HISTOPATHOLOGICAL AND BIOCHEMICAL ANALYSIS OF SELECTED ORGANS OF CATTLE INFECTED WITH *BOVINE BABESIOSIS*

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Abstract. The current study aims to investigate the effects of babesiosis infection on biochemical parameters, oxidative stress biomarkers, and the histopathological changes on the spleen, kidneys, and liver. The blood of 110 animals were collected. Biochemical screenings for antioxidant and oxidant markers, as well as kidney and liver function testing. Liver, kidney, and spleen samples were examined using histology and immunohistochemistry in this study. The Babesia organism was detected in Giemsa-stained blood smears in 42 cattle. When compared to a healthy control (44 cattle), animals infected with Babesia had significantly higher levels of serum liver and kidney function. Oxidative stress in Babesia-infected animals was an increase and a significant drop in the activity of antioxidants relative to the healthy control. In addition, the current study found that *Babesia bovis* caused significant degeneration in the spleen, kidney, and liver histopathological structures, along with an increase in collagen fiber deposition, decrease in BCL-2 positive cells and an upregulation of CD3+ cells in the spleen. The findings of this study suggest that Babesiosis can impair antioxidant defenses and increase oxidative markers. Therefore, in addition to Babesicidal drugs, antioxidants can be used as a supportive therapy for a better and faster recovery.
Keywords: *Bovine babesiosis, babesiosis, kidney, liver, spleen, histopathological, oxidative markers*

Introduction

Protozoa belonging to the genus Babesia cause bovine babesiosis, a parasitic illness. The most common species of Babesia that cause babesiosis in cattle are *Babesia bovis*, *Babesia bigemina,* and *Babesia divergens*, according to the World Organization for Animal Health (previously the Office International des Epizooties) (Alvarez et al., 2019; Attia and Khalifa, 2023).

Babesia bovis, transmitted by the tick vector Rhipicephalus microplus', infects cattle in tropical and subtropical regions (Almazán et al., 2022). Asexual reproduction takes place within the erythrocytes of the host bovine, while sexual reproduction takes place in the gut of the definitive Rhinocephalus tick, which is a key component of the parasite's intricate life cycle (Alzan et al., 2022). A large number of studies have reported the infection and prevalence of Theileria spp., Babesia spp. and Anaplasma spp. in cattle worldwide (Al-Shammari et al., 2024).

Weight loss, decreased milk output, and animal mortality caused by bovine babesiosis are direct economic losses. This makes the disease a big burden for the cattle business. The prevention and treatment of it also incur secondary costs (Guswanto et al., 2017). Without treatment, animals that recover from acute babesiosis become chronically sick and can spread the disease to other animals in the herd (Sondgeroth et al., 2014). The infection can be fatal for afflicted animals in extreme circumstances (El-Sayed et al., 2019).

Oxidative stress is a condition when there are more oxidants than antioxidants. A decrease in antioxidant enzymes or other causes of unchecked free radical generation can lead to this kind of stress. Massive harm to cellular components results from the

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uncontrolled production of free radicals. The process of lipid peroxidation culminates in malondialdehyde (MDA), which is produced when reactive oxygen species (ROS) react with polyunsaturated fatty acids within the cell membrane. As a biomarker for damage mediated by free radicals, MDA is utilized. In addition to regulating host defense mechanisms, nitric oxide influences a wide variety of intracellular parasite illnesses. Parasitic illnesses are characterized by changes in oxidative stress indicators. It is widely acknowledged that erythrocytic peroxidation has a role in the development of several hemiparasitic illnesses (De et al., 2012; Esmaeilnejad et al., 2018). When exposed to extreme oxidative stress, Babesia spp. trigger the body's natural defenses, including antioxidant systems that protect cells, tissues, and organs from further harm. It is worth mentioning that many oxidative stress biomarkers are tested to assess the efficacy of antioxidant systems and total antioxidant capacity. These biomarkers include the activity of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glucose-6-phosphate dehydrogenase (G6PD), among others. Furthermore, the levels of MDA, which indicate lipid peroxidation, protein carbonylation, which indicates protein oxidation, and DNA damage, which is measured by an alkaline comet assay, can be used to monitor the oxidation states of biomolecules (Esmaeilnejad et al., 2020).

This study aims to investigate the effects of babesiosis infection on biochemical parameters, oxidative stress biomarkers, and the histopathological aspects of the disease's impact on the spleen, kidneys, and liver. The results may provide light on the detrimental effects of babesiosis on cattle's quality of life.

Materials and methods

Animals

The current research examined slaughterhouse samples from the Al-Madinah region in Saudi Arabia's northwest province to determine whether 110 adults cattle (3–5 years) tested positive for *Babesia bovis* infection. All of the selected animals had shared an environment, lived under the same roof, and eaten the same food.

Blood sample collection

After sterilising the region, blood samples were collected from the animals' jugular veins. 5 ml from each animal were collected and divided into two sections, (2.5) ml of blood was placed in tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant for the purpose of separating the blood plasma for parasitological examination. Confirmation of positive cases was done using microscopy after staining with Giemsa stain. For estimation of biochemical parameters (2.5) ml of blood was collected in sterile non-EDTA vial for the separation of serum from apparently healthy cattle and Babesia infected cattle.

Parasitological examination

Parasitological examinations of blood were performed for all cattle using Giemsa-stained blood smears and were assessed for *Babesia bovis* infection or non-infected. For each sample, a very thin layer was spread out on a microscope slide. Following a 10-to fifteen-minute period of air drying, the slides were fixed in methanol and subsequently stained using a 10% Giemsa solution in phosphate-buffered saline (pH 7.2) were used to

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detect the blood parasites (Weiss and Wardrop, 2011). Light microscopy (OLYMPUS; Japan; CX41) with a 100× and an oil immersion objective lens was used to examine all the slides.

Biochemical examination

5 ml of blood was used to measure the biochemical parameters after the separation of the serum from the blood samples that tested positive for *Babesia bovis* and control healthy samples. A centrifuge was used to spin the whole blood samples at 3000 rpm for 10 minutes at 4 °C. Plasma and the buffy coat were taken off. The erythrocyte pellets were centrifuged, and the supernatant was collected after three washes with a 0.9% isotonic saline solution. To prepare 10% of the erythrocyte lysate, the washed pellets of red blood cells were lysed in nine litres of cold, deionized water. The serum samples of the animals were used for biochemical parameters comprising creatinine, Blood Urea Nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) through using special cassettes for each test in a chemistry analyzer (IDEXX-Vet Test, Arachem, USA). The 40 µL of serum samples were used and the procedure performed according to the chemistry guidebook.

Oxidative stress marker malondialdehyde (MDA, catalog number: MBS9718963) and antioxidant marker Reduced glutathione (GSH, catalog number: MBS724319, superoxide dismutase (SOD, catalog number: MBS2548473), and catalase (CAT, catalog number: MBS8243260) were evaluated using Elisa kits according to the manufacturer's instructions (MyBioSource, Inc. San Diego, USA).

Histopathological examination

Light microscope analysis

Representative samples from liver, Kidney, and spleen from the *Babesia bovis* infected cattle and non-infected cattle's were taken and fixed in 10 % neutral-buffered formalin. The specimens were subjected thorough tissues processing technique. For handling paraffin sections, tissues were immediately fixed in 10% formalin. The mounted paraffin sections were inserted on 37°C oven on clean slides. The sections were deparaffinized in xylene then rehydrated in descending series of alcohol concentrations to distilled water were used for rehydration. Sections prepared for stain with the haematoxylin and eosin stain (H&E) stain for identification of the general architecture and histopathological changes (Bancroft and Gamble, 2008) and Masson's trichrome stain for detection of collagen fibres to determine the degree of fibrosis (Ohkawa et al., 1979). Light microscopic examination was performed at images obtained by using 10, 20, and 40X Objective lens magnifications

Immunohistochemical (IHC) study

The avidin-biotin-peroxidase (ABC) method was used to detect splenic CD3 (in spleen tissue) and Bcl-2 (in B-cell lymphoma 2) positive cells in another set of sections that were prepared for immunohistochemistry (IHC). These samples were representative of the liver, kidney, and spleen. Coating the slides with polylysine allowed the paraffin sections to be dewaxed and rehydrated. Two or four drops of peroxide block were used to inhibit the endogenous staining. It was by means of microwaves that the antigen was extracted. Afterwards, the CD3 and Bcl-2 (B-cell lymphoma 2) monoclonal antibodies were administered by VMRD Inc. of Pullman, WA, USA, a veterinary diagnostic test kit and

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reagent supplier. Next, we used an Olympus camera to analyses the sections under 400x magnification after counterstaining them with haematoxylin (Nikon Eclipse E200-LED, Tokyo, Japan). To create negative control sections, phosphate-buffered saline (0.01 M) was used in place of the primary antibodies during incubation.

Statistical examination

The results for both the infected and control groups were reported as the mean ± standard deviation (SD) after statistical analysis using IBM-SPSS (Statistical Package for Social Science) statistics version 26.0. We used independent t-tests to statistically examine the data. The significance level for all the differences was found to be less than 0.05. All statistical graphs were performed by using Prism 10 (GraphPad Software, La Jolla, Calif).

Results

Biochemical analysis

Serum ALT and AST levels are indicators of hepatic integrity (Athanasiou et al., 2023). In the current study there was a significant elevation ($P<0.0001$) in the levels of AST (124.0 ± 4.342 IU/L) and ALT (93.33 ± 3.634 IU/L) in the infected animals with Babesia in comparison to their levels of AST (80.43 ± 8.854 IU/L) and ALT (69.86 ± 9.367 IU/L) in the control healthy group (*Figure 1a &b*).

Highly significant increases in the mean values of blood urea nitrogen (44.40 ± 2.699 mg/dL), and serum creatinine (0.8079 ± 0.09342 mg/dL) were found in infected animals with Babesia when compared with the mean values, blood urea nitrogen (25.07 ± 2.645 mg/dL), and serum creatinine (0.3273 ± 0.08899 mg/dL) in control healthy group (*Figure 1c &d*).

Identified important components that play a role in controlling oxidative stress-related chemicals, including GSH (88.76± 5.861ng/mg), SOD (87.08± 6.927 U/g), and CAT (234.7 ± 14.26 U/g), respectively. Conspicuously, these substances were significantly ($p <0.0001$) downregulated by *Bovine babesiosis* infections when compared to the control healthy GSH (143.2± 6.663 ng/mg), SOD (139.9 ± 6.293 U/g), and CAT (528.5 ± 50.93 U/g). In contrast, the MDA content was significantly ($p <0.0001$) higher (7.879± 0.6365 nmol/g) in Babesia infections compared to the healthy control group (1.377± 0.5244 nmol/g) (*Figure 1e-h*).

Confirmation of Babesia bovis infection

Out of 110 cattle, 42 had Babesia organisms detected in their Giemsa-stained blood smears. Blood films stained with Giemsa revealed intra-erythrocytic pyriform or pear-shaped piroplasms of Bacillus bovis under a microscope. Serious haemolytic anaemia characterized by changes in erythrocyte size (Anisocytosis) and form (Poikilocytosis) was observed in blood smears from calves infected with *B. bovis* that underwent increasing cases of the disease (*Figure 2a & b*). As a control healthy group, these animals (n=44) were chosen for their good health. There were no parasites or erythrocytic alterations observed in the Giemsa-stained blood smears of uninfected cattle. Control healthy animals were clinically healthy, meaning they were not infested with ticks, had not received anti-parasitic or antimicrobial therapy in the 30 days prior to the start of the study, had not undergone surgery, were not pregnant, had not recently given birth, or had

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been vaccinated. In addition, they were checked very extensively to make sure they were free from any disease. The remains 24 cattle had different diseases and excluded from the current study.

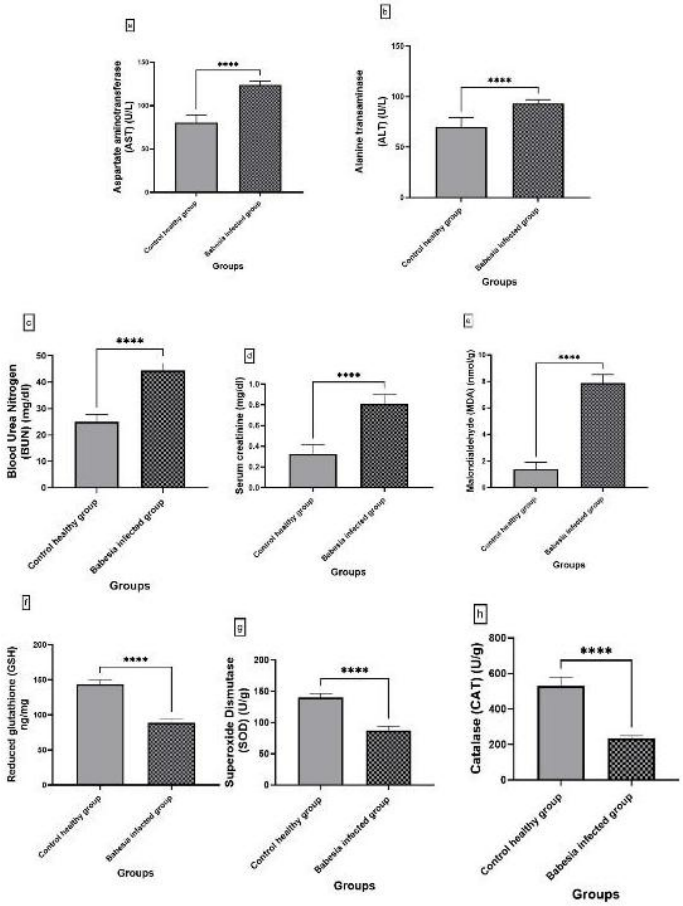

Figure 1. *Graphic representation of the biochemical parameters. Statistical analysis was carried out using T-test using SPSS computer program for all Babesia bovis infected (n=44) and control healthy groups (n=44). (a) Aspartate aminotransferase (AST), (b) Alanine aminotransferase (ALT) to determine liver damage. (c) Serum Blood Urea Nitrogen (BUN) and (d) Serum creatinine was assessed to determine kidney damage. (e) Oxidative stress markers malondialdehyde (MDA) and antioxidant markers (f) Reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Significant difference between groups at* ****$p < 0.0001$*

Histopathological examination

Spleen

Because it is more sensitive to low concentrations of chemicals than other organs, the spleen was chosen for this study to evaluate *Babesia bovis's* effects because some researchers have hypothesized that it is a crucial component of the immune system (Schneider et al., 2011). White pulp, marginal zone, and red pulp are formed by the differentiation of the parenchyma of the spleen in the healthy control group, as shown in H&E-stained sections. Two primary parts of the white pulp are the lymph nodules and the periarterial lymphatic sheath (PALS). The vascularized trabeculae's trabecular artery

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is the starting point for the PALS, which forms like a chain reaction when cells clump together. Blood sinusoids were interspersed among splenic cords that branched and anastomosed to form the red pulp (*Figure 2c & d*). In contrast, examination of Babesia bovis-infected group sections showed a disorder in the splenic architecture, which manifested as an enlargement and increased cellularity of the red pulp, a lack of zonal definition around the white pulp, and a noticeable amount of eosinophilic exudate. There was a lack of germinal center in the white pulp and cytoplasm that was highly vacuolated. The sinusoidal gaps were mostly big and filled with diseased red blood cells. When contrasted with the noninfected spleen, the infected spleen had a thinner capsule (*Figure 2e-i*).

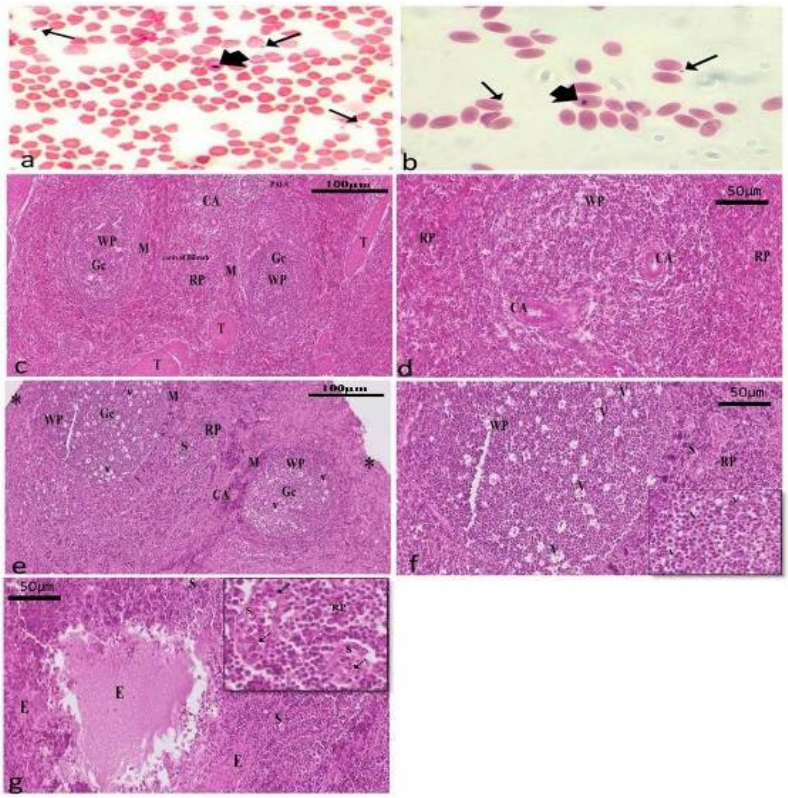

Figure 2. *(a&b) Blood smears of infected cattle in Giemsa-stained blood film of cattle showing severe haemolytic anomia with abnormalities in cell size (Anisocytosis) and cell shape (Poikilocytosis) of erythrocytes. Notice extracellular forms (↑) as well as intracellular parasites (arrowhead). Giemsa stain (100X oil immersion objective). (c -g) A photomicrograph of section of spleen of control group showing the white pulp (WP) contains well defined splenic lymphoid follicle with central germinal center (GC), central arteriole (CA), periarterial lymphatic sheath (PALS), marginal zone (M) and red pulp (RP). The red pulp contains splenic blood sinuses (S) and cords of Billroth in between. The sinuses are lined with endothelial cells (↑). A part of the fibrous trabecula (T) is also seen. (c, d, & e) of Babesia bovis infected group showing loss of architecture highly atrophied white pulp (WP) and thin capsule (*). The marginal zone (M) between white (WP) and red pulp (RP) are started to disappear with marked eosinophilic exudate (E). Degenerated lymphoid cells in the white pulp (WP) with vacuolated cytoplasm (V) were and no germinal center (Gc) is seen. Note, central artery (CA) is also seen. Most of the sinusoidal spaces (S) are large and contain infected erythrocytes (dot arrow). This disorganization was due to hyperplasia of the lymphoid tissue. The capsule of the infected spleen are thinner (*) when compared to the noninfected spleen capsule. (H & E, a & c x 10;100 µm, b, d &e x 20;50 µm, Inset x40;50 µm)*

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Masson' trichrome stain of spleen control healthy animals showed fine collagen fibers around the central artery and in-between lymphocytes of the white pulp and in the parenchyma of the splenic tissue in the red pulp. Notice trabeculae were seen thick according to Masson trichrome stain. *Babesia bovis* infected group revealed an apparent increase of the collagen fibres (Interstitial fibrosis) in the parenchyma of the splenic tissue mainly in the white pulp and red pulp surrounding dilated splenic sinusoids (*Figure 3a-f*).

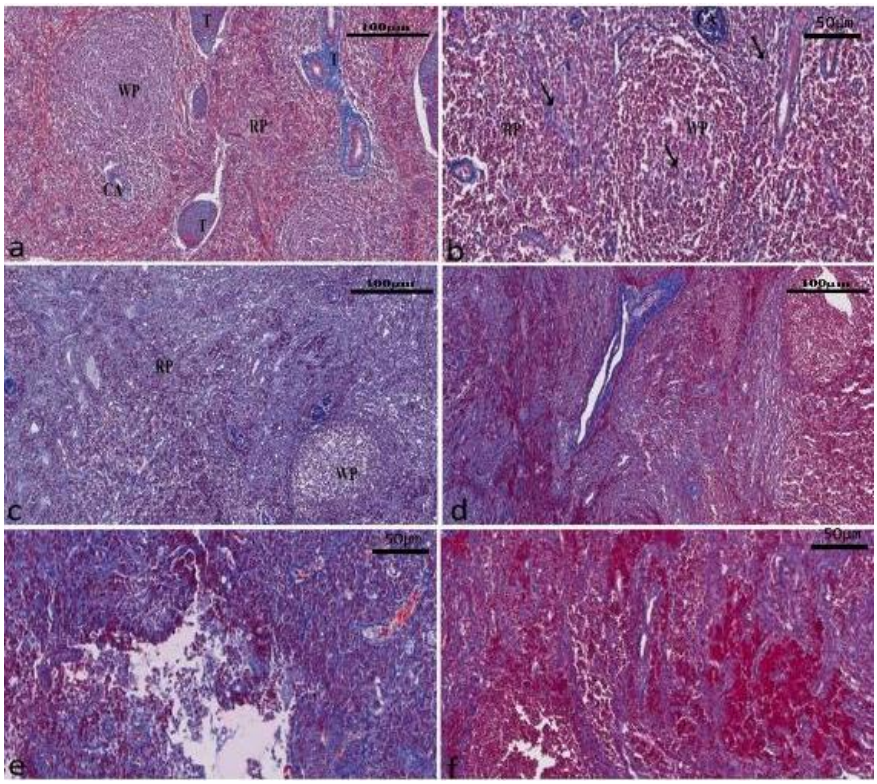

Figure 3. *A photomicrograph of Masson' trichrome stain of spleen (a& b) of control group showing fine collagen fibers (↑) around the central artery (CA) and in-between lymphocytes of the white pulp (WP) and in the parenchyma of the splenic tissue in the red pulp (RP). Notice trabecula (T) are seen. (c-f) of Babesia bovis infected group showing an apparent increase of the collagen fibers in the parenchyma of the splenic tissue mainly in the white pulp (WP) and red pulp (RP) surrounding dilated splenic sinusoids (S). (Masson' trichrome stain, a& c x 10;100 µm, b &d-f x 20;50 µm)*

The control group's healthy spleens stained strongly positive with Bcl-2 immunohistochemistry, indicating that the protein was expressed in the follicles rather than the germinal centers of the white pulp and in scattered positive cells throughout the red pulp. The Bcl-2 antibody was able to identify B cells through interactions with their membranes and cytoplasm (*Figure 4a-b*). *Babesia bovis* infected group showed an apparent decrease and a weak positive cytoplasmic brownish reaction in the marginal zone of the white pulp. Notice negative reaction in most of the red pulp were seen (*Figure 4c-d*).

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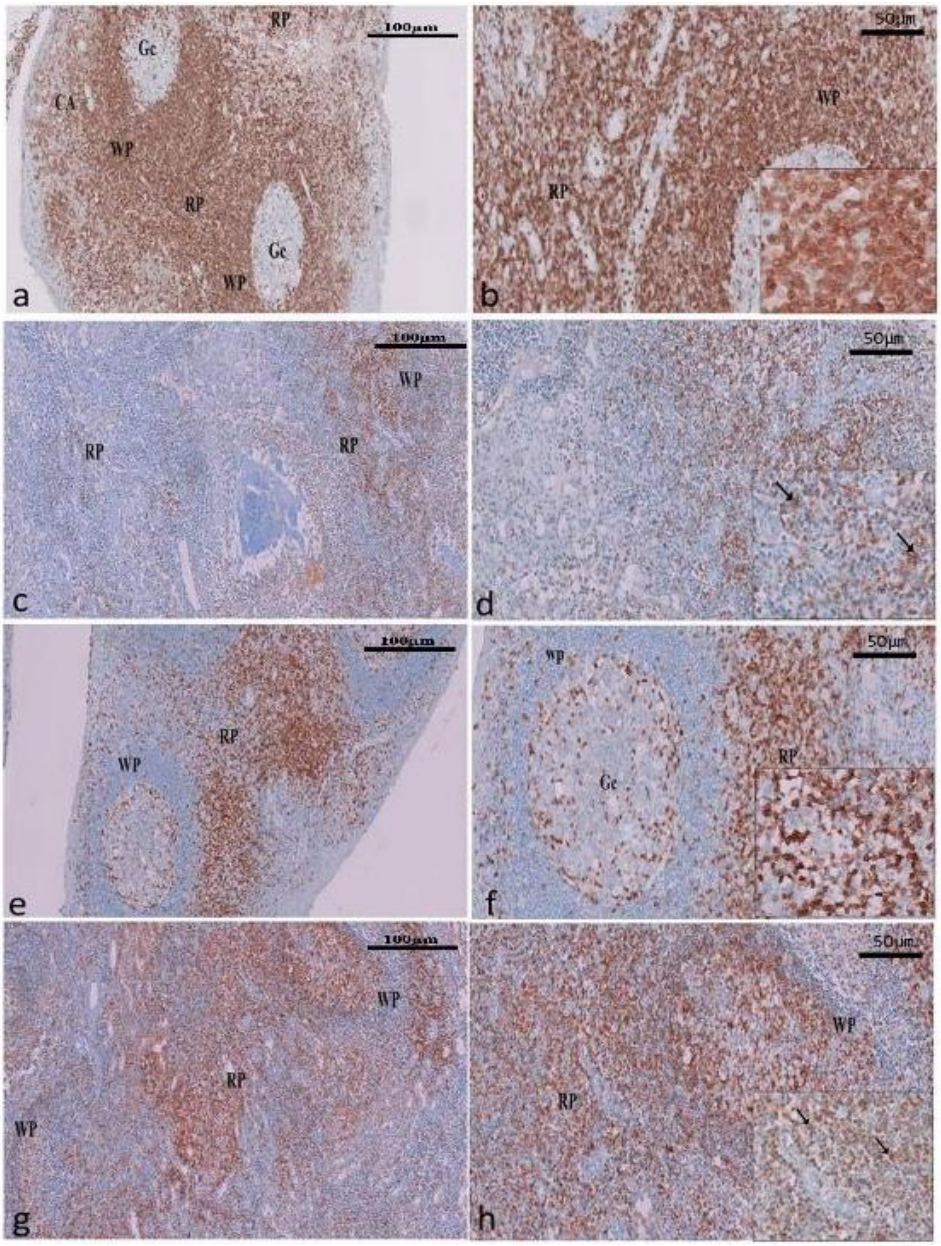

Figure 4. *A photomicrograph of BCL-2 Immunohistochemical reaction (IHC) of spleen sections (a& b) of control group showing strong positive cytoplasmic brownish reaction in red pulp (RP) and in the marginal zone (M) of the white pulp (WP). But negative reaction in the germinal center (Gc) of the white pulp. (c& d) of Babesia bovis infected group showing an apparent decrease and a weak positive cytoplasmic brownish reaction in the marginal zone of the white pulp. Notice negative reaction in most of the red pulp are seen (BCL-2 IHC, x10;100 µm, x20;50 µm, Inset x40;50 µm). CD3 Immunohistochemical reaction (IHC) of spleen sections (e& f) of control group showing the CD3 antibody recognized T cells by the cell membrane and cytoplasmic reactions. The immunohistochemical staining of CD3 cells in the spleen of control sections showed strong positive cells in the red pulp (RP). But negative reaction in the germinal center (Gc) of the white pulp (WP). (g& h) of Babesia bovis infected group showing an apparent increase of the positive cytoplasmic brownish reaction in the white pulp (WP). Notice an apparent increase of the positive reaction in most of the red pulp (RP) are seen. (BCL-2 IHC, x10;100 µm, x20;50 µm, Inset x40;50 µm)*

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The CD3 antibody identified T cells by cytoplasmic and cell membrane responses, as demonstrated by CD3 immunohistochemical staining of the healthy control group. Spleen control sections revealed a robust positive cytoplasmic brownish reaction in both the red pulp and the white pulp's marginal zone. The white pulp's germinal center, however, shows a negative reaction (*Figure 4e & f*). There was an increase scattered positive cytoplasmic brownish reaction in the red pulp and white pulp of the *Babesia bovis* infected group (*Figure 4g & h*).

Kidney

Renal cortices from the control group revealed classical architecture in H&E-stained sections, including the glomerulus, renal tubules, and interstitial tissue. Normal glomerular capillaries, a normal urinary space, dark-nucleated mesangial cells, and regular Bowman's capsule were all features of the glomeruli. Pyramidal epithelial cells lining the proximal tubules had uniformly pink cytoplasm, basal black nuclei, and a well-organized brush border that blocked the lumen. The distal tubules were bordered by cuboidal epithelial cells, which exhibited pink cytoplasm, black rounded nuclei, and thin lumina (*Figure 5a & b*). Kidneys of naturally infected cattle with *Babesia bovis* showed histological abnormalities. Wide interstitial spaces showed severe congestion. In addition, the renal tubules displayed degenerative alterations in the cytoplasm of the lining epithelial cells together with mononuclear leukocytic cellular infiltrations, resulting in the destruction of most of the kidneys. Furthermore, there was a buildup of oedematous fluid in the renal interstitial tissue and hyaline casts in the lumen of the renal tubules. In addition, the renal tubule lining endothelial cells showed signs of vacuolation. On rare occasions, the glomerular tuft will also shrink. Nonetheless, there was a noticeable abundance of eosinophilic elements within Bowman's capsule. On rare occasions, many pathological glomerular alterations were noted, including glomerular tuft shrinkage or even glomerular tuft destruction (*Figure 5c-d*).

Interstitial fibrosis, visible in the spaces between tubules and around glomeruli, was increased in the *Babesia bovis* infected group when compared to the healthy control group, according to Masson's trichrome staining of renal sections (*Figure 6a-e*).

Bcl-2 immunohistochemical staining of renal cortex sections revealed positive cytoplasmic immunoreactivity, which was weak in *Babesia bovis* infected group, moderate in the control healthy group (*Figure 6f & g*).

Liver

Sections stained with H&E showed that the histological architecture of the healthy control group was normal. Hepatocytes were shown to extend away from a central vein towards the corners of the portal area. Hepatocytes were the building blocks of the liver's parenchyma. Hepatic blood sinusoids, bordered with flat endothelial cells and Kupffer cells, separated these cords. Round vesicular nuclei and eosinophilic cytoplasm were features of the hepatocytes. Some of the hepatocytes looked to have two nuclei.

At the corners were the portal tracts, which comprised the bile ducts, portal vein, and hepatic artery (*Figure 7a-c*). Most of the central veins seemed enlarged and clogged in the *Babesia bovis* infected group, indicating significant disruption in the structure of the liver tissue. Hepatocyte enlargement and prominent fibrosis around the major vein were hallmarks of steatohepatitis. Most of the hepatocytes were enlarged and displayed numerous intracytoplasmic macro and micro vacuoles. The pericentral and periportal areas were also marked by shrunken, irregular, and darkly stained pyknotic nuclei. There

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seemed to be an increase of Kupffer cells and a small dilation of the blood sinusoids. There was thick fibrous tissue, multinucleated cells, a dilated and obstructed portal vein, and an enlarged portal region (Macrophage) (*Figure 7d-h*).

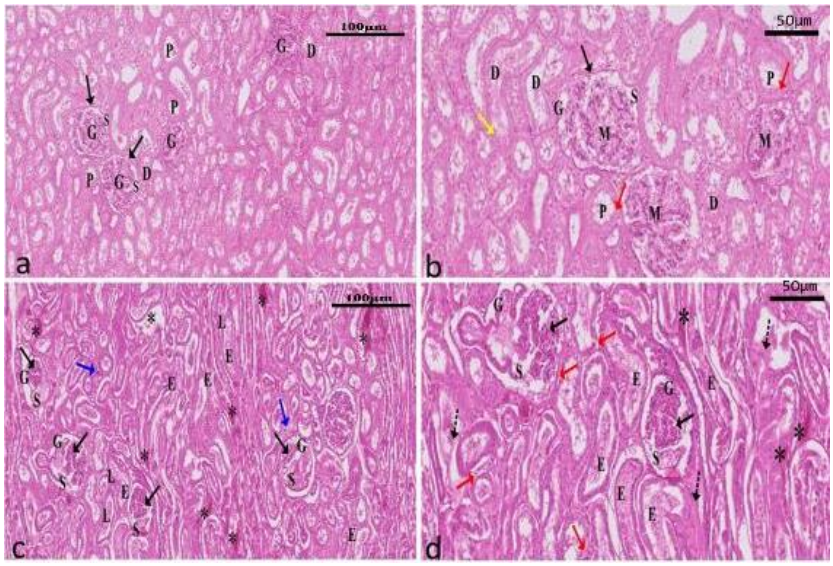

Figure 5. *A photomicrograph of section of Kidney (a& b) of control group showing renal cortex of control group, showing an organized renal cortex, normal organized renal tubules have closed lumen with well-organized brush border in proximal tubules (P). Other tubules are mildly dilated (D), tubular cells have dark rounded nuclei and homogenous dark pink cytoplasm, glomerulus (G) has mesangial cells (M) with dark nuclei and well-preserved urinary space (S). (c& d) of Babesia bovis infected showing destructive atrophied structure of the renal cortex is noticed with wide interstitial tissue spaces with severe extravasation of RBCs (*), eosinophilic exudate (dot arrow), and perivascular mononuclear leukocytic cellular infiltrations (blue↑). Large number of hyperaemic glomeruli (G) with urinary space (S) filled with acidophilic materials and shrinkage of the glomerular tuft (↑). Vacuolation (red ↑) of the epithelial cells lining the renal tubules. The lumen (L) of the renal tubules is dilated and contains eosinophilic material (E). (H & E, a& c x10;100 µm, b& d x20;50 µm)*

Masson's trichrome stain is among the most common special stains applied to liver specimens to determine the degree of fibrosis and collagen deposition. The stain imparts a blue colour to collagen against a red background of hepatocytes and other structures. Control healthy group liver examination revealed normal hepatic tissue with minimal collagen deposition surrounding central veins, portal tracts and in the thin liver septa (*Figure 8a*). Also, examination of *Babesia bovis* infected group livers showed an apparent progressive increase in collagen fiber deposition in the liver tissue surrounding central vein and porta area. Moreover, collagen deposition increased in the connective tissue septa. These septa formed bridges connecting adjacent portal areas which is a main feature of liver cirrhosis (*Figure 8b & c*).

Bcl-2 immunohistochemical staining of liver sections revealed moderate positive cytoplasmic brownish reaction in the hepatocytes and in the cells lining blood sinusoids. *Babesia bovis* infected group showing a weak positive cytoplasmic brownish reaction in the cells lining blood sinusoids. Notice negative reaction in most of the hepatocytes are seen except for few positive immuno reaction appeared in few hepatocytes (*Figure 8e-g*).

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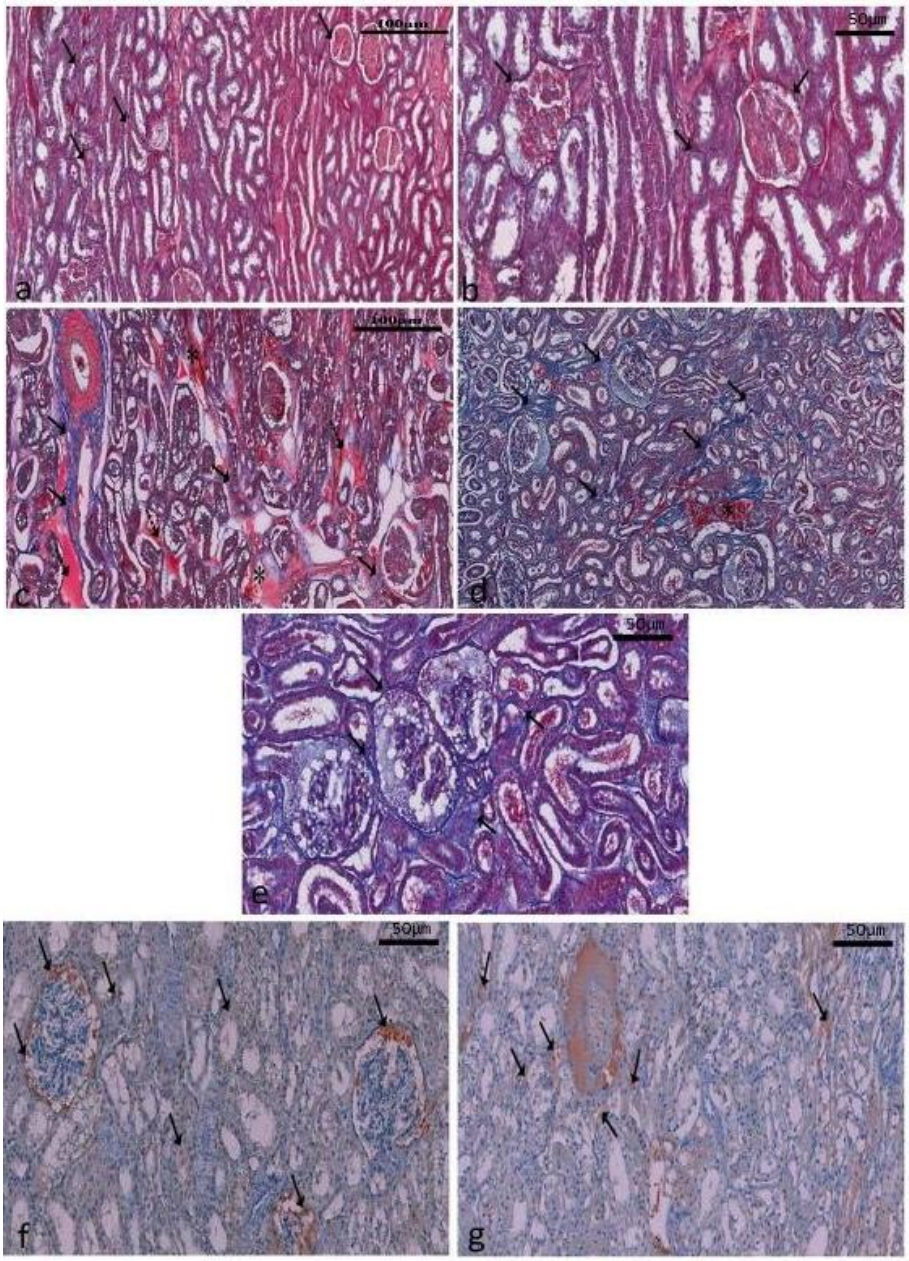

Figure 6. *A photomicrograph of Masson' trichrome stain of kidney (a& b) of control group showing fine collagen fibers (↑) in the stroma in-between the glomeruli and renal tubules. (c, d &e) of Babesia bovis infected group showing an apparent increase of the collagen fibers (↑) in the stroma in-between the glomeruli and renal tubules. Notice wide interstitial tissue spaces with severe extravasation of RBCs (*) and eosinophilic exudate (dot arrow) are seen. (Masson' trichrome stain, a, c, d x10;100 µm, b, e x20;50 µm). BCL-2 Immunohistochemical reaction (IHC) of kidney sections (f) of control group showing moderate positive cytoplasmic brownish (↑) reaction in the renal glomeruli (G) and renal tubules (RT). (g) of Babesia bovis infected group showing a weak positive cytoplasmic brownish reaction (↑) reaction in the renal glomeruli (G) and renal tubules (RT). (BCL-2 IHC, x20;50 µm)*

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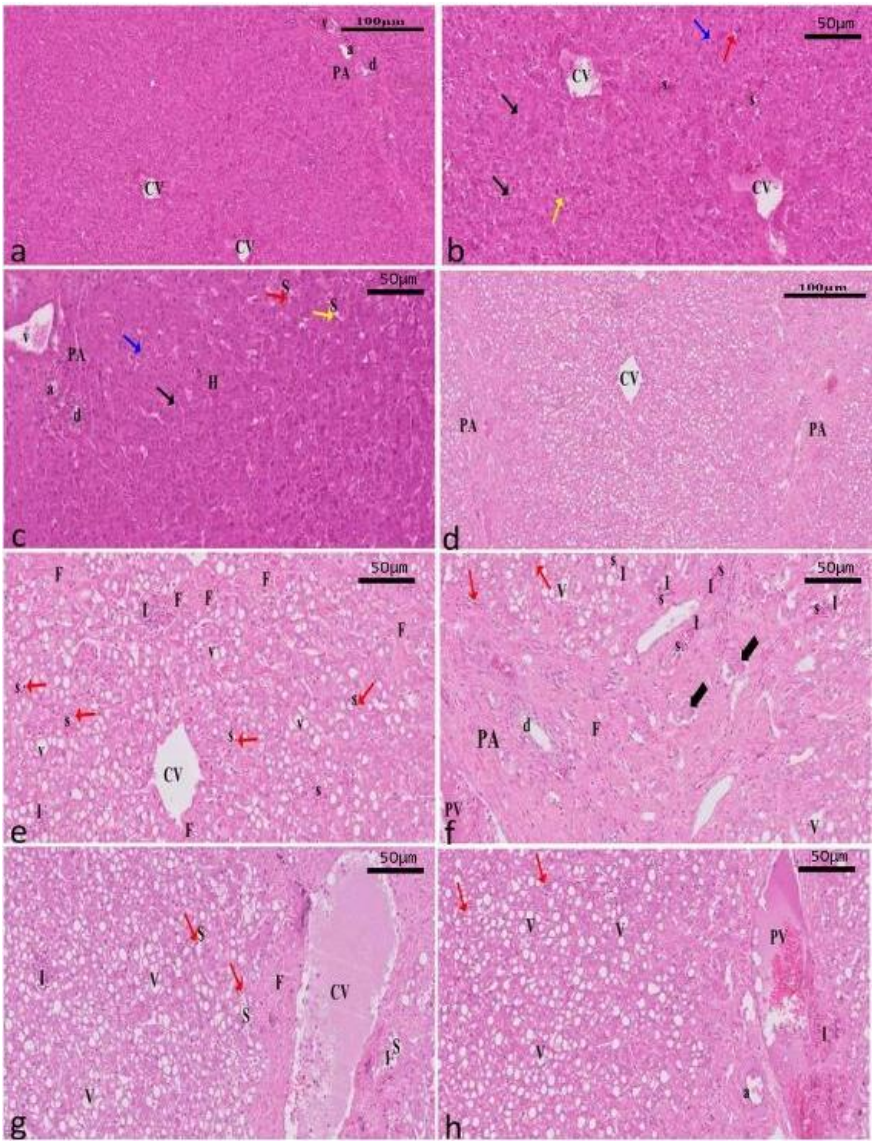

Figure 7. *A photomicrograph of section of liver (a-c) of control group showing preserved hepatic architecture with the hepatocytes radiate outwards from a central vein (CV) and at the corners of the lobules, portal area (PA) is seen. (H & E,). Firmly arranged branching and anastomosing cords of polygonal hepatocytes having rounded vesicular nuclei and acidophilic cytoplasm (black ↑) and binucleated hepatocytes (blue↑) radiating from the central vein (CV) and separated by hepatic blood sinusoids (S) and are lined by flat endothelial cells (yellow↑) and Kupffer cells (red↑). At the corners of the lobules, portal area (PA) contained branches of the hepatic artery (a), portal vein (v), and bile ducts (d) are seen. (H & E, a x10;100 µm, b &c x20; 50 µm). (d-h) Babesia bovis infected group showing marked disruption in the hepatic tissue structure as most of central veins (CV) and portal vein (v) in porta area (PA) appeared dilated and congested. Very thick fibrous (F) surrounding central vein (CV), at the portal area (PA), and in between hepatocytes are seen. Ballooning of the hepatocytes with micro and macro vacuoles (V). Dilated and congested (S) with an apparent increase of Kupffer cells (red↑). Meanwhile, the bile duct is seen with degenerated cells. Notice marked inflammatory cells infiltrations (I) and multinucleated cells (thick arrow) are seen in thick periportal and pericentral fibrous tissue (F) are seen. (H & E, d x10;100 µm, e-h x20; 50 µm)*

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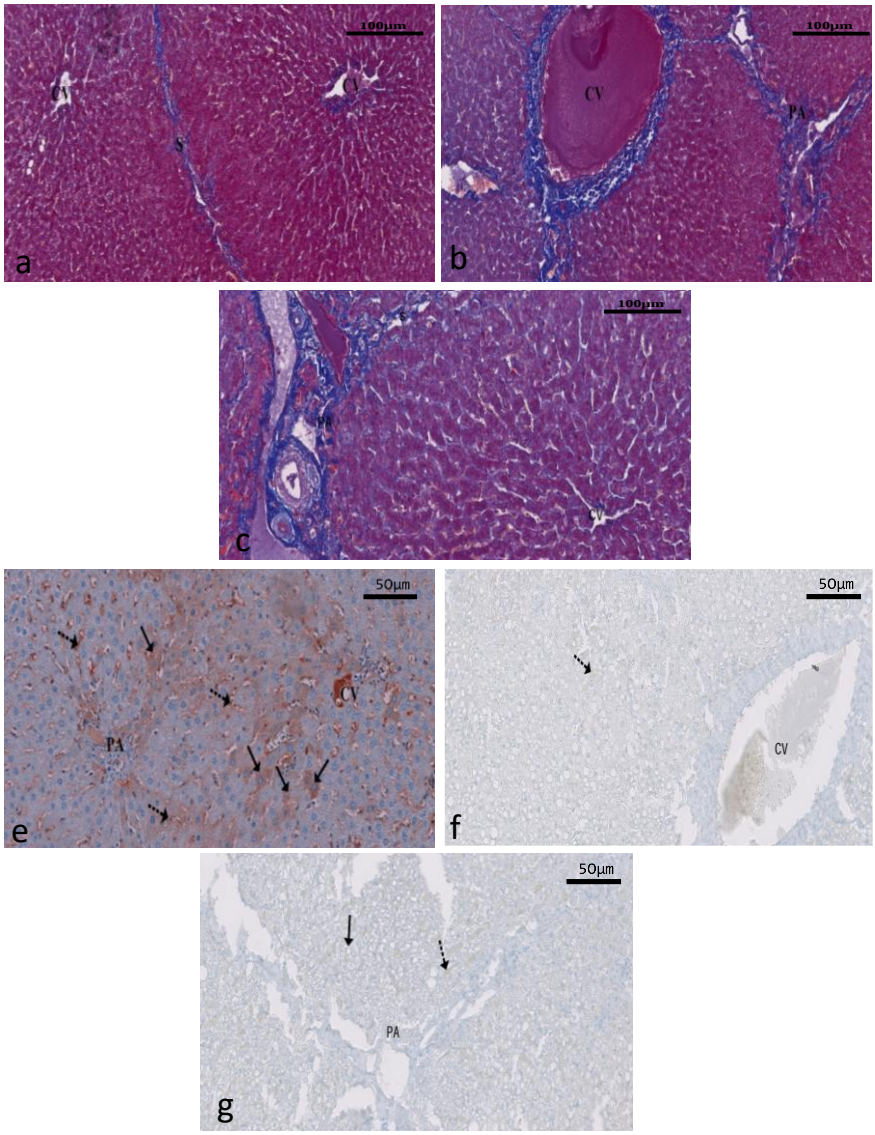

Figure 8. *A photomicrograph of Masson' trichrome stain of liver (a) of control group showing fine collagen fibers around the central vein (CV), portal area (PA), and in-between hepatocytes. Notice fine collagen fibers are seen in the septa (S). (b& c) of Babesia bovis infected group showing an apparent increase of the collagen fibers around the central vein (CV) and portal area (PA). Notice an apparent increase of collagen fibers in the septa (S) are seen. (Masson' trichrome stain, a-c x10;100 µm). BCL-2 Immunohistochemical reaction (IHC) of liver sections (e) of control group showing moderate positive cytoplasmic brownish reaction in the hepatocytes (↑) and in the cells lining blood sinusoids (dot arrow). (f& g) of Babesia bovis infected group showing an apparent increase of a weak positive cytoplasmic brownish reaction in the cells lining blood sinusoids (dot arrow). Notice negative reaction in most of the hepatocytes (↑) and mild positive brownish reactions (dot arrow) are seen. (BCL-2 IHC, a-c x20;50 µm)*

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Discussion

The dairy business is greatly impacted by bovine babesiosis, a tick-borne illness (TBD). Among bovines, the most common babesia species are *B. bigemina, B. bovis, and B. divergens*. The more pathogenic *B. bovis* is, the more severe the illness and death it causes. Both immature and fully grown animals might expect a death rate of 20–50 percent. Tick infestations can be more severe in exotic dairy cattle than in native breeds. Ixodid ticks are vectors for the hemoparasites (Wodaje et al., 2019; Masih et al., 2022).

In order to reduce economic losses caused by the parasite, it is urgently needed to develop sensitive methods for effective identification and medications for treatment of Babesia sp. Babesia sp. detection in host animals is accomplished using a variety of traditional and contemporary techniques. The gold standard is a microscopic analysis of Giemsa-stained blood smears, although this method isn't used to find carrier animals because of its limited sensitivity, which means parasitaemia may be minimal. However, it's generally enough for acute infection identification (Alvarez et al., 2019). The results of the parasitological analysis of *B. bovis* infection in cattle corroborate those of Mahmmod (2014) and Hakimi et al. (2021). These results corroborate those of earlier studies by Canever et al. (2014) and Nasreldin et al. (2020) which found that the two most common and costly tick-borne infections in cattle are caused by the intraerythrocytic pathogens, *B. bovis* and *A. marginale*. Among the *Babesia species*, one can find *Babesia bovis*. The parasite *Babesia* develops all of its stages in the red blood cells (RBCs) of the vertebrate host, and the sporozoites enter the RBCs directly (Asada et al., 2012).

Cattle infected with babesiosis had considerably higher mean serum ALT and AST values than healthy cattle. Findings from this study are consistent with those from earlier investigations (Hussein et al., 2007; Hashem et al., 2018; Aziz et al., 2020; Athanasiou et al., 2023). Babesiosis, according to their findings, causes an increase in liver enzymes because the parasite causes hepatic damage and lesions as it multiplies in the blood, which disrupts liver function. Bovine babesiosis can change liver function, which could explain the elevation in serum ALT and AST values, which are markers of hepatic function (Hashem et al., 2018; Esmaeilnejad et al., 2021; Saini et al. 2022). The liver and muscles both have very high amounts of these enzymes (Masih et al., 2022). The parasite's hepatic damage during blood multiplication and subsequent disruption of liver function might explain an increase in these enzymes in the blood (Abdel-Hamied et al., 2020; Khinchi et al., 2016). Hepatic cell degeneration, which can result in elevated AST, ALT, and LDH levels, can happen in the presence of hypoxia and severe haemolysis (Masih et al., 2022). Consequently, injury or necrosis to an organ is indicated by a high blood level of these enzymes.

Cattle afflicted with babesiosis had much higher mean values of BUN and creatinine than healthy cattle in the present investigation. Findings from this study are consistent with those from earlier investigations (Sharma et al., 2020). Babesiosis infected cows had significantly elevated BUN levels. This could be because the reticulo-endothelial system destroys red blood cells rapidly through phagocytosis. Massive haemolysis and hypoxia cause hepatic cell degeneration and glomerular dysfunction, which in turn raises the BUN levels (Aziz et al., 2020). A spike in BUN is anticipated due to the degradation and necrosis that Babesia can produce in the convoluted tubules of the kidneys (Mosqueda et al., 2012). According to Esmaeilnejad et al., an increase in BUN and creatinine levels could be due to kidney failure, muscle breakdown, or the colonization of the renal blood circulation by *B. ovis*. It has been proposed that there are numerous variables in ovine babesiosis that can compromise renal function (Esmaeilnejad et al., 2012). The toxic

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metabolites of Babesia sp. damage and modify the enzymes in the liver and kidneys, as seen by the apparent increase in creatinine, AST, ALT, and BUN levels (Sharma et al., 2016).

When there is an imbalance between systems that produce radicals and those that scavenge them, oxidative stress results. There may be a correlation between the illness pathogen and the erythrocyte peroxidation that happens in hemo-protozoan infection. When the levels of ROS in the blood rise above what the body's antioxidant defenses can handle, an oxidative process begins, which can damage cells and tissues. New research on blood parasites has discovered significant evidence that oxidative damage from red cell lipid peroxidation is a cause of the resulting anaemia. Critical in preventing ROS damage are antioxidant components like SOD and GPx (Zaidi et al., 2005; Nazifi et al., 2011; Omar et al., 2015).

The antioxidant enzyme activities of GSH, SOD, and CAT were significantly reduced, and MDA was significantly elevated in the Babesia-infected cattle compared to the healthy control group, indicating oxidative stress in the present study. Findings from this study are consistent with those from earlier investigations (Salem et al., 2016). It seems that Babesia has a significant impact on the oxidant/antioxidant system, leading to the generation of ROS in large quantities, including MDA. As a result, the antioxidant system becomes overwhelmed and red blood cells are directly damaged (Kucukkurt et al., 2014).

In oxidation reactions that lead to the creation of GSSG, GSH acts as a reductant and is an antioxidant that does not involve enzymes. Depletion of the antioxidant reserve may be indicated by low GSH levels because GSH protects cells from ROS and free radicals that form during oxidative stress. Protein biosynthesis, immunological function, buildup of lipid peroxidation products, and detoxifying capacity are some of the linked functions that may be affected due to GSH depletion (Liu et al., 2022; Averill-Bates, 2023). Babesia infection was found to lower GSH concentrations in this study. This finding corroborate previous reports showing a decrease in GSH levels in parasite-infected animals. The cells that produce anti-oxidative agents are damaged by parasites, according to Kucukkurt et al. (2014), and a host's antioxidant reserve is significantly reduced when infected with Babesia.

When protecting the body from haemoparasitic disorders like babesiosis, the spleen-the biggest secondary lymphoid organ-is essential. Babesia infections cause immunomodulatory responses to be initiated despite the spleen's severe injury (Xue et al., 2021; Zafar et al., 2022). Macrophages, B and T cells, and immunoglobulins and substances that mobilize immunological functions are all housed in the spleen (Bronte and Pittet, 2013; Golub et al., 2018). Infected animals suffer haemolysis when babesia parasites, similar to those of malaria and Theileria, penetrate their red blood cells. In the early stages of an infection, the spleen's mononuclear phagocytes are likely to come into contact with contaminated red blood cells or merozoites, which will set off an innate immune response and encourage a targeted adaptive response. The spleen's phagocytic activity likely explains its function in infection control (Ganzinelli et al., 2018; Abdalla, 2021). Anatomical analysis of B. bovis-infected naturally occurring cattle revealed a disarray in the splenic architecture, as seen by the red pulp's enlargement and increased cellularity, the white pulp's lack of zonal definition (marginal zone), and a notable amount of eosinophilic exudate. There was a lack of germinal center in the white pulp and cytoplasm that was highly vacuolated. The sinusoidal gaps were mostly big and filled with diseased red blood cells. When contrasted with the noninfected spleen, the infected spleen had a thinner capsule. These findings are consistent with Henning et al. (2020) and

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Dkhil et al. (2014). Splenomegaly, defined as an enlarged spleen, was linked to the red and white pulp expanding in infected gerbils. An increase in hematopoietic support and a subsequent rise in macrophage numbers (macrophages increase as a result of erythrophagocytosis) cause this reaction (Dkhil et al., 2014). In the current study, there was an apparent increase of CD3 positive cells in the splenic tissue of the *Babesia bovis* infected animals as compared to control healthy animals. Similarly, Schneider et al. (2011) who reported that changes in T-lymphocyte numbers after *Babesia bovis* infection may represent immunotoxicity caused by Babesia. The white pulp and red pulp showed an apparent rise in the immunopositively reactivity of CD3 T-lymphocytes. Therefore, the possible explanations for the present findings include oxidative stress brought on by Babesia infection.

The liver is an important organ in babesiosis since it is where the host immune system develops to combat the pre-erythrocytic stages of Babesia parasites and where the parasites themselves asexually grow (Dkhil et al., 2010). Because of its central role in the elimination of metabolic byproducts and xenobiotics, the kidney is also a prime target of preclinical investigations (Marcos et al., 2022). The excretory system may become dysfunctional during babesiosis. Babesiosis is known to cause proximal tubule alterations, mesangial hyperplasia, and glomerulonephritis as the disease progresses, as reported in the literature (Luciano et al., 2013; Jasik et al., 2023).

The biochemical study revealed hepatic and renal damage in the form of raised serum levels of liver and renal enzymes, this picture described by numerous authors with multiple hepatorenal toxic agents that cause liver and renal fibrosis and degeneration (Lee et al., 2012; Hamoda et al., 2014; Prasanna et al., 2020). In the current study, the histological examination of the liver and kidney of the infected animals were confirmed the biochemical changes.

In the current study, histological examination of liver of *Babesia* infected group by H&E revealed lost normal hepatic architecture that has been replaced by multiple intracytoplasmic macro and micro vacuoles with shrunk, irregular, and darkly stained pyknotic nuclei. These findings are consistent with those of Hamoda et al. (2014) and Dkhil et al. (2010). Hamoda et al. (2014) discovered that rats died five days after infection. Histopathological examination revealed the presence of Babesia in its piroplasma form in the pathological liver and kidney tissues of the rats. According to Mosqueda et al. (2012), gerbils infected with Babesia rodhaini exhibit cytoplasmic vacuolation in their liver tissue as a result of their immune response This is mostly caused by significant abnormalities in lipid inclusions and fat metabolism that occur in clinical situations (Otsuka et al., 2002). Additionally, the majority of the kidneys studied by Hamoda et al. were found to be in a damaged state. This was due to a combination of factors such as perivascular mononuclear leukocytic cellular infiltrations (primarily macrophages and lymphocytes), acute diffuse proliferative glomerulitis, acute glomerular hemorrhage, thrombi, congestion and stasis in glomerular capillaries, acute glomerular hemorrhage, acute tubular necrosis, and rapid glomerular hemorrhage (Hamoda et al., 2014). The histological examinations conducted by Kuleš et al. (2018) revealed primarily degenerative alterations in the proximal tubules of the canine kidneys infected with babesiosis caused by *B. canis*. Cell vacuolization, tubular epithelial cell detachment from basal membrane, and, in certain regions, cell necrosis and even tubule disintegration were all components of the devastation. There was evidence of hemoglobin deposits and necrotic debris in the tubule lumen. Hypoxia was identified by the authors as the root cause of renal tubular necrosis, which in turn caused anaemia in babesiosis patients as a

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result of erythrocyte destruction. Hypoxia owing to vasoconstriction was also detected (Kuleš et al., 2018).

Through the use of Masson trichrome staining, the distribution of collagen in the liver, kidneys, and spleen was identified. Collagen buildup between liver lobules was clearly visible in the livers of rats treated with Masson trichrome, a hallmark of hepatic cirrhosis. This explained by Dkhil et al. (2010), who found hyperplasia of Kupffer cells in infected gerbils' liver sections, the reason for the increased deposition of collagen fibers and fibrosis is this. Also, according to Winiarczyk et al. (2019), babesiosis is a parasite infection that can spread from dog to dog. It causes an increase in TGF-β levels, which in turn causes collagen to be deposited in dogs that have renal damage as a natural consequence of the infection caused by *Babesia canis*.

Following inflammatory cell activation and proliferation, programmed cell death (apoptosis) plays a critical role in down-regulating immune responses. The proto-oncogene Bcl-2 controls cell death in a variety of organisms. Inactivating Bcl-2 in mice causes the lymphoid system to vanish, suggesting that Bcl-2 is involved in immune system maintenance (Othman et al., 2013). The present study found that compared to healthy control animals, those infected with *Babesia bovis* had a lower number of BCL2 positive cells in their liver, kidney, and splenic tissue samples. Moreira et al. provided an explanation for our findings by stating that parasites secrete antigens that control host cell death. There was also a decrease in the parasite model of Bcl-2 mRNA, an antiapoptotic protein (Moreira et al., 2016).

Conclusion

Babesia infection impacts biochemical markers, as seen by elevated levels of AST, ALT, BUN, and creatinine in this study. Babesiosis appears to impact the kidney and liver function of the animals that contract it, according to these results. Histopathological examination of the affected cattle's liver and kidneys provided documentation of these biochemical characteristics, which were then compared to those of healthy, uninfected cattle. The current study's significant data might aid in the disease's diagnosis and therapy (symptomatic). Babesiosis may cause hepato-renal damage in animals, according to this study's findings, by lowering antioxidant levels (GSH, SOD, and CAT) and raising oxidative indicators (MDA). So, it's safe to say that infected cattle are more likely to experience oxidative damage due to the Babesia infection. The development of bovine babesiosis seems to entail oxidative stress. For this reason, in addition to Babesicidal medications, antioxidants should be taken as a supplementary treatment for a speedier and more complete recovery. Our study found that the spleen underwent histological and immunohistological structural changes following Babesia infection, which likely leads to immune system modifications when compared to the spleens of healthy control animals.

Conflict of interest. There are no conflicts of interest.

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