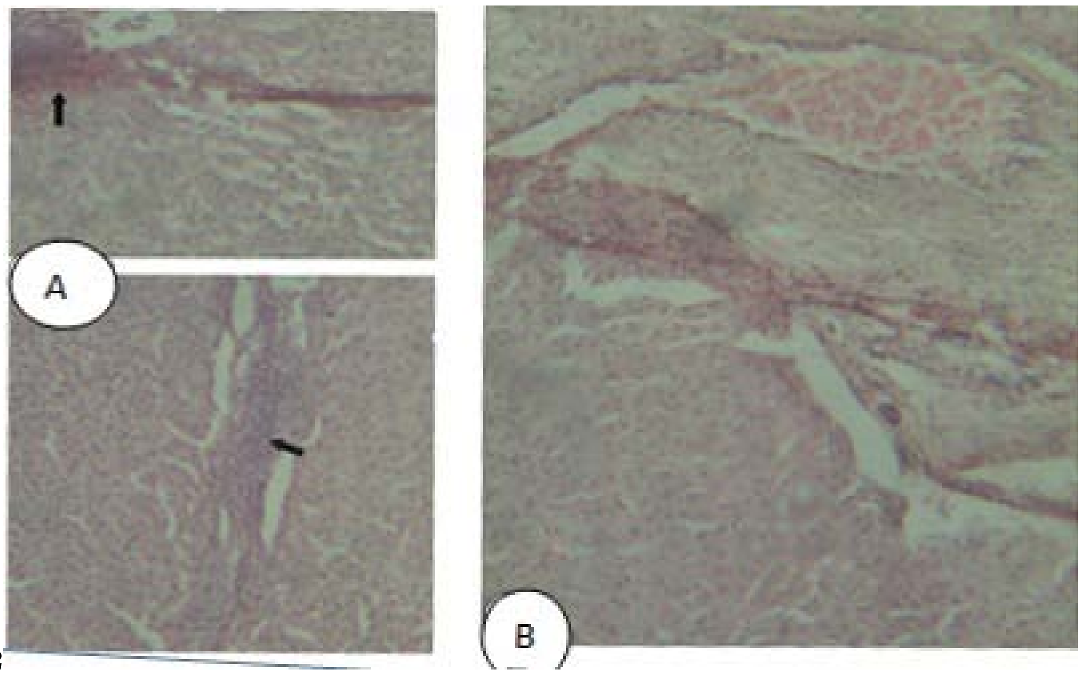
2 

Figure 3: Figure 2 :

1

The pathological examination revealed that 47(78.33%) acute and 13(21.67%) chronic liver lesions proportion with 8(61.54%) cattle, 3(23.08%) sheep and 2(15.38%) goats, were suffering with chronic liver fluke infection. On the other hand 35(58.33%) livers were only positive for fasciola and fasciola indicative lesions, whereas 25(41.67%) of livers showed lesions that indicate co-infection (Table: 2).

Figure 4: Table 1 :

2

Variables		Levels	No. examined	No. positive (%)	P value
Body condition score	Moderate		201	51(25.37)	
		excellent	83	9(10.84)	0.00
Age		Young	94	16(17.02)	
				0.20	
		Adult	190	44(23.16)	
		Cattle	77	39(50.65)	
Species		Sheep	99	16(16.16)	0.00
		Goats	108	5(4.63)	
		Total	284	60 (21.13)	

-

Lesion type	Animal spp.	Status of fasciola infection (%)			P value
		Only fasciola	Co-infection	Subtotal	
	Cattle	20(64.52)	11(35.48)	31(66.00)	
	Sheep	7(53.85)	6(46.15)	13 (27.67)	
Acute	Goats	1(33.33)	2(66.67)	3(6.38)	
	Subtotal	28 (59.57)	19 (40.42)	47 (78.33)	
	Cattle	5(62.50)	3(37.50)	8(61.54)	0.00
	Sheep	1(33.33)	2(66. 67)	3(23.08)	
Chronic	Goats	1(50.00)	1(50.00)	2(15.38)	
	Subtotal	7(53.85)	6(46.15)	13(21.67)	
Total	-	35 (58.33)	25(41.67)	60 (21.13)	

Figure 5: Table 2 :

3

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	56.76±1.08 x	14.68±0.51 y	147.96±4.09 z
	Hyperplastic cholangitis	61.00±2.40 j	17.60±2.47	396.45±21.06 e
	Hepatocyte necrosis	87.00±4.78 k	24.99±0.88 b	161.88±7.41
	Hepatocyte fibrosis	59.95±0.04	21.30±1.74 c	179.45±2.00 f
	Biliary tract fibrosis	52.17±2.90	11.86±0.45	373.07±47.34 g
Group B	Biliary cirrhosis	53.55±2.34	11.80±0.74	384.20±15.96 h
	Fatty necrosis	60.90±1.31 j	19.35±0.86 c	274.70±36.74 i
	Eosinophilic hepatitis	111.16±6.79 m	28.90±2.45 d	160.14±4.57
	Hepatocyte degeneration with telangiectasis	105.44±6.75 n	25.60±1.13 b	149.28±4.14

[Note: Comparisons of some serum liver enzymes among group-A/controls, group-B/ affected with liver fluke only, and group-C/ co-infected cattle (Mean±S.E)]

Figure 6: Table 3 :

4

only and group-C/ co-infected sheep (Mean ± S.E)

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	86.89±2. 53 x	19.58± 1.04 y	140.60±6.12 z
	Hepatocyte necrosis	213.13±6.80 j	40.10±1.94 a	166.10±16.68
Group B	Biliary cirrhosis	82.33±1.15	18.9± 0.27	297.70±20.69 f
	Eosinophilic hepatitis	311.38±16.67 k	44.28 ±3.39 c	131.74±2.40
	Hepatocyte degeneration with Telangiectasis	204.68±9.10 m	39.32±2.79 d	117.38±2.89
		F= 75.20, p=0.00, r=0.276	F= 15.96, p=0.00, r=0.339	F= 12.53, p=0.00, r=0.475
	Hyperplastic cholangitis	88.10±1.24	15.95±3.52	247.05±2.39 h
	Hepatocyte fibrosis	241.8 ±3.47 n	28.70±1.93 b	202.20±12.06 i
Group C	Hepatocyte necrosis	218.13±4.64 j	38.10±1.4 a	171.21±14.57
	Fatty necrosis	83.95±2.31	27.70±6.31	252.25±1.89 p
	Biliary tract fibrosis	73.55±4.88	22.65±4.22	291.40±11.06 q
	Hepatocyte degeneration with Telangiectasis	207.48±7.89 m	38.36±2.86 d	101.18±4.6
		F=48.21, p=0.00, r=0.083	F= 8.43, p=0.00, r=0.173	F= 28.62, p=0.00, r=0.436

Figure 7: Table 4 :

5

	F= 18.41, p=0.01		F=12, p=0.02	F= 21.62, p=0.02
	r=0.421		r=0.367	r=0.089
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Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	62.65±3.32 x	20.54±1.02 y	121.88±9. 56 z
	Hyperplastic cholangitis	74.50±3.46	24.60±1.46	802.20±51.48 e
	Biliary tract fibrosis	68.00±1.09	29.00±2.45	701.50±106.70 f
Group B	Eosinophilic hepatitis and hepatocyte necrosis	208.50±44.24 j	80.85±8.33 c	125.75±17.75
	Hepatocyte degeneration with Telangiectasis	215.50±46.58 k	54.85±5.92 d	118.55±1.02
		F=4.17, p=0.02, r=0.813	F=7.45, p=0.01, r=0.821	F=4.63, p=0.01, r=0.230
	Hyperplastic cholangitis	74.28±2. 81	23. 34±4.14	792.12±14.32 e
Group C	Fatty necrosis	82.41±6.45 m	26.85±2.5	462. 64±10.71 h
	Hepatocyte degeneration with Telangiectasis	212.50±42.64 k	57.15±2.76 d	114.52±4.26
		F=1.67, p=0.04, r=0.103	F=4.25, p=0.02, r=0.216	F=1.46, p=0.04, r=0.108
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[Note: Note : Rows with superscripts x , y and z are significantly different (Fcalcul.>Ftabul.; p?0.05; 0< r<1) from(j , k , m and n),(b , c and d) and(e , f , g , h and i), respectively; and similar superscripts in different rows indicate equal significance from respective healthy mean values Note : Note]

Figure 8: Table 5 :

6

Figure 9: Table 6 :

7

Enzymes	Levels	Ruminants	Mean ± S.E	Range
	Young control	cattle sheep goat	57.07±1.35 85.41±6.22 73.5±4.99	30.40 56.00 42.00
AST	Young infected	cattle sheep goat	88.11±10.29 153.05±32.27 127.40±38.89	98.70 327.40 220.00
	Adult control	cattle sheep goat	56.03±1.86 88.00±3.93 54.54±3.45	35.40 58.90 37.00
	Adult infected	cattle sheep goat	92.42±4.07 214.63±18.79 140.25±46.20	139.80 270.80 203.00
	Young control	cattle sheep goat	15.06±0.65 20.95±1.94 22.61±1.57	16.60 22.60 15.23
ALT	Young infected	cattle sheep goat cattle	22.76±3.01 28.87±2.96 38.08±7.47 13.9045±0.87	33.60 29.30 39.90 18.00
	Adult control	sheep	18.55±1.04	15.40
		goat	18.56±1.20	12.00
	Adult infected	cattle sheep goat	24.04±1.84 38.99±2.42 53.50±16.42	25.20 37.66 66.20
	Young control	cattle sheep goat	167.43±3.80 162.70±8.36 144.33±10.73	102.10 96.00 90.06
	Young infected	cattle sheep goat	207.39±34.33 221.34 ± 23.55 474.92±148.40	318.00 221.80 748.80
ALP		cattle	119.00±3.65	57.50
	Adult control	sheep	124.03±6.63	83.69
		goat	108.32±13.67	165.70
	Adult infected	cattle sheep goat	183.49±11.39 156.88±16.59 303.23±186.46	319.70 190.00 778.40

Figure 10: Table 7 :

8

Lower Bound	Upper Bound

Figure 11: Table 8 :

9

Mean	Sig.	95% Confidence Interval

Figure 12: Table 9 :

Figure 13:

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