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Original Research

Histological and Immunohistochemical Assays of *Trypanosoma evansi* Infected Camel Hepatic Tissues

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Abstract

Camel Trypanosomiasis is one of the most significant illnesses influencing the livestock economic development. The present study reports observations of histological and immunohistochemical alterations of *Trypanosoma evansi* infection in the liver samples of camels (*Camelus dromedarius*) from Al-Madinah district in Saudi Arabia . Liver of camels showed presence of red blood cells among the hepatic cells, infiltration of connective and fibrous tissue between the liver's lobules, and a reduced number of hepatic cells. Upon Masson trichrome staining the infected liver showed elevated deposition of thick collagen fibers that were conspicuously stained blue. Immunohistochemical study of the -SMA-positive cells in the *T. evansi* infected camels also exhibited considerable growth of the brownish cells between the sinusoidal walls and around the portal area. Moreover, increase numbers of -SMA-positive cells in the tissue zone around the portal vein, artery, and bile duct branches were observed. Overall, the infection stimulates hepatic CD8+ and CD4+ T cells in the liver, which is a well-known component of the immune response cascade, according to the study. The acquisition of milk, meat, and other products is crucial for camels. Therefore, it is essential to examine the camels' health, risks, and pathological effect of related diseases.

Keywords: liver, *Trypanosoma evansi*, camel, histopathology, immunohistochemistry

Introduction

Trypanosomiasis, also known as surra (from the Hindi word for "rotten"), is one of the most significant diseases afflicting animals in tropical and semitropical regions. It is brought on by the hemoparasite *Trypanosoma evansi*. Despite the fact that some wild animals as well as camels and horses are particularly susceptible to trypanosomiasis, infections and clinical cases have been reported in numerous different domesticated mammals. *T. evansi* can become endemic when introduced to a new area since it is distributed mechanically by several tabanids and other flies. Populations lacking or having weakened immunity

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may incur high rates of illness and mortality in such an endemic condition. Surra, thus causes economic losses by adversely affecting productivity of domestic animals caused due to lower weight gains, decreased milk supply, reproductive losses, and the expense of treatment in addition to illness and fatalities [1, 2].

The parasite *Trypanosoma* is widely distributed and infects mammals throughout the world [3]. Due to the parasite's reliance on the host for glucose and oxygen for growth and reproduction, the host experiences degenerative alterations as a result of a reduction in both of these substances. Cellular damage brought on by toxicants discharged by the parasite or as a response from the immune system can bring forward the later alterations in various organs of the host. Despite being a hemoprotozoa, visceral forms of *T. evansi* have been recognized in various organs, primarily the heart. Additionally, the optic lobes, cerebrum, liver, kidneys, lungs, and optic nerves also showed parasite presence [4]. Anemia, recurrent fever, weight loss, emaciation, enlargement of the hind limbs, and hemostatic abnormalities are among the clinical signs of *T. evansi* infection [2]. Moreover, Trypanosomiasis signs are severe and fatal, particularly in the later stages of infection as the disease progresses from a chronic stage to a lethal acute stage, along with gradual physical deterioration, significant exhaustion, enlargement of lymph nodes, and eventually death [5].

T. evansi shows a predisposition towards the host's connective tissues. Hence in connective tissue of the host, it tends to shatter the collagen bundles and annihilate the main connective tissue cells; the fibroblasts. The subsequent result is additional tissue damage occurred due to cytoplasmic and mitochondrial enzyme release into the serum [1].

The host organs that respond most noticeably during the acute stage of disease are the lymph nodes and spleen, with plasma cells prevailing. While the immune system loses the benefit of its lymphoid cells in the advanced phases of infection, this may be the cause of the extensive lymphoid tissue hyperplasia that characterizes *T. evansi* infection [6]. The study of *T. brucei* species-infected laboratory animals revealed circulating and tissue-mediated immune complexes in the animals. A major fraction of their antibody is directed against the parasite [7].

T. evansi parasitizes on the blood tissue of the host and is entirely susceptible to the immune system of animal. Contrary, the parasite is able to avoid host-specific immune responses by virtue of its ability to make persistent alterations in variable surface glycoproteins (VSGs) [8]. Suramin is an ordinarily used drug for the treatment of surra in horses [9]. Understanding the mechanism of *T. evansi* host immune evasion would enable to create novel control techniques since there is currently no available vaccination to prevent surra [10].

This condition causes significant productivity losses worldwide and, if not treated in time, can be fatal [11]. Due to variations in diagnostic techniques (blood smears stained with Giemsa, hematocrit centrifugation, serologic testing, and a more recent method of molecular analysis using PCR), trypanosomiasis is more or less common in various Red Sea countries [12]. The goal of the current research was to examine the histological changes in the livers of *Trypanosoma* infected camels from the Saudi Arabian Al-Madinah region.

Materials and Methods

Animals and Sample Collection

Camel (*Camelus dromedarius*) liver samples were carefully gathered from slaughterhouses in the Al-Madinah district of Saudi Arabia's northwest province. For additional processes, 10% formalin was used to store samples. An ethylene diamine tetraacetic acid (EDTA) tubes were used to draw 5 mL of blood from jugular vein of each camel, samples were placed in a cold box and moved to the laboratory for examine operations.

Confirmation of *T. evansi* Infection

Freshly drawn blood was used to perform a direct thin blood smear then stained with Giemsa stain on a clean glass slide for the presence of *Trypanosoma* under an oil immersion objective lens (100 magnification) of light microscope (BX53, OLYMPUS, Tokyo, Japan), the obtained samples were evaluated for *Trypanosoma* infection or non-infection [13].

Histopathological Preparation of Liver Tissues

Positive *Trypanosoma spp.* samples of liver were cut into 1 cm-long sections and fixed in 10% neutral buffered formalin (NBF). Samples were then dehydration and paraffin blocks were prepared. Using a rotary microtome, the blocked tissues were carved into slices that were 4-5 m thick. After being picked with glass slides and dewaxed in descending alcohol concentrations (100%, 95%, and 70%), the cut portions were flattened on a water bath. Hematoxylin and eosin (H&E) were used to stain the paraffin liver slices on slides, which is a typical histological procedure for identifying different tissue types and morphologic alterations. The Masson trichome stain (MTS), which is used to distinguish between collagen and smooth muscle for fibrosis details, was also applied to the tissue samples. Following alcohol dehydration, xylene cleaning, and mounting with DPX for additional evaluation [14]. The Masson trichrome stain can be a useful tool to differentiate myofibroma from smooth muscle lesions [15].

Immunohistochemical Examination (IHC)

The paraffin-covered liver slices were hydrated and paraffin-free. The endogenous peroxidase activity was blocked by a 5-minute incubation with 3% hydrogen peroxide. Veterinary Diagnostic Test Kits and Reagents (VMRD Inc), Pullman, WA, USA, sold the CD4+ and CD8+ monoclonal antibodies that were used to incubate these paraffin-free tissue slices overnight. Each primary antibody was applied to distinct tissue sections. The biotinylated goat anti-mouse immunoglobulin serum (bought from Dako Denmark A/S) was then coupled to the monoclonal antibody for 30 minutes. After 5 minutes of exposure to 3-diaminobenzidine tetrahydrochloride solution (DAB), followed by rinsing with distilled water, a brown reaction was produced. Sections were countered-stained with hematoxylin and then examine under a microscope [16].

Results

Confirmation Tests of *T. evansi* Infection in Camels

Blood smears from normal camels exhibits normal blood cells with no hemoparasites. In contrast, the typical picture of the long slender forms of *T. evansi* showed among infected blood smears.

Histopathological Results with Hematoxylin and Eosin Staining

Hematoxylin and eosin (H& E) staining of sections of camel liver infected with *T. evansi* revealed coagulative necrosis, loss of hepatic architecture, hemosiderin infiltration as granular brown deposits, and fatty degeneration in hepatic cells around the central vein as symptoms of infection, according to comparative histological observations of section of non-infected normal camel liver (Fig. 1(a, b)). The central vein was recognized to be slightly dilated and obstructed,

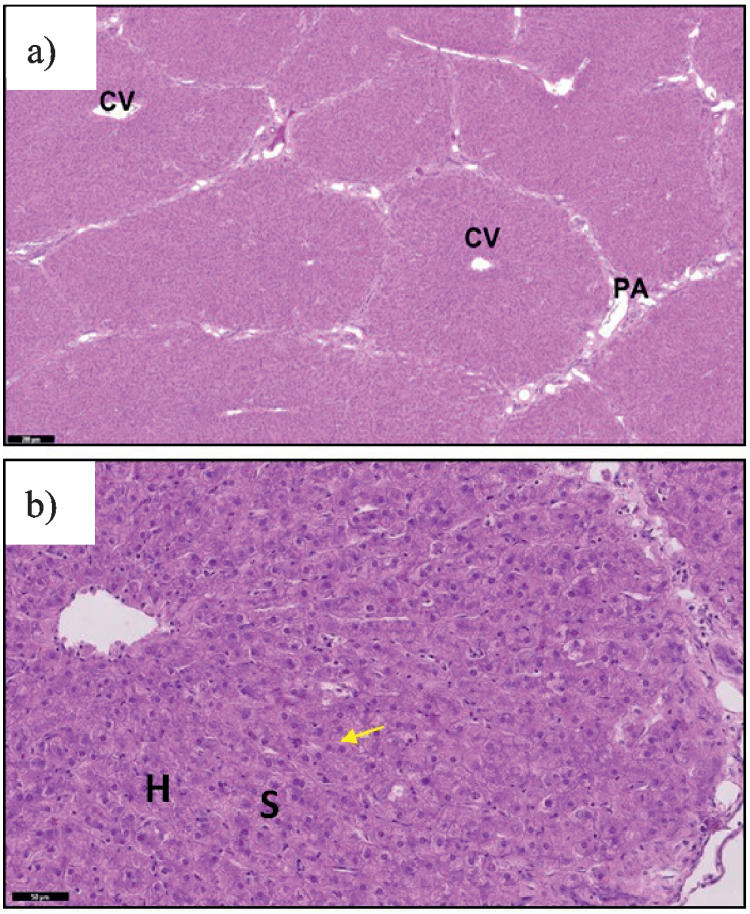

Fig.1. Photomicrographs of hematoxylin and eosin (H&E)-stained sections in the liver of normal non infected camel. a): Normal hepatic structure with central vine (CV) and portal area (PA). b) Hepatocytes (H) have rounded vesicular nuclei with prominent nucleoli and acidophilic granular cytoplasm. Some hepatocytes are binucleated. The blood sinusoids (S) are regularly arranged and lined by thin flat endothelial cells with flattened nuclei and Kupffer cells with ovoid nuclei (yellow arrow).

and there was also some sign of red blood cell infiltration. Other noteworthy histological changes brought on by the virus were inflammation, hemorrhage, and Kupffer cell hyperplasia. Moreover, sections displayed infiltration of fibrous tissue and connective tissue between the lobules of the liver (liver cirrhosis), in addition to hemorrhage among the hepatic cells. (Fig. 2(a, c)).

Observations with Masson Trichrome Stain (MTS)

Following MTS treatment, the *T. evansi*-infected camel liver sections and healthy, uninfected liver sections showed noticeably thicker, corrugated collagenous fibers. Cord injury is brought on by increased collagen in connective tissue. Some of the hepatic lobes displayed

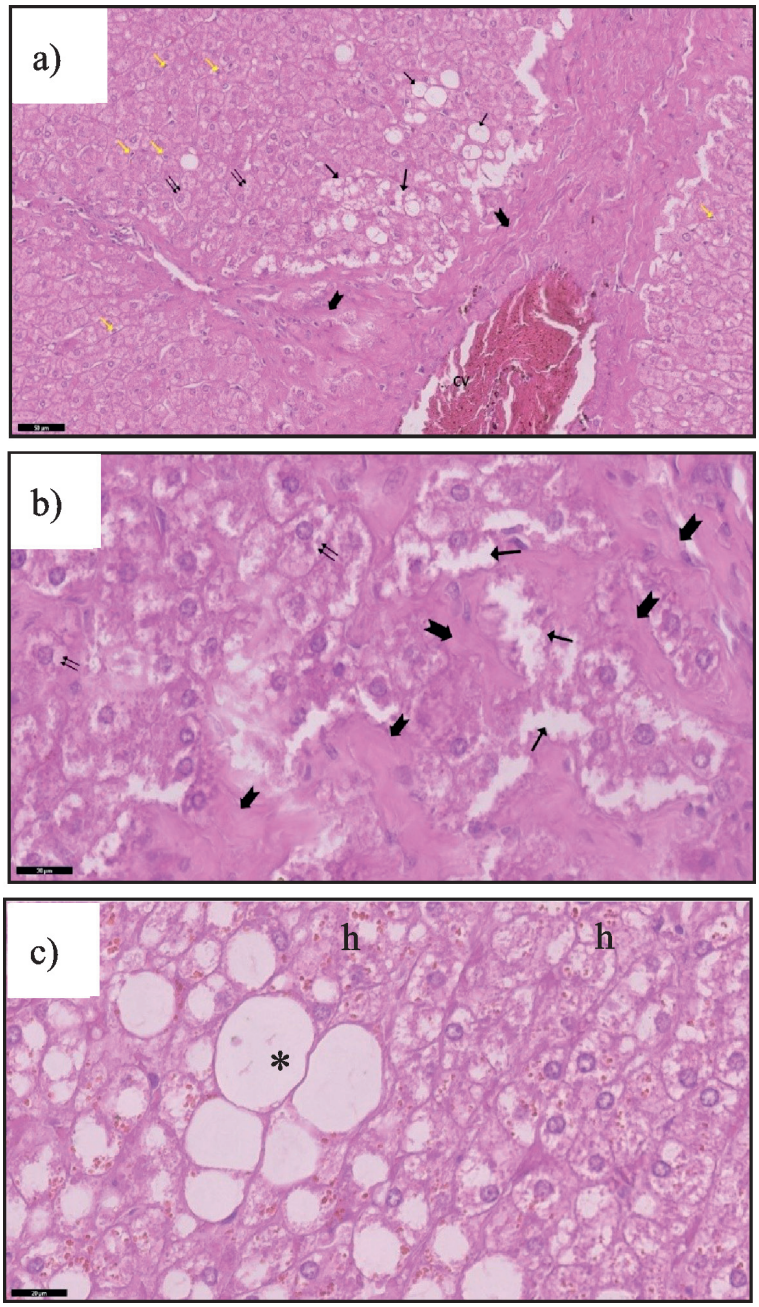

Fig. 2. Photomicrographs of hematoxylin and eosin (H&E)-stained sections in the liver tissue of camel infected with *T. evansi.* a) Exhibits the hepatic cord lacking any distinct identity and loss of the radiating from the central vein (CV), Most of the hepatocytes show marked hydropic degeneration (↑↑), Congestion of the central vein (CV) and massive collagen fibers deposition surrounding the central vein (bifid black arrow), loss of normal hepatic architecture with a part of the dilated central vein, notice the hypertrophied scattered Kupffer cells (yellow arrow) in dilated blood sinusoids are seen. b) Some pericentral hepatocytes appear with foamy cytoplasm (↑), degeneration of hepatocytes in the form of large vacuolation. c) Most of hepatocytes appear with hemosiderin granules (h), fatty changes marked by an asterisk (*).

sinusoidal space fibrosis. The portal tract has a dense layer of collagenous fibers visible. Complete and partial periportal intersecting bands were discovered, resulting in a pseudo-lobular structure. The central vein was dilated collagen fibers surrounding it as seen in (Fig. 3b). The changes can be compared to a normal liver section stained with MTS as shown in (Fig. 3a).

α-SMA Immunohistochemical Study

Alpha Smooth Muscle Actin or α–SMA is a widely utilized marker of activated hepatic stellate cells (HSCs). In the normal non-infected liver, hepatocytes reacting with anti α-smooth muscle-actin (α-SMA) antibody were recognized around the central vein and the portal area. Notice α-SMA-positive brownish cells appeared surrounding the vascular structure in the portal area (portal vein and branches of the hepatic artery) and branches of bile ducts as in (Fig. 4a).

SMA positive expression was seen strongly and widely in the *T. evansi*-infected camel liver tissue, but only along the sinusoids. The majority of these positive expressions, which represent active HSCs, were found in the peripheral regions of the hepatic lobule close to the portal area. All of the proliferating bile ducts, hepatic artery branches, and portal vein had positive brownish -SMA cells. In several regions of the infected tissue, there were numerous and widely distributed -SMA positive cells surrounding the central vein. In areas of expanding septa and sinusoidal gaps of lingering hepatic parenchyma, a considerable number of -SMA-positive cells were seen. However, α-SMA positive cells were enclosed in the peripheral area of the regenerative plates in areas demonstrating steatosis (vacuolated hepatocytes). Although most α-SMA positive cells stretched lengthy cytoplasmic processes along the endothelium lining, they were wide-ranging in shape and size. The greater expression of α-SMA positive cells was found at the site of necrosis in the infected camel tissue with *T. evansi* (Fig. 4b).

CD-4 Immunohistochemical Study

As shown in (Fig. 5a), the immunohistochemically CD4 treated section of the normal liver displayed a negative brownish reaction of CD4 T- lymphocytes (T. cell). It's interesting to note that the hepatic tissue of the infected camel group showed an evident increase

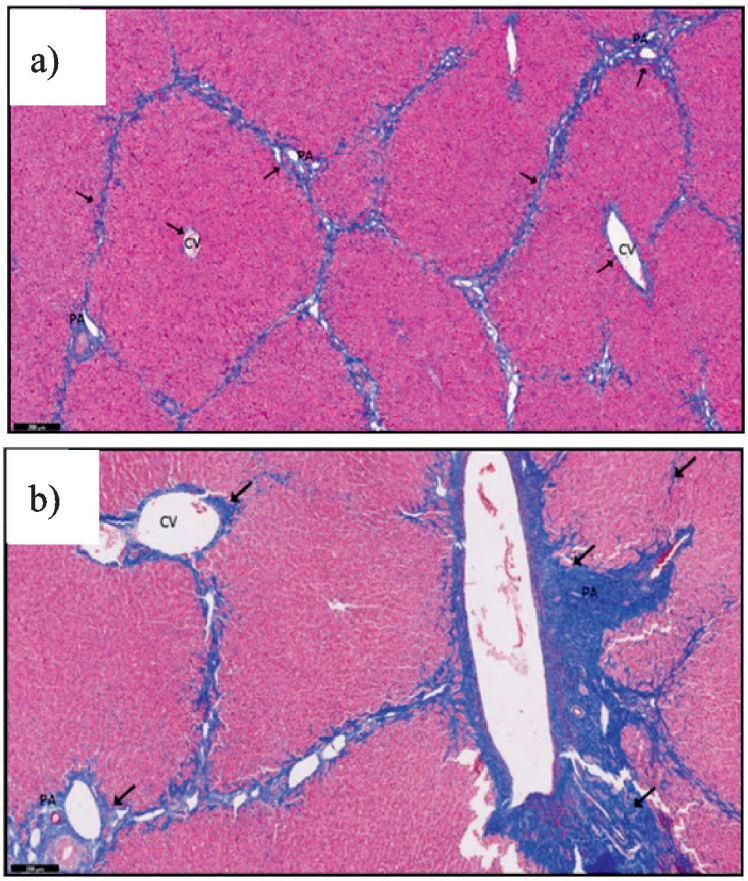

Fig. 3. Photomicrographs of Masson trichrome (MTS) in the liver of normal non-infected and infected camel. a) Normal liver showing the fine blue stained fibers (↑) between the radially arranged hepatocytes around the central vein (CV) and at the portal area (PA). b) Liver infected with *T. evansi* shows increase deposition of dense blue stained collagen fibers (↑) around the central vein (CV) and at the portal area (PA).

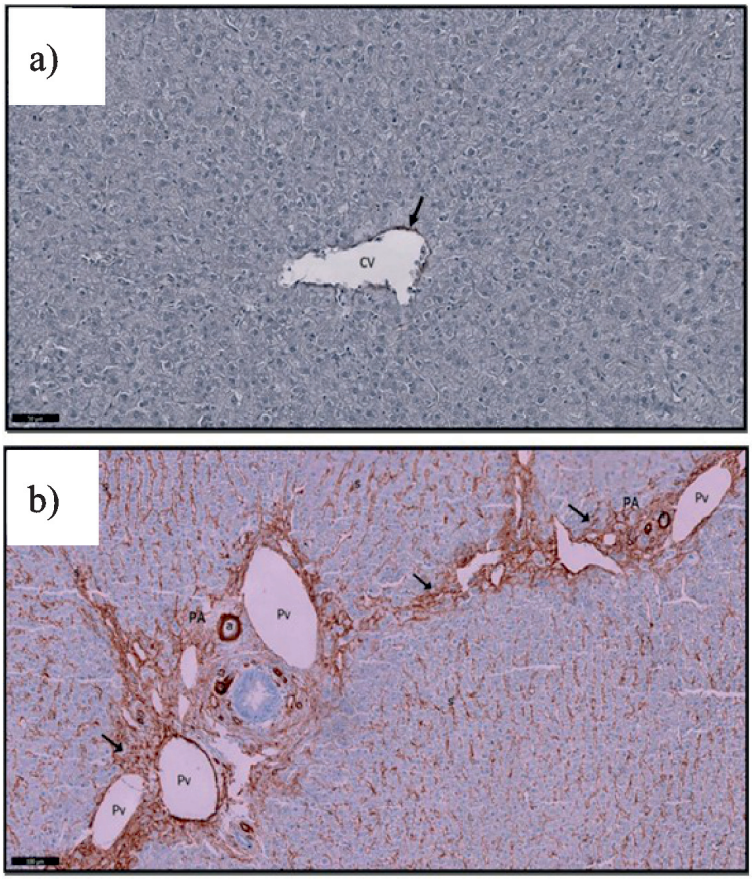

Fig. 4. Photomicrographs of α -SMA Immunohistochemically treated sections in the liver of normal non-infected and infected camel. a) Normal liver analysis of α -SMA showing few positive brownish cells (↑) surrounding the central vein (CV) in Fig. a) liver infected with *T. evansi* shows an apparent marked increase of the positive brownish cells (↑) in between the sinusoidal walls (S) and surrounding the portal area (PA). b) Notice extensive α-SMA-positive cells in the portal area (PA) surrounding portal vein (PV), artery (a), and branches of bile ducts (d).

of positive brownish immune reaction around the central vein and in the portal area, indicating significant inflammatory cell infiltrations (Fig. 5b).

CD-8 Immunohistochemical Study

Additional CD8 T-cell immunohistochemistry study in a healthy, untreated liver exhibited negative brownish reaction (Fig. 6a). On the other hand, the *T. evansi*-infected group displayed an increase in positive immune reaction between the hepatocytes, around the central vein, and at the portal area, indicating significant inflammatory cell infiltrations (Fig. 6b).

Discussion

A number of microscopic changes were discovered when *T. evansi*-infected camel liver tissue was examined histologically. These modifications included the existence of neighboring pericentral and periportal cells and absence of distinct polygonal forms in hepatic cells as well as the existence of undefined intercellular gaps between hepatocytes. The hepatic cells around the central vein exhibit necrosis, hemosiderin, and fatty degeneration. Additionally, it was discovered that the cell membranes were extensively damaged due to the cytoplasm's heavy vacuolation. Infiltration of connective and fibrous tissue between the hepatic lobules was also found in this investigation. Moreover, hemorrhage, inflammatory sinusoid regions, and an aggregation of inflammatory cells near the central canal and portal regions, red blood cells may also be present.

These pathological alterations are most likely brought on by trypanosome proliferation, which in the due course is accompanied by amplified oxygen consumption. This subsequently causes a hypoxic state and further chain of degenerative changes [17]. According to certain investigations [13, 18], the toxins generated by *T. evansi* were responsible for these pathological changes in the hepatic tissue.

According to a previous study [19] by Rodrigues et al., in 2009, the infected hepatic tissue in horses had the following notable features: (a) mild lymphoplasmacytic periportal hepatitis; (b) Kupffer cell hypertrophy; and (c) hemosiderosis.

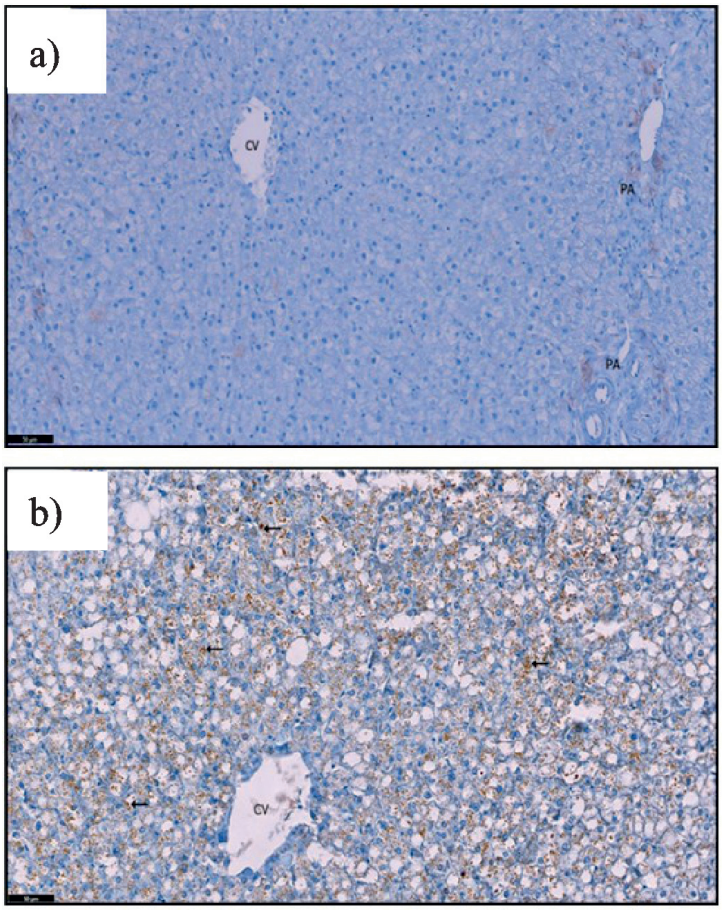

Fig. 5. Photomicrographs of Immunohistochemical stained sections in the normal non-infected and infected liver analysis CD4 T-cell. a) Negative cells around central vein (CV), and at the portal area (PA) in normal non-infected. b) Infected liver camels with *T. evansi* positive cells in-between the radiated hepatocytes, around central vein (CV).

In one study, rats infected with *T. brucei* showed damaging and irreversible changes, such as fatty change, binucleated and calcified cells [20]. Another study [13] observed fatty degeneration in camels and hepatocytes with rounded, pointed, and characterized vacuoles. This may be brought on by *trypanosome* infection or by metabolic byproducts of trypanosomes, as well as by the presence of haemosiderin from blood cell lysis. Similar research suggested that the blurriness of the intercellular gap was caused by the deformed geometries of liver cells [21].

Hepatocyte apoptosis was evident, and the adjacent portal tract cells were seen harmed as well. The enlarged, blocked sinusoids were stuffed with blood cells. Hepatic cord placement was obviously uneven. Invading and accumulating inflammatory cells surrounded the portal canal and bile duct. Hepatic cells lost their polyhedral structure and took on a more or less spherical shape. The cytoplasm was heavily stained and vacuolated by eosin. Kupffer cells grew larger and occasionally shifted in place. There is visible liver cell loss, and the adjacent portal tract cells are damaged. Blood cells are crammed into dilated, clogged sinusoids. The position of the hepatic cords is noticeably asymmetrical. Around the bile duct and the portal canal, inflammatory cells intrude and collect. Hepatic cells develop a more or less rounded form after losing their polyhedral configuration. Eosin deeply stains and vacuolates the cytoplasm. Kupffer cells enlarge and, occasionally, shift out of place [22].

Domestic animals frequently contract hepatitis and the pathological alterations that arise from a parasite infection or the movement of helminth larvae [23]. *Strongylus* spp., *Ascaris* spp., *Fasciola* spp., and *Schistosoma* spp. are only a few of the parasite larvae that have been observed migrating across the hepatic parenchyma and have been linked to local routes of hepatocellular necrosis and inflammation. In a subsequent step, connective tissue replaces these necrotic channels, leaving fibrous scars on the capsule's surface [24].

The migration of various parasite larvae such as *Strongylus* spp., *Ascaris* spp., *Fasciola* spp., and *Schistosoma* spp. throughout the hepatic parenchyma is seen and is reported to cause local pathways of hepatocellular necrosis associated with inflammation. These necrotic pathways are in a subsequent manner replaced by connective tissue which creates fibrous scars on the surface of the capsule [24].

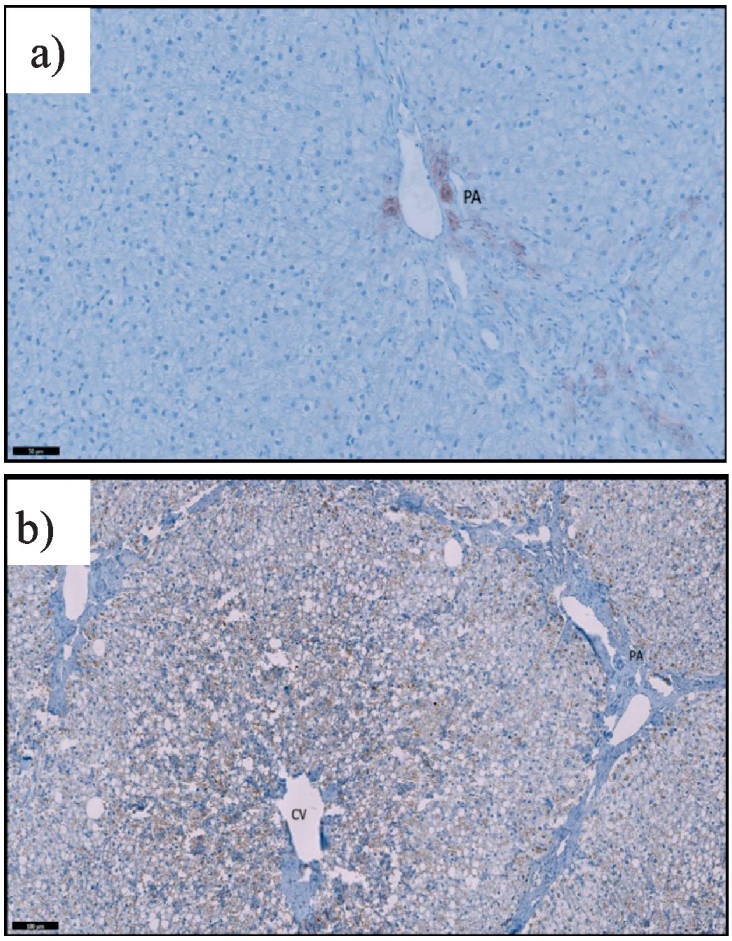

Fig. 6. Photomicrographs of Immunohistochemical stained sections in the normal non-infected and infected liver analysis CD8 T-cell. a) CD8 T-cell negative cells around central vein (CV), and at the portal area (PA) in control figure from normal uninfected liver tissue section. b) Infected liver camels with *T. evansi* shows positive brownish IHC reaction in-between the radiated hepatocytes surrounding central vein (CV) and at the portal area (PA).

The ability of Masson's trichrome stain to give collagen fibers a blue stain in contrast to the red backdrop of hepatocytes and other associated structures makes it a popular tool for studying liver tissues [25]. Type 1 collagen may get stained. In hepatic tissue, collagen Type-1 is typically found in the portal tracts and vessel walls. MTS, however, can also highlight the presence and dispersion of reactive fibrosis brought on by hepatic injury. In order to identify and highlight the pattern and progression of injury, MTS is also used to evaluate and determine the phases of chronic hepatic disorders. One illustration is primary sclerosing cholangitis, which is characterized by periductal fibrosis and perisinusoidal fibrosis along with steatohepatitis [26]. Acute liver inflammation causes fibrotic reactions at the site of inflammation [27], as well as the recruitment and activation of leukocyte populations, in contrast to the aforementioned chronic modifications. The resolving fibrosis seen during acute injury acts to protect surviving hepatocytes by decreasing pro-apoptotic signaling and increasing tolerance to a range of toxins [27]. Injured tissue is visited by leukocytes, which discharge growth factors and inflammatory cytokines that regulate the fibrotic process. HSCs, which are potent producers of extracellular matrix components like type I collagen and smooth muscle actin, are activated and proliferate as a result of these cytokines [28]. HSCs normally have a non-proliferative phenotype; however, when exposed to infections or encounter a hepatic injury, they are activated and possess the ability to transdifferentiate from being vitamin A-storing cells to myofibroblasts. Myofibroblasts are the cells responsible to produce collagen. As a result, it is generally agreed that these cells constitute the most significant cell type in liver fibrosis [29, 30].

Pathologists were formerly obligated to employ electron microscopy and other histochemical staining methods, such as hematoxylin and eosin, to identify potential changes brought on by specific pathogenic organisms [31]. A significant change was brought about by the broad availability and specificity of antibody reagents. These tests were important in the identification of infectious disease agents in tissue sections due to their high sensitivity and relative simplicity, which had an impact on immunohistochemistry methods [32, 33]. The use of immunohistochemistry (IHC) assays for

infectious disease detection has a number of benefits, including direct morphologic localization with high sensitivity that helps in the testing of fixed tissues and cells and identifying the infection-causing parasite during chronic stages.

It has proven successful in the past to diagnose *T. evansi* in tissues taken from camels using immunohistochemistry testing techniques. An apparent noticeable increase of the positive brownish cells between the sinusoidal walls and surrounding the portal area is shown by immunohistochemical examination of -SMA-positive cells in the *T. evansi*-infected hepatic tissue of camels in the current investigation. There are numerous -SMA-positive cells in the region around the portal vein, arteries, and bile duct branches. Regarding the hepatic immune response, it was found in the current study that the infection results in activated CD8+ and CD4+ T cells in the liver. Here, a range of anatomical, immunological, and environmental factors play a significant role in the balance between immune responses and tolerance in the liver. These highly vascularized hepatic microvessels are known as hepatic sinusoids. The membrane segments of the sinusoids' lining epithelial cells can protrude, allowing stromal and parenchymal cells to interact physically with the cells moving in the vase lumen. The liver has a variety of cell types in the Disse space [32], which is an area close to the sinusoids. Between the hepatocyte cords and the sinusoid wall is the Disse space. This integrated cellular contact network gains from the sinusoids' extraordinarily low blood pressure because of their advantageous position, which influences the liver's biochemical and immunological functions [34-36]. The liver's primary metabolic functions are well understood, but it also contributes significantly to the local and systemic immune response [22].

Conclusions

Camel is one of a prominent element of the pastoral economy all across the world especially in arid and semi-arid regions where it is highly regarded. people mostly rely on camel milk and meat for food and many purposes. Camel trypanosomiasis is a significant economic burden in many parts of Africa, Asia, and South America. The disease still causes devastating epidemics, albeit they are less frequent now than they were a few years ago. The camel population is increasing accompanied by the development of animal health difficulties, Therefore, it is necessary to focus attention is on analyzing the changes associated with pathogen infection related to camels.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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