Review on camel liver pathology and Its major diagnostic approaches

Dinaol Belina¹,², Bulto Giro², Hagos Ashenafi², Tilaye Demissie², Yimer Muktar¹,²

¹Haramaya University, College of Veterinary Medicine, P.O. Box 138, Diredawa, Ethiopia; Addis Ababa University College Veterinary Medicine and Agriculture, Department of Pathology and Parasitology P.O. Box 34, Bishoftu, Ethiopia. ²Addis Ababa University, College Veterinary Medicine and Agriculture, Department of Pathology and Parasitology P.O. Box, 34, Bishoftu, Ethiopia.

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Abstract

The physiological functional reserve and regenerative capacity of liver can be lost by diseases affecting liver. Camel liver infection is an imperative disease that leads to great losses to camel production and condemnation of large numbers of livers in slaughter houses. A number of diseases can affect camel liver but toxic substances, infectious diseases, parasitic hepatitis, fatty liver, and tumors are considered as the usual causes. Clinical signs and pathological lesions of liver diseases are usually nonspecific hence, the disease is frustrating to diagnose and often difficult to treat. It is tricky to know the primary cause and even to differentiate the location of liver pathology (hepatic vs pre/post-hepatic). Hence its tentative clinical diagnosis should be confirmed by special techniques and diagnostic approaches such as necropsy, histopathology, serum liver enzyme (ALT, AST, GGT and ALP) tests, molecular pathology tests like IHC, PCR and in situ hybridization, and diagnostic cytology. Molecular pathology tests are employed in specific antigen detection, nucleic acid amplification and localization of cells containing specific nucleic acid sequences. Thus, an up to date and special diagnostic approach towards differentiation and confirmation of liver pathology requires the combined application of the above tests coupled to anatomic-pathology of the organ.

Keywords: Anatomic-pathology, camel, cytology, liver pathology, molecular pathology tests, serum enzyme tests

INTRODUCTION

Liver is considered the most important organ for animal health production and reproduction as many of the metabolic activities of the body occurred in the liver. The liver of dromedary camel is situated in the intra-thoracic part of the abdominal cavity, occupying most of the right hypochondriac and epigastric regions. As in other domestic animals in dromedary camels liver is kept in position by the pressure of the neighboring organs and two groups of ligaments, a visceral group and a parietal group (Siddig, 2002). The liver possesses considerable functional reserve and regenerative capacity. In healthy animals including camels more than two thirds of the hepatic parenchyma can be removed without significant impairment of hepatic function and normal hepatic mass can be regenerated in a matter of days (Radostits, 2000). Hepatocellular injury is one of the pathologic condition affecting domestic animals including camels. Liver infection is an imperative disease that affects all kinds of meat producing animals, leading to great losses to livestock production and national income due to condemnation of livers in the slaughter houses as it represents 2.8% to 5.7% of the dressed carcass weight.
The influx of acute or chronic inflammatory cells in to the liver is termed hepatitis. Inflammatory cells may be limited to the sites of entry (portal tracts) or spill over into the parenchyma and intracellular enzymes escape (Talukder, 2001). Besides this, infected liver constitute a good media for bacterial multiplication, transportation of microorganisms with the parasites occurs during the different stages of its life cycle. Anaerobic necrotic lesions of the liver produced by immature flukes occasionally provide a suitable environment for the germination of spores of Cl. novyi type B bacteria in the liver. The bacteria will release toxins into the blood stream resulting in what is known as black disease in camels (Sohair and Eman, 2009). The clinical signs of liver disease are usually non-specific and include anorexia, depression, weight loss and vomiting. Jaundice, when present, may assist the clinician to localize the disease process to the hepatobiliary system (provided that haemolytic anaemia is not the cause). However, jaundice is not always present in animals with liver disease and a variety of different etiologies can cause hepatobiliary disease and jaundice. Other clinical signs include: fever, abdominal effusion and CNS signs (Maddison, 2006).

The causes of liver disease in camel are numerous but primarily liver is affected by: Toxic substances, Infectious diseases, parasitic hepatitis, Fatty liver, Tumors and etc. It is difficult to examine liver because of its location in the cranial abdomen, and its obvious malfunction occurs only after the liver has lost approximately two thirds of its functional capacity. Therefore, hepatic failure is seen only when there is extensive damage to the hepatic parenchyma or there is obstruction to biliary drainage (Pearson, 1999; Schrotter et al., 2000). According to Green and Flamm (2002) most hepatitis allows escape of intracellular enzymes into the blood stream as the damage enhances permeability of membranes of the liver cells. The major intracellular enzymes involved in camels are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT is found primarily in the liver with a small amount in muscle. Its highest cellular concentration occur in the cytosol; therefore, the enzyme is released following acute and diffuse hepatocellular damage (Maddison, 2006); whereas the AST is present in both the mitochondria and cytosol of hepatocytes. The cytosolic and mitochondrial forms of AST are true isoenzymes and immunologically distinct. On the other hand, Cholestasis (e.g., biliary obstruction or hepatic infiltration) obstructed bile ducts cause the induction of synthesis of alkaline phosphatase (ALP) and gamma-glutamyltranspeptidase (GGT).

Abnormal liver tests may indicate an abnormality of the liver and provide clues as to the nature of the problem. However, in an asymptomatic diseased animal, mild abnormalities may not be clinically significant. A systematic approach in evaluating the animal and ordering further tests will help to identify underlying disease (Howard et al., 1998). Liver performs different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver. All laboratories usually employ a battery of tests for initial detection and management of liver diseases and these tests are frequently termed “Liver function tests”, although they are of little value in assessing the liver function per se. The role of specific disease markers, radiological imaging and liver biopsy is not underestimated (Thapa and Walia, 2007). However, liver disease is frustrating to diagnose and often difficult to treat, because it is difficult to differentiate the location of liver pathology (hepatic vs post-hepatic) using a single test, yet such differentiation can be important in deciding the appropriate diagnostic or therapeutic step to take next. Surgical correction of post-hepatic obstruction may be feasible whereas surgery is indicated in primary hepatocellular disease to obtain biopsies. Thus, when liver disease is suspected after a general clinical examination, special techniques and diagnostic approaches such as Necropsy Examination, Clinical pathology tests (total bile acid and liver enzyme), Molecular pathology tests like Immunohistochemistry (IHC), Polymerase chain reaction (PCR) and In situ Hybridization (ISH), ultrasonography and magnetic resonance image (MRI) incorporation is better to determine further the status of the liver (Ayman, 2008). Diagnostic cytology is also helpful in identification and examination of individual cells involved in camel liver pathology.

Therefore, the objective of this seminar paper is to provide a review paper on camel liver pathology, causes and its major diagnostic methods.

**Major Causes of Camel Liver Pathology**

Liver disease is relatively common but often occurs in the absence of specific clinical signs. The liver has great powers of regeneration and more overt clinical signs associated with its failure do not appear until 70-80% of the functional capacity is lost. Obscure signs of liver disease are therefore much more common than overt signs of liver failure (BCMA, 2011). The liver is subject to many of the same pathologic conditions that affect other body organs. The inflammation of the liver refers to us hepatitis, which can be due to reactions to chemical agents, drugs, and toxins; disorders such as autoimmune diseases and infectious mononucleosis that cause secondary hepatitis; and by hepatotropic viruses that primarily affect liver cells or hepatocytes (Hennessy and Porth, 2004). Signs of liver failure include central nervous system disturbances and are usually acute, even if the underlying liver disease develops over a protracted period. This hepatic encephalopathy is associated with toxic blood levels of ammonia and intestinal amines, which would normally be detoxified by the liver (Mudron et al., 2004). Concerning camel disease, camels are formerly considered resistant to most of the diseases (Sohair and Eman, 2009).
commonly affecting livestock, but as more research is conducted, camels are found to be susceptible to a large number of pathogenic agents which contribute to hepatobiliary disease, such as hepatic insufficiency. The major liver disease in ruminants that may also affect camel include: Infectious: septic abscesses, Chlamydia, Salmonella, and Listeria spp., tuberculosis, Johne’s disease, Parasites: sarcosporidiosis, Fasciola hepatica, Ascaris spp, Metabolic: hepatic lipodisosis, immune responses, neoplasms, Toxins: blue-green algae, pyrolizidine alkaloid containing plants, mycotoxins (moldy hay, moldy tall fescue), cotton seed meal, Klein grass, Chemicals: iron, copper, phosphorus, arsenic, carbon tetrachloride, hexachloroethane, gossypol, cresols, coal tar pitch, nitrite, chlorinated naphthylenes, Drugs: like halothane, Extrahepatic bile duct obstruction: calculi, abscesses, Congenital: hepatic fibrosis, portosystemic venous shunting of blood, and congenital hyperbilirubinemia of Corrydale, and Idiopathic: hepatic fatty cirrhosis (Douglas, 2004).

Toxic Substances

Liver is the most common site of toxic injury for two reasons: the liver receives approximately 80% of its blood supply from the portal vein which drains blood from the GI tract. Thus, ingested toxic substances, including plant, fungal, and bacterial products, as well as metals, minerals, and other chemicals that are absorbed into the portal blood, are transported to the liver in high concentrations. Second, the liver possesses the enzymes capable of biotransformation of a variety of endogenous and exogenous substances for elimination from body; this process may also bioactivate some substances to a more toxic form, thereby causing hepatic injury. The common causes of toxic hepatitis in cameldes and other animals are either inorganic poisons such as copper (Junge et al., 1989), phosphorus, arsenic, possibly selenium or organic poisons like carbon tetrachloride, hexachloroethane, Gossypol, cresols and coal tar pitch, chlorophorm and some anaesthetic agents (Groom et al., 1995) and copper diethylaminequinoline sulfonate. Poisonous plants include Senecio, Crotalaria, Panicum meftusum and water-damaged alfalfa hay. Alfatoxin contaminated stored camel food can also be toxic when its concentration is above 2.5 mg/kg of feed and is considered lethal when found in a concentration of 6.2 mg/kg feed (Osman et al., 2004). Ingestion of some insects such as sawfly larvae (Lophyromatamentu) and insecticides can be toxic to the liver (Byars, 2003); as high incidence of toxicity due to Dazinon is reported by Agab (2003), from the misuse of this insecticide by the camel herders through administration via drinking water. Accidental accessibility of the camels to urea fertilizer in the farm as it is obtained for soil fertilization is also a cause for ureal poisoning.

Infectious Hepatitis

Infectious Hepatitis usually caused by infectious agents such as bacteria and viruses (Hennessy and Porth, 2004). Routes of infection into the liver can be haematogenous, direct penetration, and ascending via the biliary system. The most common route is haematogenous because the liver receives both arterial and venous blood. The severity of inflammation is dictated by the nature (acute or chronic) of infectious agents. Among which viral agents such as: infectious canine hepatitis virus, rift valley fever virus are reported in dromedary camels in Sudan, Egypt and Kenya (Ayman, 2008), herpes virus. Wessels born disease virus and infectious equine anaemia virus are also common; bacterial agents: Bacillary haemogloniaemia caused by Cl. haemolyticum, infectious necrotic hepatitis caused by Cl. novyi type B are reported in dromedary camels by Seifert (1992), Tyzzer’s disease caused by Bacillus piliformis, leptospirosis of L. grippotyphosa and liver abscesses caused by Fusobacterium necrophorum or Corynebacterium psuedotuberculosis are also recognized to affect camel liver (Rosa et al., 1989). Acute or chronic infectious hepatitis enhances influx of inflammatory cells into the liver and the inflammatory cells may be limited to the sites of entry (portal tracts) or spill over into the parenchyma. There are two mechanisms of liver injury in viral hepatitis: direct cellular injury and immune responses against viral antigens in infected hepatocytes. On the other hand, a Physiological alteration of hepatocyte by infectious agents has a significant effect on other body systems which may be due to the development of cirrhosis, portal hypertension, liver failure and etc. If infectious hepatitis occurs with fulminant hepatitis, necrosis may wipe out entire lobules or destroy central and midzonal regions sparing perportal region of lobules (Thapa and Walia, 2007).

Parasitic Hepatitis

Parasitic liver affections in meat-producing animals are one of the major factors that reduce the national income, either directly through condemnation of the pathologically affected livers, or indirectly by their effect on the animal growth and so its meat production (El-Hallawany and Abdel-Aziz, 2012). Hepatitis caused by migration of helmith larvae such as Ascaris spp, Strongylus spp, Fasciola spp and Schistosoma spp, through the liver is common in domestic animals. The migration of the larvae throughout the hepatic parenchyma causes local tracks of hepatocellular necrosis accompanied by inflammation. The tracks are eventually replaced with connective tissue leading to the production of fibrous scars on the capsular surface. Some Cestode including members of the genus Taenia occur within the hepatobiliary system of domestic animals and may result
in hepatic infection (Neil et al., 2010). Hydatid liver disease caused by E. granulosus is also one of the most important liver problems in animals and man worldwide (Belina et al., 2012). For instance, an incidence of 59.8% of hydatidosis is reported in dromedary camels from different parts of Sudan (Omer et al., 2004). The hepatic migration of the immature flukes of F. hepatica, a trematode commonly found in sheep, cattle and occasionally other species including camels, produces hemorrhagic tracks of necrotic liver parenchyma (Sohair and Nasr, 2009). These tracks are grossly visible in heavy infestations usually as dark red. The infective metacercariae usually migrate the liver capsule and hepatic tissue. This migration usually cause direct trauma with hemorrhages, necrosis and subsequent granulation tissue eventually ensuing liver cirrhosis (El-Hallawany and Abdel-Aziz, 2012). A variety of signs can follow this migration like acute peritonitis, hepatic abscesses and baccillary hemoglobinuria resulting from the proliferation of Cl. hemolyticum or Cl. Novyi in the formed necrotic tissue, the so called black liver disease. Mature flukes reside in the larger bile ducts and cause cholangitis or cholangiohepatitis which may lead to stenosis of the duct (Stuart, 2012).

Fatty Liver

The presence of excessive lipid within the liver is termed as fatty liver. Fatty liver occurs when the rate of triglyceride accumulation within hepatocytes exceeds either their rate of metabolic degradation or their release as lipoproteins. Fatty liver is not a specific disease entity but it occurs as a sequel to many perturbations of normal lipid metabolism which can be due to excessive entry of fatty acid into liver, abnormal hepatocyte function, excessive dietary intake of carbohydrate, increased esterification of fatty acids to triglycerides, decreased apoprotein synthesis and subsequent decreased production and release of lipoprotein and impaired secretion of lipoprotein from the liver (Smith, 2002). The other mechanism for this is activation of free radicals. For instance, Carbon tetrachloride (CCL₄) is converted to the toxic free radical CCL₃, principally in the liver; causing autocatalytic membrane phospholipid peroxidation, with rapid breakdown of the ER. Hence decline in hepatic protein synthesis of enzymes and plasma proteins; swelling of the smooth ER and dissociation of ribosomes from the smooth ER have occurred. Thus, there is reduced lipid export from the hepatocytes, as a result of their inability to synthesize apoprotein to form complexes with triglycerides and thereby facilitate lipoprotein secretion; the result is the “fatty liver” of CCL₄ poisoning (Althaian, 2013). Liver degenerative changes in camels including cloudy swelling, hydropic degeneration, fatty change and amyloidosis are also described (TejSingh et al., 2006). Liver diseases such as hepatic lipidosis with biliary hyperplasia, cholangiohepatitis, hepatic necrosis, lymphoplasmacytic cholangitis, pericholangitis, septic phlebitis and hydropic degeneration are notorious in Llamas and alpacas (Ayman, 2008). According to Torquinst et al. (1999), Females are more affected with fatty liver in llamas and alpacas than males, even though the sex distribution is not different from that of the camelid population in the diagnostic laboratory’s data base. In view of this author, all affected females are pregnant and lactating in the age of 6 to 10 years and anorexia and recent weight loss are common.

Tumors of the Liver

Damage to the cellular genome is a common feature for virtually all neoplasms (tumors), despite the facts that neoplasms arise in a broad variety of tissues and that diverse agents such as viruses, mutagenic chemicals, and radiation induce their outgrowth. The genetic damage produced by carcinogens is believed to be random, and many mutations may be inconsequential (Cullen et al., 2002). Primary hyperplastic and neoplastic proliferation of the hepatobiliary system arise from hepatocytes (e.g. hepatocellular nodular hyperplasia or hepatocellular carcinoma), epithelium of the bile ducts (cholangiocellular hyperplasia or carcinoma) and gall bladder (carcinoma), and mesenchymal elements such as connective tissue and blood vessels. Multifocal lymphoma is reported in a 7-year old female dromedary camel which evaluated for inappetence, weight loss, polyuria, and polydipsia. Up on IHC staining the neoplastic cells shows uniformly CD3-positive, indicating a T-cell lymphoma (Simmons et al., 2005). On the other hand, in acute and chronic liver diseases there is occasionally neoplastic associated photosensitization. This is an injury of the cutaneous tissues resulting from activation of photodynamic pigments by ultraviolet light present in the sun rays. It is caused by the increased circulating concentration of phylloerythrin, a photodynamic derivative of chlorophyll, which is normally detoxified and excreted by the liver (Galitzer and Oehme, 1978). In addition to this, Cholangiocarcinoma (CC), which is a malignant tumor arising from bile duct epithelium, is described in other domestic animals and recently in camel (Birincioglu et al., 2008). It is more often originates in intra hepatic bile duct epithelium than in extrahepatic bile ducts or the gall bladder (Ayman, 2008). The incidence of CC increases with age and most cases occur in animals over 10 years of age; neither a breed nor sex prevalence is accounted in animals including camel (Bergman, 1985). Intrahepatic CC mainly affects older domestic animals, particularly dogs and cats, though there is a doubt about intrahepatic CC in camel (Ayman, 2008). However, Birincioglu et al., (2008) describes it for the first time from a case of 18-year old male camel slaughtered at the Incirliova Abattoir in Aydin, Turkey; as shown below (Figure 1).
Major Diagnostic Approaches

Necropsy Examination

Morphological diagnosis is powerful and allows for the accurate classification and diagnosis of the majority of disease states within pathologically altered tissues. Necropsy may be defined as the systematic examination of an animal carcass aimed to search for lesions. Necropsy provides a firsthand look on what really happened along the course of the disease. It provides an opportunity to examine everything, both inside and outside. Obviously, the necropsy allows a thorough visualization of all internal organs (Melissa, 2013). Necropsy examination is often performed to determine the cause of an unexpected death. However, a thorough and systematic postmortem examination also used to confirm a clinical diagnosis, identify the etiology of disease process, explain apparent unresponsiveness to treatment or reveal unrecognized disease process. Integration of necropsy with clinical signs and laboratory data ultimately enhance the clinician’s understanding of the disease process and sharpen diagnostic skill (Lowenstine, 1986). Liver is the largest visceral organ in the body. To deal with liver necropsy, first examine the intact, and cut surfaces of the liver and note for color, texture, size and consistency. Several slices of the liver is made for closer inspection. By cutting and opening of the gall bladder, quality and color of bile is examined. While evaluating anatomic liver pathology at necropsy, organisms like liver flukes and lesions of different sizes and consistency can be detected (Sohair and Nasr, 2009). For instance, in examination of CC in camel liver, the liver is enlarged, firm, and yellow-greenish. Grayish-white and centrally depressed multiple lesions are observed on the serosal surface of the liver. Similar lesions may also be appreciated in the cut surface of the liver. They may range from 0.5 to 3.0 cm in diameter and are generally distinct from the hepatic parenchyma (Birincioglu et al., 2008). On the other hand, in liver affected by fascioliasis and secondary bacterial complication the liver is appeared hard, dark and brown in color with presence of multiple soft abscesses (ranged from 3-10mm in diameters) on the liver surface. On cut section, a viscous yellow material oozed from the cut ends. The abscesses may be surrounded by hyperemic zone (Figure 2) (Sohair and Nasr, 2009).

Fibrosis and Cirrhosis of liver are the usually findings in abattoir surveys. In the case of fibrosis, fibrous tissue is formed in response to inflammation or direct toxic insult to the liver. The initial stage of fibrosis develops around portal tracts or the central veins or within the spaces of disse. With continuing fibrosis, liver is subdivided into nodules of regenerating hepatocytes surrounded by scar tissue, termed cirrhosis. Cirrhosis is characterized by: bridging fibrous septa in the form of delicate bands or broad scars replacing multiple adjacent lobules, disruption of architecture of the entire liver and Parenchymal nodules created by regeneration of encircled hepatocytes (Talukder, 2001).

Histopathological Examination

The microscopic examination of tissues is an extremely valuable component of the post-mortem examination. During or after the gross microscopy, small pieces of tissues (usually 1-3 cm is adequate) are removed from all of the organs that are examined. It is important to collect both normal and abnormal looking tissues because tissues that appear normal may be found otherwise at the microscopic level. Also, including both normal and abnormal appearing tissues from the same organ allows for comparison of healthy and diseased tissue and may help in understanding the development or progression of
the disease (Melissa, 2013). According to Birincioglu et al., (2008), in 18-year-old male camel microscopic lesions of CC is characterized as, the tumor consists of gland-like structures and or solitary islands of neoplastic cells irregular in size and shape and often surrounded by prominent sefta of fibrous connective tissue. Most of the tumor cells exhibit marked anisocytosis, anisokaryosis, and pleomorphism, and they are eosinophilic or basophilic cytoplasm. The nuclei are roughly round or ovoid and contain 1-3 prominent nucleoli. On the other hand, in liver affected by mixed fasciollasis and bacterial infection the hepatic blood vessels are dilated and engorged with blood, the hepatic cords disorganized and distorted while the hepatocytes of parenchymal cell reveal necrosis and degeneration with histopathological examination (Figure 3a).

The necrotic lesions emphasize deep eosinophilic cytoplasm with karyorrhexis and karyolysis of their nuclei. Moreover, the degenerative changes manifested by vaculolization of the hepatocytes particularly around central vein (Figure 3b). The hepatic sinusoids show presence of mononuclear inflammatory cells in their lumina as well as the kupffer cells are swollen and increase in number. Sometimes, multiple variable size abscesses are detected in the hepatic parenchyma. The core of the abscess consists of homogenous structureless mass of necrotic cells surrounded by heavy aggregations of inflammatory cells mainly neutrophils, histiocytes, eosinophils and lymphocytes and bounded by fibrous connective tissue capsule. Focal infiltration of inflammatory cells may be also observed in the capsule. Whereas, focal micro abscesses consist of lymphocytes, histiocytes, eosinophils and polymorphonuclear leukocytes surrounded by thin layers of fibrous connective tissues are the findings in the hepatic parenchyma in mixed fasciola and bacterial infection (Sohair and Nasr, 2009).

Figure 3: a. Liver showing distortion and individualization of the hepatic cells; b. Liver showing dilated central vein and paracentral fatty change of the adjacent hepatocytes (H & E X400).


Serum liver Enzyme Test

Serum liver enzymes are measured as biochemical parameters to provide information about hepatocellular injury or cholestasis but do not define how much functional liver is present (Douglas, 2004). There are two major categories of liver enzymes: leakage enzymes and cholestatic enzymes. Leakage enzymes are enzymes that leak into the plasma when hepatocyte injury or death occurs and their high activities in serum is an indication of hepatocellular injury. Commonly measured leakage enzymes include: AST, ALT, Sorbitol dehydrogenase (SDH), and Lactate dehydrogenase (LDH). Cholestatic enzymes are those induced by biliary obstruction or hepatic infiltration like ALP and GGT (Salem and Hassan, 2011).

Aminotransferases

Aminotransferases are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes are AST-formerly serum glutamate oxaloacetic transaminase,SGOT and ALT-formerly serum glutamic pyruvate transaminase,SGPT catalyze the transfer of α amino acids of aspartate and alanine, respectively to α keto group of ketoglutaric acid (Thapa and Walia, 2007). Of the numerous methods used for measuring their levels, the most specific method couples the formation of pyruvate and oxaloacetate.

ALT is found primarily in the liver with a small amount in muscle. The highest cellular concentrations occur in the cytosol and it is released following acute and diffuse hepatocellular damage as ALT is specific marker of hepatocellular injury in camel, although occasionally severe muscle damage may also raise serum ALT activity (Salem and Hassan, 2011). In general, serum levels are not regarded as significant unless they are at least two to three times above normal. Liver can be regarded as a sympathy organ – it reacts in sympathy whenever any other organ in the body is damaged. Hence, small to moderate increases in ALT may occur in hypoxia, GI disease, sepsis, hyperthyroidism, diabetes mellitus, pancreatitis, hyperadrenocorticism and other non hepatic disorders. ALT may also be moderately increased in animals on anticonvulsant therapy and glucocorticoids and those with biliary stasis (Maddison, 2006).

AST is present in both mitochondria and cytosol of hepatocytes. It also found in tissues like the heart, skeletal muscle, kidney and brain. The cytosolic and mitochondrial forms of AST are true isoenzymes but immunologically distinct (Rosalki and McIntyre, 1999). Large increases in mitochondrial AST occur in serum after extensive tissue necrosis. AST considers a nonspecific index for camel liver investigations as it elevated in skeletal or cardiac muscle disease as well as in liver disease. Mitochondrial AST is also increased in chronic liver disease (Salem and Hassan, 2011). According to Tapasya and Chosdol (2007), the activity of ALT and AST in serum at any moment reflects the relative rate at which they enter and leave circulation. In cases of camel hepatitis there is significant increase in the activity of ALT and AST. Significant decrease in A/G ratio due to decrease in
serum albumin and increase in serum globulin concentrations with insignificant change in BUN are observed in all cases of hepatic affection (Salem and Hassan, 2011). Hepatic sinusoids are primary site for clearance aminotransferases, so they are absent in urine or bile.

**Cholestatic Enzymes**

Cholestatic enzymes are enzymes for which their synthesis increased as a result of bile retention, biliary epithelium damage or administration of drugs. Bile retention usually results from intrahepatic or extrahepatic bile duct obstruction. For diagnostic purpose commonly measured cholestatic enzymes are ALP and GGT (Douglas, 2004). In normal condition average values of ALP vary with age and are relatively high in neonatal and at puberty but lower in middle age and higher again in old age in small animals. However, Serum ALP concentration is higher in lactating sows than their suckling and weaning calves though some controversy exist between different scholars (Omer et al., 2007). ALP is a family of zinc metaloenzymes, with a serine at the active center and not liver specific. ALP isoenzymes are present in all tissues with high activity in liver, bone, kidney, intestine, and placenta. However, the highest level of ALP occurs in cholestatic disorders. Elevation occurs as a result of both intrahepatic and extrahepatic obstruction to bile flow. Its degree of elevation does not help to distinguish between the two though ALP levels are likely to be very high in EHBA. The mechanism by which ALP reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete ALP made in bone, intestine and liver (Thapa and Walia, 2007).

In acute viral hepatitis, ALP is usually either normal or moderately increased. Tumors may secrete ALP into plasma. Hepatic and bony metastasis can also cause elevated levels of ALP. Other camel liver diseases like infiltrative liver diseases, cholestasis, abscesses, granulomatous, hepatic necrosis and amyloidosis increase serum activity of ALP (Salem and Hassan, 2011). Mildly elevated levels of ALP may be seen in cirrhosis and hepatitis of congestive cardiac failure. Even though it has not been well diagnosed in camel, corticosteroids and drugs like cimetidine, frusemide, phenobarbitone and phenytoin induce a marked increase in a specific hepatic isoenzyme in dogs (Douglas, 2004). Low levels of ALP occur in hypothyroidism, pernicious anemia, zinc deficiency and congenital hypophosphatasia and wilson’s disease. When wilson’s disease complicated by hemolysis the ratio of ALP and bilirubin declines. This might be the result of replacement of cofactor zinc by copper and subsequent inactivation of ALP. Regardless of the cause of acute hepatic failure a low ratio of ALP to bilirubin is associated with a poor prognosis (Thapa and Walia, 2007). GGT is a membrane bound glycoprotein which catalyses the transfer of gammaglutamyl group to other peptides, amino acids and water. Large amounts of GGT are found in the kidneys, pancreas, liver, intestine and prostate. In liver disease GGT activity correlates well with ALP levels but rarely the GGT levels may be normal in intra hepatic cholestasis as in some circumstances like familial intrahepatic cholestasis. In EHBA GGT is markedly elevated and in the case of hepatic lipodosis of camel GGT is only substantially increased when concurrent conditions such as pancreatitis and cholangiohepatitis are present (Salem and Hassan, 2011). Clinicians may faced a dilemma when they see elevated levels of ALP to differentiate between liver diseases and bone disorders; in such situations measurement of GGT is helpful as GGT is raised in cholestatic disorders and not in bone diseases (Rosalki and McIntyre, 1999).

**Molecular Pathology Tests**

Molecular methods of disease diagnosis allow the early and/or specific detection of inherited, infectious and malignant liver diseases process at a molecular level and discover possible avenues for treatment. Molecular approaches increasingly lead to a better understanding of the pathogenesis of the various liver diseases and also have an impact on diseased animal management, including the presymptomatic identification of animals at risk, the correct staging of the disease and the follow-up of animals undergoing therapy (Hubert, 2007). The use of IHC, ISH, PCR and recombinant DNA technology for drug development and the possibility of gene therapy as a treatment modality, are some to list for this approach (Howard et al., 1998).

**Immunohistochemistry (IHC)**

IHC is a method for localizing specific antigens in tissues and cells based on antigen-antibody recognition. IHC provides supplemental information to the routine morphological assessment of tissues during diagnosis, prognosis, and prediction of disease status and biology. IHC stains use monoclonal or polyclonal antibodies raised against the pathogen to target areas in tissue where a pathogen is present, coupled with a technique for flagging the pathogen. In this either a fluorescent dye or a chemical chromogen is used. It offers fast detection of antigens / antibodies associated with specific diseases and disease biomarkers (Dabbes, 2002). The diagnostic capacity of IHC in liver pathology is well described with HCC which is known for its histomorphologic heterogeneity. The comparative morphologic evaluation of HCC and their mimics is often a challenging issue. Some of these diagnostic challenges can be attributed to: the variety of neoplasms that can arise from the hepatic cells, as the liver is a target for metastases. Hence it is
difficult to make a distinction of a poorly differentiated HCC from CC and metastatic carcinomas; however, various IHC markers such as α-1-antitrypsin, Carcinoembryonic Antigen (CEA), factor XIIIa, ferritin, and albumin have been advocated for the identification of these tumors. Conversely, their ability to distinguish HCC from other malignancies has been limited (Sawan, 2009). Diagnostic findings are sometimes absent in biopsies of autoimmune liver diseases, and consideration of various entities has to be entertained in the differential diagnosis. Chronic inflammatory infiltrate which often plasma cell rich, is a common feature in all autoimmune liver diseases. There is evidence to suggest that the identification of the predominant immunoglobulin (Ig) subclasses within plasma cells in liver biopsies may be useful in the differential diagnosis of autoimmune liver diseases. Even though there has not been widely encountered in the differential diagnosis between autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC) is a very challenging from histopathological perspective. However, study of immune phenotype of plasma cells shows IgG is predominant in plasma cell infiltrate of AIH, while IgM prevailed over IgG in PBC cases upon qualitative analysis of IHC slides in human cases (Moreira et al., 2010) which may also be true for camels. Milne and Horne (1990), explain predominance of IgA and IgG in plasma cells in “chronic active hepatitis”, while IgM and IgG represents the main Ig classes of PBC cases. As a diagnostic tool there are differences in sensitivity of IHC tests based on staining method. For instance, the avidin–biotin (AB) based method; rely on the strong affinity of avidin or streptavidin for the vitamin biotin (Marc Key, 2003). However, endogenous biotin background is a Problem when using microwave pre-treatment Antigen-Retrieval’ method from liver tissue (liver is rich in biotin).

Polymerase Chain Reaction (PCR)

PCR is a molecular technique that amplifies nucleic acid extracted from patient’s sample, producing millions of copies of an isolated portion of nucleic acid (DNA/RNA). The extracted nucleic acid added to a reaction mix and subjected to heating and cooling cycles, (If the sample contains RNA, the RNA is converted to cDNA before PCR) (MSAC, 2004). When Formalin-fixed, paraffin-embedded (FFPE) tissues are used the advantages of PCR as diagnostic test are: tissue architecture is examined, only small amounts of tissue are required and tolerate the degradation of target nucleic acids. Ideally PCR is a quantitative assay with high sensitivity, specificity, and reproducibility, fast, easy and accurate (Clementi, 2000). However, sample taking, handling, extraction and processing all affect the sensitivity from field samples and reduce the diagnostic potential. Infection due to Cytomegalovirus, all hepatitis, Chlamydia, Mycobacterium etc.are easily detected by PCR; however, the main criterion for sample used for PCR is that it must be analyzed for nucleic acid (Hoffmann, 2004). Sample for PCR can be body fluids like bone marrow, blood, or skin cell, saliva, hair etc.

A study on Anti-infectivity of camel polyclonal antibodies against hepatitis C virus (HCV) in hepatoma to analysis a camel naïve polyclonal IgG and α-lactalbumin activity. In order to determine the inhibitory effects of Lactoferrin (Lf) on HCV replication in infected HepG2 cells, where Lf of: camel, human, bovine and sheep treatment is performed at different concentrations using RT-nested-PCR. Results show that camel Lf has the ability to inhibit HCV replication at concentrations start from 200μg/ml after four days of treatment, but failed to block the HCV replication at concentrations less than 150 μg/ml inside the infected cells. On the other hand, human, bovine and sheep LFs are able to completely inhibit the replication of HCV at concentration starts from 0.5 mg/ml, while at concentration of 0.25 mg/ml, those proteins are failed to prevent HCV replication inside infected HepG2 cells (EL-Fakharany et al, 2013). Since liver is a sympathy organ – it reacts in sympathy whenever any other organ in the body is damaged. Camel brucellosis and trypanosomosis (IAEA, 2007) are among such diseases having indirect effect on the liver. In line with this female animals positive for B. melitensis by serological and bacteriological tests, undergo a treatment with oxytetracycline and streptomycin combination for investigation. In the study apart from the significant increase in hemoglobin concentration and ALT activity with only slight liver infection and a decrease in glucose level of the treated animals; there are no significant changes in the hematological and biochemical parameters (Omer et al., 2011). However, “increase in liver enzymes (SD, ALT and AST) is explained in previously published scholars from camels infected with B. melitensis and B. abortus”. In view of this scholars
high levels of liver enzymes and hypoglycaemia attributes to liver damage during the brucella infection in camel (El-Boshy et al., 2009). After four months of treatment with oxytetracycline and streptomycin; however one of the female animal shows joint enlargements and gives abortion. Following this PCR is employed for confirmation of abortion, while PCR results are negative in this female. Hence it appears that the abortion might be due to a cause other than brucellosis and the treatment eliminates brucella organism (Omer et al., 2011). In addition to this real-time PCR detects B. abortus DNA in camel with a sensitivity of 91.7 % (Gwida et al., 2011).

PCR is also very useful for study on the dynamics of parasite populations in animals like trypanosome and the trypanosome infection in camel and other host species has some sequel on their liver. A research in Thailand on analysis of a genetic diversity of trypanozoon species (T. equiperdum, T. brucei and T.evansi), through a single PCR bases on a repeated DNA to discriminate 54 Thall and T.evansi isolates originated from 6 hosts species; reveal that T.evansi isolates have a high degree of heterozygosity at the locus related to the repeated coding sequence. In this the PCR products are ranging from 578 bp to 884 bp, corresponding to 3 to 6 repeats respectively; and 4 group-specific genotypes of T.evansi are demonstrated based on numbers of repeats in allele. Accordingly T.evansi clusters are not highly homogenous and, show genetic divers (IAEA, 2007).

In Situ Hybridization(ISH)

In situ hybridization is a term that collectively refers to histochemical techniques that reveal the location of cells that contains specific nucleic acid sequences. In its broadest applications, ISH has been used to detect DNA and all types of RNA. Fundamentally, all ISH techniques involve the hybridization of single nucleic acid strands that incorporate a label with complementary strands in cells. The hybridization events are made visible using various histochemical techniques, including, most often, autoradiography, enzyme cytochemistry, or fluorescent molecules (Baskin, 2008). It provides invaluable information regarding the localization of gene expression in heterogeneous tissues and detects the amount of mRNA contained in a single cell. The appearance of cells and their architectural arrangement in tissue examined with histological section represents only a fraction of information; missing all of the cellular proteins and gene expression, which help to ultimately determine the biological behavior of cells, as well as provide clues to the origins and pathogenesis of disease states (Hicks et al., 2004). In ISH protocols there are two basic labeling approaches, the radioactive (isotopic) and nonradioactive. Radioactive ISH procedures use liquid autoradiographic emulsion or autoradiographic film where as the nonradioactive technique uses standard histochemical and immunocytochemical methods in order to identify the cellular location of ISH events. In nonradioactive method all reagents are prepared with RNase-free reagents and slides are handled with gloves. DNA probes can provide high sensitivity to RNA probes (oligonucleotides vs riboprobes); however, DNA probes do not hybridize as strongly as RNA probes to the target mRNA molecules (Baskin, 2008). Fluorescent in situ hybridization (FISH) is the ISH which uses special fluorescent dyes that only attach to specific genes or parts of particular chromosomes. As a diagnostic test in study to demonstrate polysomy (duplication of two or more chromosomes) in greater than 5 cells of cytologic specimens FISH gives a sensitivity of 41% and a specificity of 98% for the diagnosis of CC and it doubles the sensitivity of conventional cytology in 125 cases positive for primary sclerosing cholangitis (PSC). It is highly sensitive (73%) in a high grade dysplasia for the diagnosis of CC with specificity of 95% (Chapman et al., 2010). In research to localize HCV positive of chronic hepatitis (a PCR amplified HCV cDNA is detected), a researcher applies ISH techniques for further diagnosis. In this strong perinuclear signal with or without cytoplasmic reaction is detected. Among 32(74.4%) positive liver biopsies (HCV genomic RNA strands localized) using RT in situ PCR method, only 9 are positive by standard ISH indicating lower threshold, and higher sensitivity in the rate of detection of the HCV positive RNA strand following the addition of PCR amplification. The detection rate by RT in situ PCR is estimated to be as low as a single copy per cell in comparison to ten copies per cell detected by standard ISH. In hepatitis C infection, hepatocytes as well as kupfer cells contain less than ten copies of viral genomes per cell. Hence there is high diagnostic importance behind the utilization of RT in situ PCR over standard ISH in HCV genomic detection (Mokhtar et al., 2000). Moreover, episomal and integrated viral DNA can only be differentiated by ISH because of the staining pattern (Baskin, 2008). Hepatocyte specific analysis of telomere length by quantitative fluorescence in situ hybridization q-FISH is conducted by Plentz et al. (2004), blindly without the knowledge of tumor pathology or grade of ploidy. The analysis reveals significantly weaker telomere signals in hepatocytes of HCC compared to hepatocytes of regenerative nodules or noncancerous surrounding liver tissue. Accordingly, the telomere length in hepatocytes of HCC is shorter compared to regenerative nodules at any age, indicating that shortened hepatocellular telomeres are an age independent marker of HCC. However, in regenerative nodules there is a correlation between patient age and telomere length being slight in younger. In short hepatocyte telomere shortening is a prognostic marker of HCC risk development.

Diagnostic Cytology

Diagnostic cytology involves the examination of cells which have either naturally exfoliated or have been artificially removed from a body cavity or a tissue mass. It
is valuable in establishing a diagnosis, identifying the disease process, directing therapy, forming a prognosis, and/or determining what diagnostic procedure should next be performed. The diagnosis depends on the distinct characteristics of individual cells on a smear regardless of the architectural pattern characterizing the tissue of origin. It can be performed quickly, easily and inexpensively with little or no risk in most cases (Agarwa and Raamamoorathy, 2005). The Solid tissue Cytology method is used for liver pathology diagnosis. The main thing here is to obtain a significant number of well stained intact cells that reflect the composition of the lesion. Touch impressions made from freshly excised liver removed during surgery or necropsy is used as a sample or when the liver develops fibrosis, scrapings is better than imprints to obtain a more representative sample. Romanowsky-type Stains are cytological staining which stain organisms and the cytoplasm of cells excellently. These Stains are sufficient to differentiate neoplastic and inflammatory changes though nuclear and nucleolar detail is poorly /cannot be perceived. On the other hand Papanicolaou Stains clearly illustrate cell structure and nuclear characteristics, but cannot demonstrate bacteria and other organisms. These stains are also able to demonstrate whether the lesion is inflammatory or neoplastic in nature (Salem and Hassan 2011). In study conducted by El-Baky and Salem (2011), to diagnosis camel liver pathology by diagnostic cytology hepatic smears or impression has examined; for instance from naturally infected camels with *T.evansi*. Fatty degeneration of the liver and, round and sharp delineated vacuoles in the hepatocyte is reported from this authors finding (Figure 5a1). The vacuoles vary in size from small to large ballooning vacuoles causing hepatocyes distention. Large numbers of lymphocytes and Kupffer cells with fibroblasts indicator mixed infection. The fatty degeneration manifested by lipid accumulation inside the hepatocytes is due to tissue hypoxia resulted from anemia and vascular damage. Other hepatic smears shows necrosis of hepatocytes with poor delineation, light colors and lacy appearing areas in their cytoplasm (Fig. 5a2). This necrosis of hepatocytes is due to liberation of *T. evansitoxins* in plasma and tissue fluids that induce an increment of lysosomal secretion followed by autolysis of the cell. From cytological smears lymphocytic hepatitis is characterized by increased numbers of small lymphocytes and plasma cells without increased numbers of macrophages (Fig. 5b1) while chronic active hepatitis (granulomatous inflammation) is characterized by a mixed infiltrate of large numbers of macrophages beside lymphocytes, plasma cells with fibroblasts and occasionally eosinophils (Figure 5b2). Hepatic cirrhosis is observed with both types of this hepatitis and may appear as a fibrocytic bundles (Salem and Hassan, 2011). Inflammation of the liver as neutrophilic (suppurative) or lymphocytic is noticed and it is usually due to cholangitis resulted from ascending bacterial infections. Reactive fibrocytes are commonly seen a long with severe inflammation and careful should be taken not to over interpret this reactivity as a neoplastic activity. On the other hand, granulomatous inflammation of the liver is not uncommon in camel liver pathology (Saker et al., 1991).

**CONCLUSION AND RECOMMENDATIONS**

Diseases of various etiological agents are the major problems faced by camel producing communities. Most of these diseases either directly or indirectly affect the liver. Liver is considered the most important organ for animal health production and reproduction as many of the metabolic activities of the body occurred in the liver. Losses of camel liver in slaughter house due to disease are economically significant. Liver diseases are frustrating to diagnose and often difficult to treat. Therefore, When liver diseases are suspected after a general clinical examination, special techniques and diagnostic approaches such as Necropsy with histopathological
Examination, biochemical analysis (aminotransferases and cholestatic enzymes), Molecular pathology tests like IHC, PCR and ISH, and diagnostic cytology are effective to determine further the status of the liver and to arrive at confirmatory diagnosis. The major liver diseases in camel include: infectious hepatitis, parasitic hepatitis, toxic hepatitis, and liver tumors, metabolic (fatty liver) and etc. These diseases together with idiopathic causes aid in liver fibrosis, cirrhosis and finally liver failure.

In view of the above, liver pathology and its associated causes deserve an appropriate and comprehensive up to date diagnosis starting from anatomopathology. Accordingly, the following recommendations are forwarded: Financial losses due to camel liver pathology should be taken in to consideration, different diagnostic tests must be supplemented to arrive at confirmation in respect to the location and primary causes for the underlying pathological lesion of camel liver, future studies encompassing diagnosis and characterization of camel liver pathology should be conducted in order to estimate the extent and magnitude of the problem as well as apply the various diagnostic techniques.

LIST OF ABBREVIATIONS

AIH Autoimmune hepatitis
ALP Alkaline phosphatase
ALT Alanine amino-transferase
AST Aspartate amino-transferase
BUN Blood urine nitrogen
CC Cholangiocarcinoma
CEA Carcinoembryonic Antigen
EHBA Extrahepatic Biliary Atresia
FFPE Formalin-Fixed, Paraffin-Embedded
FNA Fine Needle Aspiration
GCT Gamma-glutamyltranspeptidase/transferase
HCC Hepatocellular Carcinoma
HCV Hepatitis C virus
LDH Lactate Dehydrogenase
Lf Lactoferrin
PBC Primary Biliary Cirrhosis
PSC Primary Sclerosing Cholangitis
SDH Sorbitol Dehydrogenase

REFERENCES
