ELUCIDATING HISTOLOGICAL, BIOCHEMICAL, AND ANTIOXIDANT PROFILES IN CAMELS NATURALLY INFECTED WITH *THEILERIA CAMELENSIS*

ALBOHIRI, H. H.

Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia (e-mail: hhalbahiri@uj.edu.sa; phone: +966-50-762-0527)

(Received 3rd Feb 2025; accepted 24th Mar 2025)

Abstract. The present study set out to investigate the extent of tissue damage in camels naturally infected with *Theileria camelensis*. A total of 114 camels were screened for *T. camelensis* infection. Hematological and biochemical investigations were conducted to assess liver and kidney function, along with oxidant/antioxidant markers. The kidney and spleen were analyzed histologically and immunohistochemically, and the lactate dehydrogenase (LDH) level in spleen tissue was measured as a marker of spleen injury. Blood smear analysis revealed that 28 camels tested positive for *T. camelensis* infection. Infected camels exhibited significantly elevated creatinine levels, blood urea nitrogen, and liver enzymes. Additionally, a significant reduction in glutathione level was noted, accompanied by a marked increase in malondialdehyde, reflecting oxidative stress. Tissue degeneration was evident in both kidney and spleen, with increased collagen fiber deposition and a reduction in B-cell lymphoma 2 (Bcl-2) positive cells. The immune response against *T. camelensis* was indicated by elevated LDH levels and CD3 cell expression in the spleen. Overall, *T. camelensis* infection negatively impacted multiple organs, particularly the kidneys and spleen in dromedary camels.
Keywords: *camels, Theileria camelensis, histopathology, biochemical parameters, immunohistochemistry, oxidative stress*

Introduction

Hemoprotozoan diseases affect 80% of the world's ruminants, causing massive losses to the worldwide dairy industry. Theileriosis, an ailment transmitted by ticks and caused by the genus *Theileria*, is characterized by the presence of a unique set of organelles called the apical complex (Riaz et al., 2023). Theileriosis is a parasite disease caused by *Theileria* spp. (*Piroplasmida*, *Theileridae*). It spreads through ixodid ticks and affects ungulates in captivity, the wild, and on farms. Negative effects on animal welfare and economic health are caused by ticks carrying protozoa in tropical and subtropical locations (Ali et al., 2024).

Two species of *Theileria* have been found in camels, one of which is *T. dromedarii*, while the other is *T. camelensis*. The former has been documented in Somalia, Egypt, Turkmenistan, and Saudi Arabia (Alanazi et al., 2020; Ismael et al., 2014; Nourollahi Fard et al., 2012). The dromedary camel is culturally and economically significant, especially in Arabian countries. In addition to thriving in their extreme habitat, these pseudo-ruminants serve multiple purposes, including but not limited to milk, meat, wool, felt, racing, transportation, and even tourism (Faye, 2020).

Camels infected with *Theileria* show indicators ranging from gastrointestinal tract to systemic health, such as fever, severe emaciation, and intermittent bouts of diarrhea. Other symptoms include enlargement of superficial lymph nodes, particularly superficial cervical lymph nodes (Abouzaid et al., 2022). Despite the presence of *Theileria* infection, camels often show no visible signs of illness and can appear healthy (Perveen et al., 2021; Salman et al., 2022; Youssef et al., 2015).

To shed light on animal health, performance issues, and fitness, hematology has seen the extensive application (Abouzaid et al., 2022; Youssef et al., 2015). Oxidative stress

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

triggered by free radicals plays an important role in the pathogenesis of theileriosis in camels. Anemia in theileriosis may have multiple causes, one of which may be oxidative stress and lipid peroxidation (El-Deeb and Younis, 2009). Hence, detecting oxidative stress and antioxidant markers could provide light on the infection's impact on host tissues and the health of infected camels.

Spleen is one of the most important lymphoid organs, which plays a role in immunological response, phagocytosis of old erythrocytes, and hemopoiesis (Maina et al., 2014). *Theileria* infection not only impacted the liver but also impaired kidney function (Abouzaid, 2022). So far, the histological alterations of the spleen and kidney of naturally infected camels with *T. camelensis* have not been adequately established in the literature. Therefore, this study is necessary to fill this current gap and to give baseline data for future research in this field.

Therefore, the objective of the present study was to demonstrate the histopathological changes in spleen and kidney of camels naturally infected with *T. camelensis*. In addition, it sheds light on the immunopathological responses in the development of tissue damage. Moreover, the study was conducted to evaluate the oxidative status as an indicator of oxidative damage in the erythrocytes of camels naturally infected with *T. camelensis*.

Materials and methods

Animals and sample collection

A total number of one hundred fourteen (114) camels (*C. dromedarius*) aged three to five years from the central slaughterhouse in Jeddah City of the western province of Saudi Arabia. Camels were investigated for the presence of *T. camelensis* infection. All animals were tested by veterinarians under the ethical handling and veterinary inspection guide from the Ministry of Municipalities and Rural Affairs in Saudi Arabia (MMRA, 2008). Spleen and kidney tissues and blood samples were transported to the laboratory for examination.

Confirmation of Theileria camelensis infection

Two blood samples were obtained from the jugular vein; the first blood sample was collected into clean and dry sterile tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) as an anticoagulant for parasitological examination and hematological analyses assays. The parasitological examinations of blood were carried out for all camels using the confirmed method for diagnosing the infection (Ismaeil et al., 2023), giemsa-stained thin blood smears were performed and assessed for the presence of *T. camelensis* and other Hemoprotozoa (Soulsby, 1982). Light microscopy (BX51, OLYMPUS, Tokyo, Japan) with a 100× and an oil immersion objective lens was used to examine all of the slides as previously described (Hamed et al., 2011). Results from blood smears that did not reveal any infection were used as the control samples.

Hematological and biochemical examination

Hematological analyses were completed immediately using EDTA tubes of camel blood samples to determine the count of red blood cells (RBC, 10^6/mL), hemoglobin (Hb, mg/dL), hematocrit (HCT, %), and WBCs (10^3/mL) by using a hematology analyzer (Abacus Junior Vet5, Budapest, Hungary).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

The second blood samples were collected in plain vacutainer tubes and used for obtaining serum. Whole blood samples were centrifuged at 3000 rpm for 10 min at 4°C to obtain clear sera for measuring serum parameters by analytical kits from MyBioSource, Inc. San Diego, CA, USA, alanine aminotransferase (ALT) (Cat No. MBS169579), aspartate aminotransferase (AST) (Cat No. MBS2540582), creatinine (Cat No. MBS8420180) and serum blood urea nitrogen (BUN) (Cat No. MBS2563700). The oxidative stress marker, Malondialdehyde (MDA) (Cat No. MBS9718963) and the antioxidant reduced glutathione (GSH) (Cat No. MBS724319) were evaluated using Elisa kits from MyBioSource, Inc. San Diego, CA, USA.

A portion of the spleen was weighed and homogenized in the assay buffer on ice for preparation of 10% W/V homogenate which was then centrifuged at 4°C for 15 min at 10000 g. A MyBioSource kit (Cat No. MBS9718969) was used to determine lactate dehydrogenase (LDH) in the spleen tissue supernatant. The protein content was measured in each spleen supernatant sample according to the protein quantification kit. The final enzyme activity was measured in units per gram of protein. All procedures were performed according to the manufacturer's instructions. T80 UV/VIS PG instrument Ltd. of the United Kingdom spectrophotometer was used for the determination of all parameters.

Histopathological examination

Representative samples from the spleen and kidney tissues of the *T. camelensis* infected camels and healthy camels were taken and fixed in 10% neutral-buffered formalin. The specimens were subjected to thorough tissue processing techniques. Sections at approximately 4 μm in thickness were prepared for staining with the hematoxylin 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 the detection of collagen fibers to determine the degree of fibrosis as previously described (Van De Vlekkert et al., 2020) by using light microscopic (OLYMPUS, Tokyo, Japan).

Immunohistochemical (IHC) study

For the Immunohistochemical investigation, Paraffin spleen and kidney at approximately 4 μm in thickness sections were dewaxed and were used for immunoperoxidase staining that was performed on a Benchmark XT (Ventana, Tucson, AZ, USA), Autostainer Plus (Dako, Carpinteria, CA, USA). The immunohistochemical staining was performed according to previously described procedures with rabbit monoclonal primary antibodies against Bcl-2 and CD3, VMSI Clone SP66 (rabbit, Cat NO. 790-4604) and CD3 (2GV6) (rabbit, Cat NO. 790-4341, respectively. The IHC for both Bcl-2 and CD3 investigations were performed in spleen sections and only Bcl-2 was investigated in kidney sections. The stained sections were examined using an Olympus camera (Nikon Eclipse E200-LED, Tokyo, Japan) (Kendrick et al., 2014; Meyer et al., 2011; Perry et al., 2014).

Histopathological analysis

The colorimetric analysis of images as the density of Masson' trichrome blue stain and the brown color in immunohistochemical staining were determined by Trigit application (Tjandra et al., 2023).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 26.0 was used for automated data analysis. The infected and control healthy groups' data were summarized using the mean ± the standard deviation (SD). Data were statistically examined with independent t-tests. When the *p* (probability) value was less than 0.05, the disparity was deemed statistically significant. All statistical graphs were performed by using Prism 10 (GraphPad Software, La Jolla, California, United States).

Results

Microscopic verification of T. camelensis infection

The Giemsa-stained blood smears examination revealed that 24 out of 114 camels (21.05%), had normal blood cells free from hemoparasites. And 28 out of 114 (24.56%) camels' blood smears represented *T. camelensis*. The erythrocytic form of *Theileria* infected red blood cells was identified by a chromatin dot on one side (*Fig. 1A, B*) and the lymphocytic forms of *Theileria* within the infected cells were identified by a swarm of light-bluish entities constituted the schizont stages (*Fig. 1C, D*). Meanwhile, examination of the rest 62 camels' blood smears revealed mixed infection and they were excluded from the study.

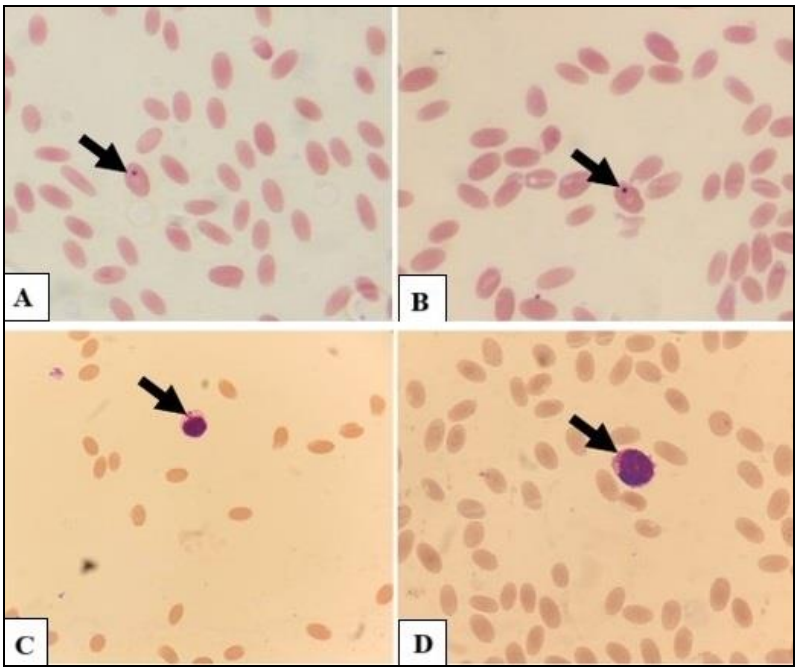

Figure 1. *Micrographs of Giemsa-stained blood smears from T. camelensis infected camels showing Theileria erythrocytic form inside the red blood cells (A& B) and the Schizonts form in lymphocytes (arrows) (C& D) (X 100)*

Hematological and biochemical analysis

Data represented as mean values and standard deviation (SD) of the hematological and biochemical parameters in clinically healthy camels and *Theileria* infected groups. Data presented (*Table 1)* represented the hematological parameters and evaluated that

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

the RBCs count, Hb and HCT values were highly significantly decreased ($P < 0.0001$) in *Theileria* infected group recording (4.25 ± 0.64), (7.89 ± 0.95) and (25.32 ± 2.14), respectively, as compared to healthy group detected values for RBCs count, Hb and HCT (7.00 ± 0.93), (15.42 ± 1.55) and (44.16 ± 2.93), respectively. Hematological parameters recorded fold changes from the control values by -0.61, -0.51 and -0.57 for RBCs count, Hb and HCT values, respectively. Meanwhile, WBCs count showed a highly significant increase in the infected group (27.21 ± 3.21) as compared to the healthy one (3.68 ± 0.73) and recorded 7.39 fold change from the control value.

As regards the biochemical tested parameters represented (*Fig. 2*) data showed highly significant increase ($P < 0.0001$) in the activities of the liver enzymes AST (123.80 ± 4.44 IU/L) and ALT (93.39 ± 3.65 IU/L) (*Fig. 2A, B*) and the serum levels of blood urea nitrogen (44.70 ± 2.83 mg/dL), and creatinine (0.78 ± 0.07 mg/dL) (*Fig. 2C, D*) in *Theileria* infected camels when compared with the corresponding values of AST (80.13 ± 8.43 IU/L), ALT (67.79 ± 10.02 IU/L), blood urea nitrogen (25.29 ± 2.59 mg/dL), and serum creatinine (0.33 ± 0.09 mg/dL) in control healthy camels.

As for the oxidative stress markers and antioxidant status, the *Theileria* affected camels showed highly significant ($P < 0.0001$) elevated level of MDA (7.608 ± 0.4201 nmol/g) and highly significant ($P < 0.0001$) decreased level of the reduced glutathione (GSH) (86.49 ± 5.57 ng/mg) as compared with the corresponding values of MDA (1.584 ± 0.41 nmol/g) and GSH (139.60 ± 6.39 ng/mg) respectively in the healthy camels control group (*Fig. 2E, F*). Also, the tissue damage enzyme indicator LDH determined in spleen tissues of the tested groups, data (*Fig. 2G*) indicated that the infected camels with *T. camelensis* had a considerably ($P < 0.0001$) higher level of the spleen LDH activity (323.80 ± 4.444 U/g protein) compared to its activity in the healthy control animals (165.90 ± 3.686 U/g protein).

Histopathological examination

Spleen

Normal splenic architecture, with white pulps and red pulps separated by marginal zones, was revealed by examination of the H & E-stained sections of the control group. There were marginal zones and lymphoid tissue (white pulps) embedded in a red pulp with many blood vessels in the spleen. Peri Arteriolar Lymphatic Sheath (PALS) and marginal zones made up the white pulp. A dark band surrounding the central arteriole was used to depict the PALS. There were germinal centers in a few follicles. Acidophilic cells with vesicular nuclei are found in the germinal center. Numerous lymphocytes with compact, darkly pigmented nuclei made up the lymphoid follicles. Splenic cords that branched and anastomosed, with blood sinusoids in between, made up the red pulp (*Fig. 3A, B*). Contrarily, analysis of infected camels with *Theileria* revealed lymphoid follicle atrophy and an altered splenic architecture. Thick capsules of connective tissue were observed. Most of the lymphoid follicles lacked a germinal center and had an unclear marginal zone. The image showed red pulps that had grown and expanded, with enlarged and crowded venous sinuses. Depletion of cells in the atrophying follicle, with pyknotic nuclei present in most cells. Apoptosis and degeneration were indicated by the numerous vacuolated cells with shattered nuclei that were found in the white pulp. Swollen and clogged splenic sinuses were found within the red pulp (*Fig. 3C-E*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

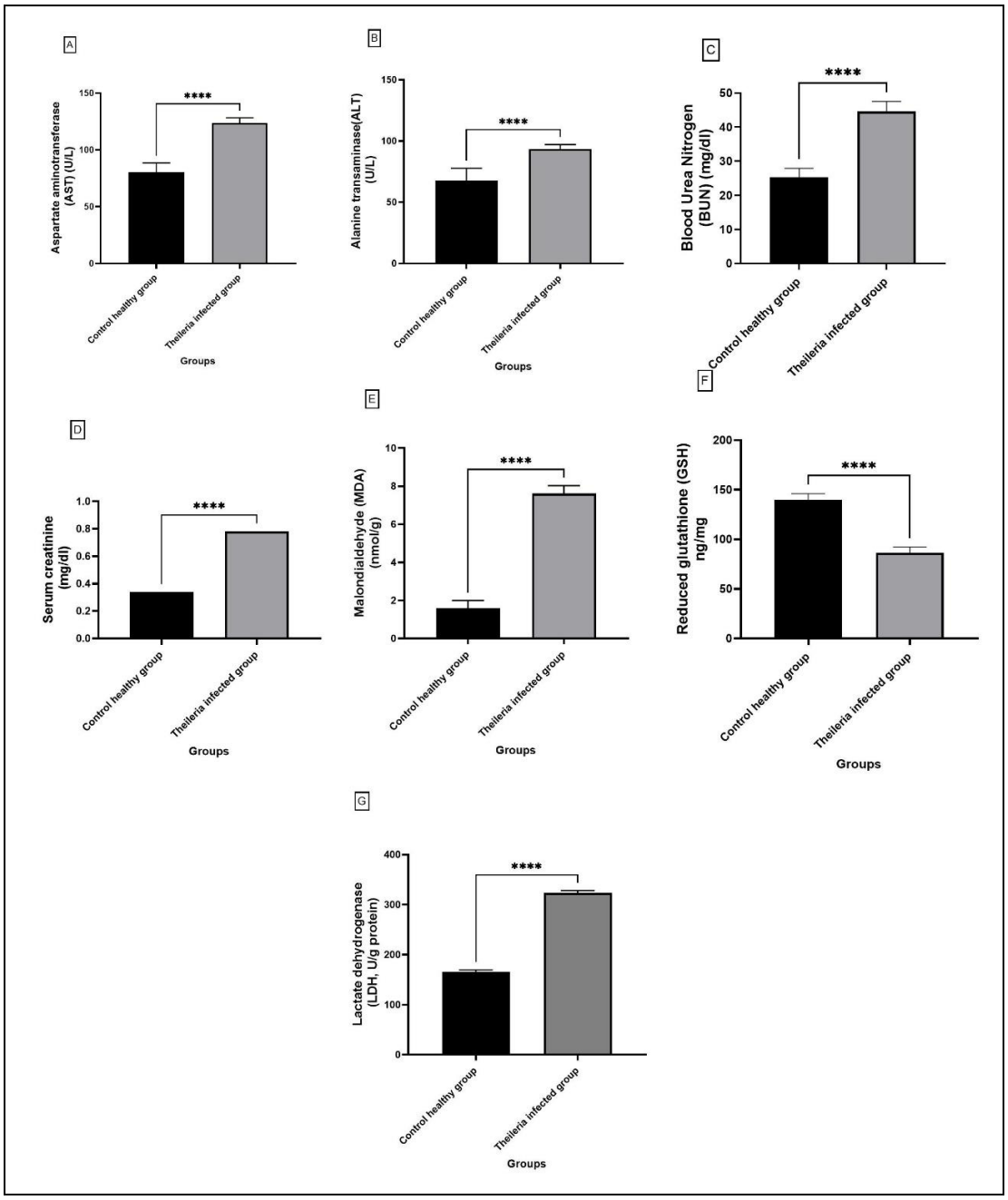

Figure 2. *Histogram representation of the serum biochemical parameters for the control healthy (n = 24) and naturally infected Theileria (n = 28) camel's groups. In serum (A) Aspartate aminotransferase (AST), (B) Alanine aminotransferase (ALT) (C) Serum Blood Urea Nitrogen (BUN), (D) creatinine (E) Malondialdehyde (MDA), and (F) Reduced glutathione (GSH). In spleen tissue (G) Lactate dehydrogenase (LDH). Data represented as mean values and standard deviation (SD). The significant difference between groups at ****$p < 0.0001$*

Masson' trichrome stained spleen sections from the control group 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. Also, thin trabecula was seen. While the *Theileria* infected group showed a highly significant increase in collagen fibers and thick trabecula were seen as compared to the control (*Fig. 4A-F*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

Table 1. *The hematological parameters for the control healthy and the naturally infected camel's groups*

Groups / Parameters	Control healthy group	*Theileria* infected group	Fold change	Normal range (Faye and Bengoumi, 2018)
RBCs (10^6/ml)	7.00 ± 0.93	4.25 ± 0.64****	-0.61	5.00 - 10.70 ± 0.90 10^6/mm^3
WBCs (10^3/ml)	3.68 ± 0.73	27.21 ± 3.21****	7.39	6.86 ± 2.26 - 20.70 ± 3.60 10^3/mm^3
Hb (g/dl)	15.42 ± 1.55	7. 89 ± 0.95****	-0.51	9.20 ± 0.50 - 15.50 ± 2.40 g-dl
HCT%	44.16 ± 2.93	25.32 ± 2.14****	-0.57	43.00 ± 3.50 - 21.50 ± 1.10%

Data represented as mean values and standard deviation (SD). Control healthy (n = 24) and naturally infected *Theileria* (n = 28). The significant difference between groups at ****p < 0.0001

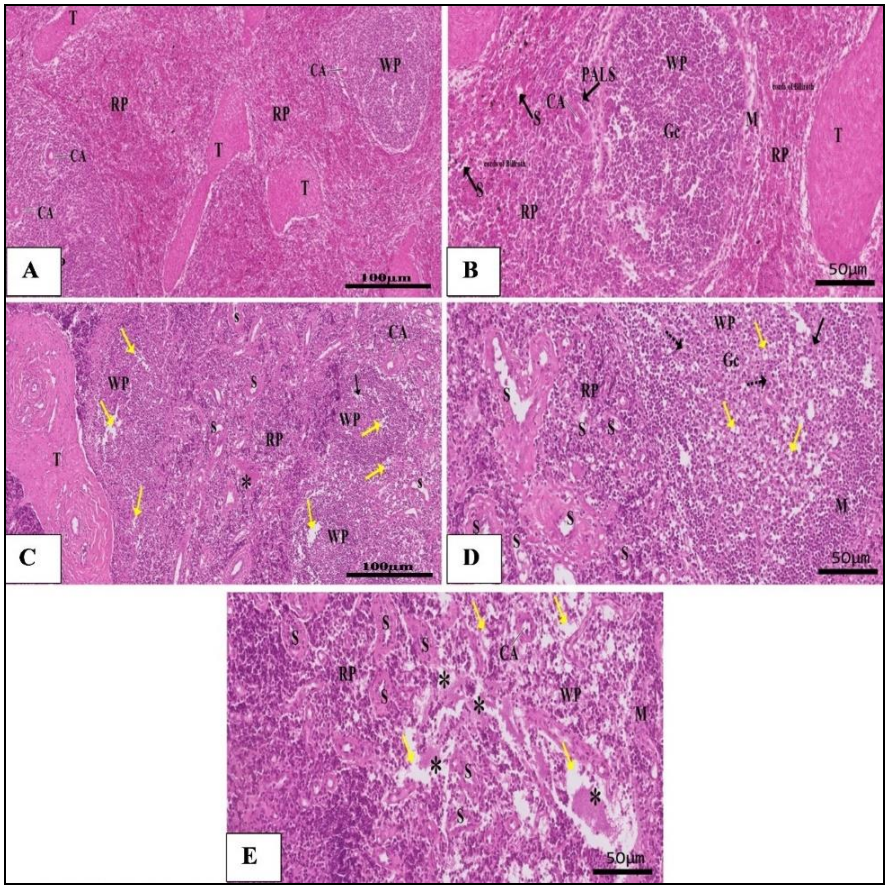

Figure 3. *Photomicrographs of camel spleen sections showing in control group (A& B) the white pulp (WP) contains well defined splenic lymphoid follicle with central germinal center (GC), central arteriole (CA), periarterial lymphatic sheath (PALS), marginal zone (M) and red pulp (RP). The red pulp contains 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. (H & E, A X 10;100 µm, B X 20;50 µm). In Theileria infected group (C-E) sections showing loss of architecture and highly atrophied white pulp (WP). Note thick trabeculae (T). Degenerated lymphoid cells in the white pulp (WP) with pyknotic nuclei and vacuolated cytoplasm (yellow ↑) were frequently seen indicating apoptosis, extravasation of RBCs (dot arrow) in-between lymphocytes, and areas of eosinophilic materials are seen. The marginal zone (M) is indistinct, and no germinal center (Gc) is seen. Note, central artery (CA) is also seen. The red pulp (RP) has marked dilated and congested splenic sinusoids (S). (H & E, C X 10;100 µm, D&E X 20;50 µm)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

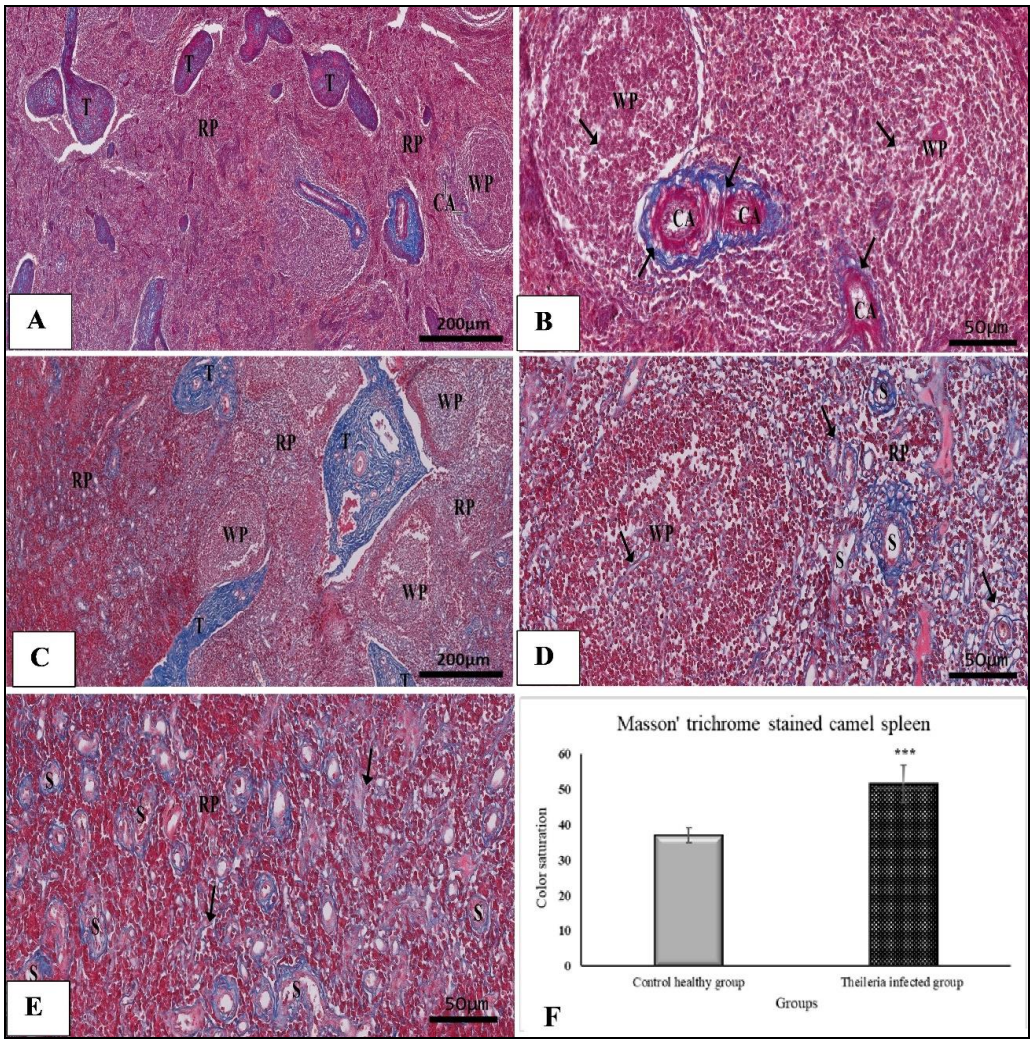

Figure 4. *Photomicrographs of Masson' trichrome stained camel spleen sections showing in control group (A& B) the fine collagen fibers (↑) around the central artery (CA) and in-between lymphocytes of the white pulp (WP) and in the parenchyma of the splenic tissue in the red pulp (RP). Notice thin trabecula (T) are seen. (Masson' trichrome stain, A X 5;200 µm, B X 20;50 µm). In Theileria infected group (C-E) sections showing an apparent increase of the collagen fibers (↑) in the parenchyma of the splenic tissue in the white pulp (WP) and red pulp (arrow). The red pulp (RP) shows an apparent increase of the collagen fibers surrounding dilated splenic sinusoids (S). Notice thick trabecula (T) are seen. (Masson' trichrome stain, C X 5;200 µm, D &E X 20;50 µm). (F) Comparison between spleen sections of Theileria infected group and control healthy group in the Masson' trichrome blue stain density. Data were mean ± SD. ⁎p < 0.0001 vs. control*

The Bcl-2 immunohistochemical staining of the control healthy group revealed strong positive cytoplasmic brownish reaction to the antibodies in the red pulp and in the marginal zone of the white pulp. But the negative reaction was recorded in the germinal center of the white pulp. Meanwhile, the *Theileria* infected group sections showed a highly significant decrease in the brownish reaction compared to that of the control group reflecting a weak positive cytoplasmic brownish reaction in the marginal zone of the white pulp. In addition to negative reactions in most of the red pulp were seen (*Fig. 5A, B, E*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

The immunohistochemical reaction to CD3 cells in spleen of control group revealed that the CD3 antibody was positively recognized T cells in the cell membrane and cytoplasmic reactions. There was strong positive brownish reaction in the red pulp. But negative reaction in the germinal center of the white pulp was seen. While *Theileria* infected group showed a highly significant increase of the positive cytoplasmic brownish reaction to CD3 antibody in the white pulp (WP) and in most of the red pulp (RP) (*Fig. 5 C, D, F*).

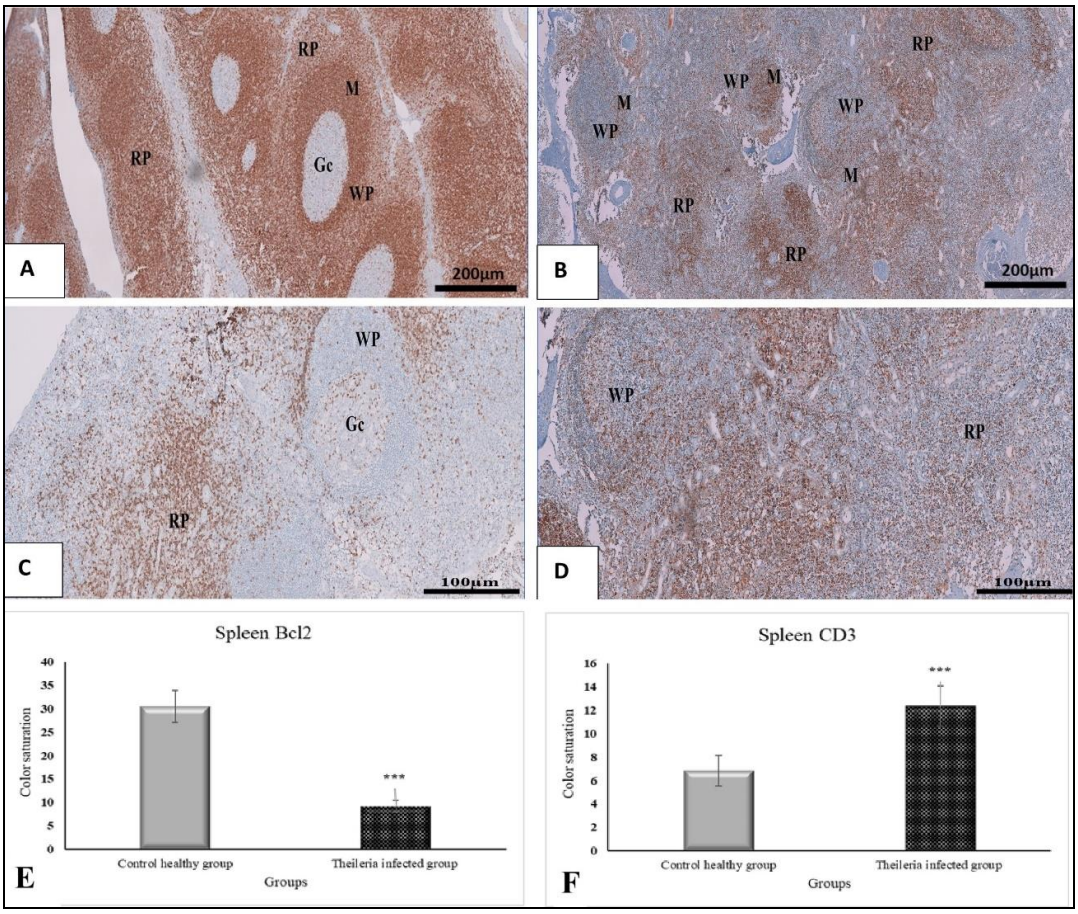

Figure 5. *Photomicrographs of spleen sections showing in control group (A) a strong positive cytoplasmic brownish positive reaction to Bcl-2 antibodies in red pulp (RP) and in the marginal zone (M) of the white pulp (WP). But negative reaction to Bcl-2 antibodies in the germinal center (Gc) of the white pulp. In Theileria infected group (B) sections showing a weak positive cytoplasmic brownish reaction to Bcl-2 antibodies in the mesangial layer (M) of the white pulp (WP). Notice negative reaction in most of the red pulp (RP) are seen. (Bcl-2 IHC, X 5;200 µm). (E) Comparison between spleen sections of Theileria infected group and control healthy group in the brownish reaction to Bcl-2 stain density. Data were mean ± SD. ⁎p < 0.0001 vs. control. Photomicrographs (C-D) A photomicrograph of spleen sections showing in control group (C) that 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). In Theileria infected group (D) sections showing an apparent increase of the positive cytoplasmic brownish reaction to CD3 antibody in the white pulp (WP). Notice an apparent increase of the positive reaction in most of the red pulp (RP) are seen. (CD3 IHC, X 10;100 µm). (F) Comparison between spleen sections of Theileria infected group and control healthy group in the brownish reaction to CD3 stain density. Data were mean ± SD. ⁎p < 0.0001 vs. control*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

Kidney

Examination of the H&E-stained sections of the renal cortices of the control group showed classical architecture of the glomerulus, renal tubules, and interstitial tissue. The glomeruli appeared with regular Bowman`s capsule and mesangial cells with dark nuclei, normal glomerular capillaries, and normal urinary space. Proximal tubules were lined by pyramidal epithelial cells with basal dark nuclei and homogenous pink cytoplasm and closed lumen by a well-organized brush border. Distal tubules were lined by cuboidal epithelial cells that had dark rounded nuclei and pink cytoplasm with narrow lumina were also seen (*Fig. 6A*).

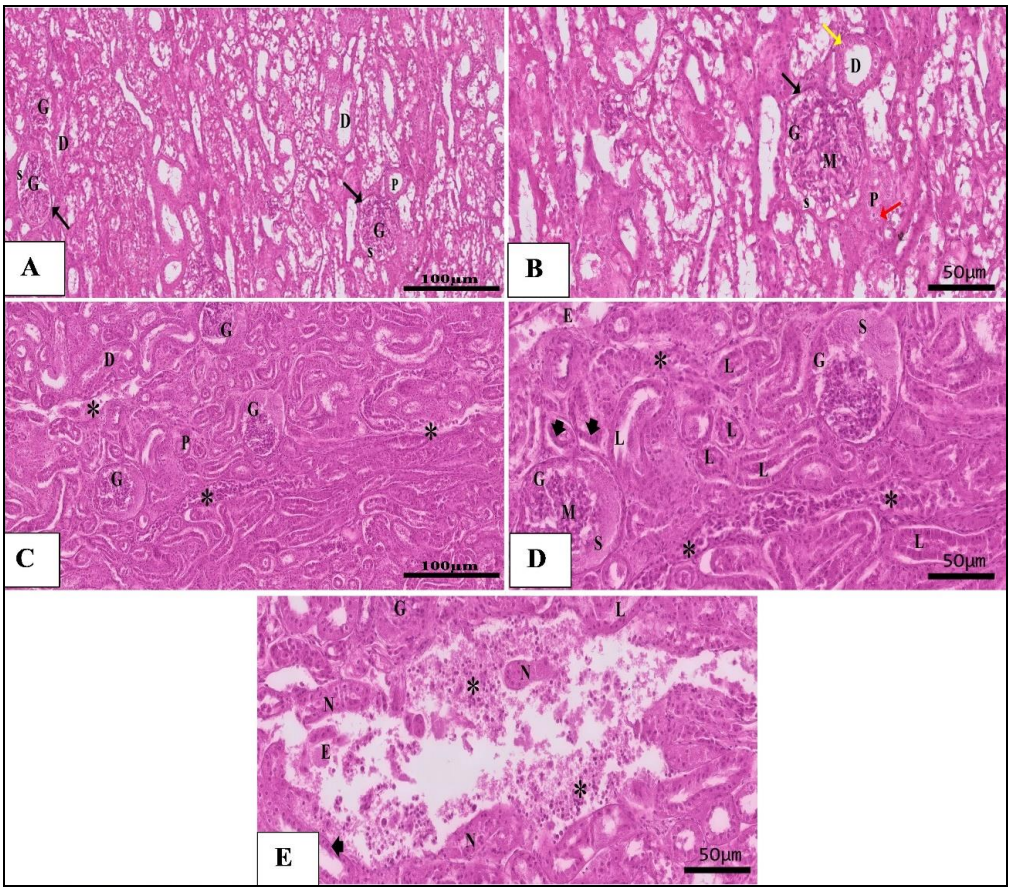

Figure 6. *Photomicrographs of camel kidney sections showing in control group (A& B) an organized renal cortex of control group, the normal organized renal tubules have closed lumen with well-organized brush border in proximal tubules (P). Other tubules are mildly dilated (D), tubular cells have dark rounded nuclei and homogenous dark pink cytoplasm, glomerulus (G) has mesangial cells (M) with dark nuclei and well-preserved urinary space (S). (H & E, A X 10;100 µm, B X 20;50 µm). In Theileria infected group (C-E) sections showing a destructive atrophied structure of the renal cortex. Large number of hyperemic glomeruli (G) without urinary space (S) filled with acidophilic materials and proliferating mesangial cells (M). Epithelial cells lining the renal proximal (P) and distal (D) tubules. The lumen (L) of the renal tubules is dilated and contains eosinophilic material (E). Wide interstitial tissue spaces with marked inflammatory cells (*). (e) Large number of hyperemic glomeruli (G), necrotic (N) and crowded proximal and distal renal tubules appear thin containing darkly stained pyknotic nuclei (arrowhead), and some tubules with absent lumen (L) and filled with eosinophilic material (E). Wide interstitial tissue spaces are seen with marked inflammatory cells (*) and contain eosinophilic material (E). (H & E, C X 10;100 µm, D-E X 20;50 µm)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

H&E-stained sections from the *Theileria* infected group showed a destructive atrophied structure of the renal cortex. A large number of hyperemic glomeruli without urinary space were filled with acidophilic materials and proliferating mesangial cells. Moreover, some glomeruli appeared necrotic and crowded proximal and distal renal tubules appeared thin containing darkly stained pyknotic nuclei, and some tubules with absent lumen and filled with eosinophilic material were noticed. The lumen of the renal tubules was dilated and contained eosinophilic material. Wide interstitial tissue spaces with marked inflammatory cells were seen (*Fig. 6B-E).*

Masson's trichrome staining of renal sections showed that, compared with the control healthy group, interstitial fibrosis was highly significant increased in the *Theileria* infected group, this fibrosis was seen in between the tubules and around glomeruli (*Fig. 7A-F*).

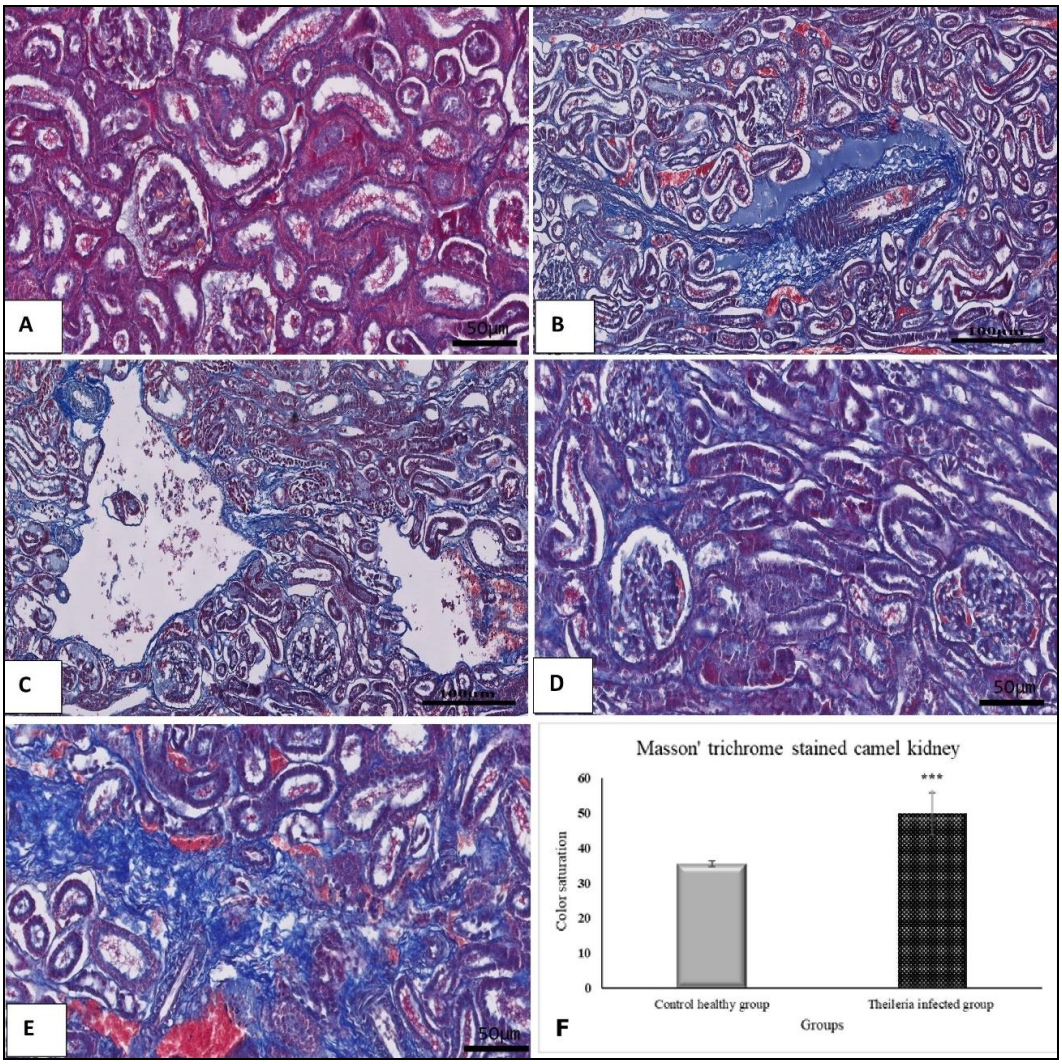

Figure 7. *Photomicrographs of Masson' trichrome stained camel Kidney sections (A) of control group showing fine collagen fibers in the stroma in-between the glomeruli and renal tubules. In Theileria infected group (B-E) sections showing an apparent increase of the collagen fibers in the stroma in-between the glomeruli and renal tubules. (Masson' trichrome stain, AX 20;50 µm, B-CX 10;100 µm, D-E CX 20;50 µm). (F) Comparison between Kidney sections of Theileria infected group and control healthy group in the Masson' trichrome blue stain density. Data were mean ± SD. ⁎p < 0.0001 vs. control*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

Bcl-2 immunohistochemical staining of renal cortex sections revealed positive cytoplasmic immunoreactivity, which was highly significant weak in the *Theileria* infected group as compared to the control healthy group (*Fig. 8A-C*).

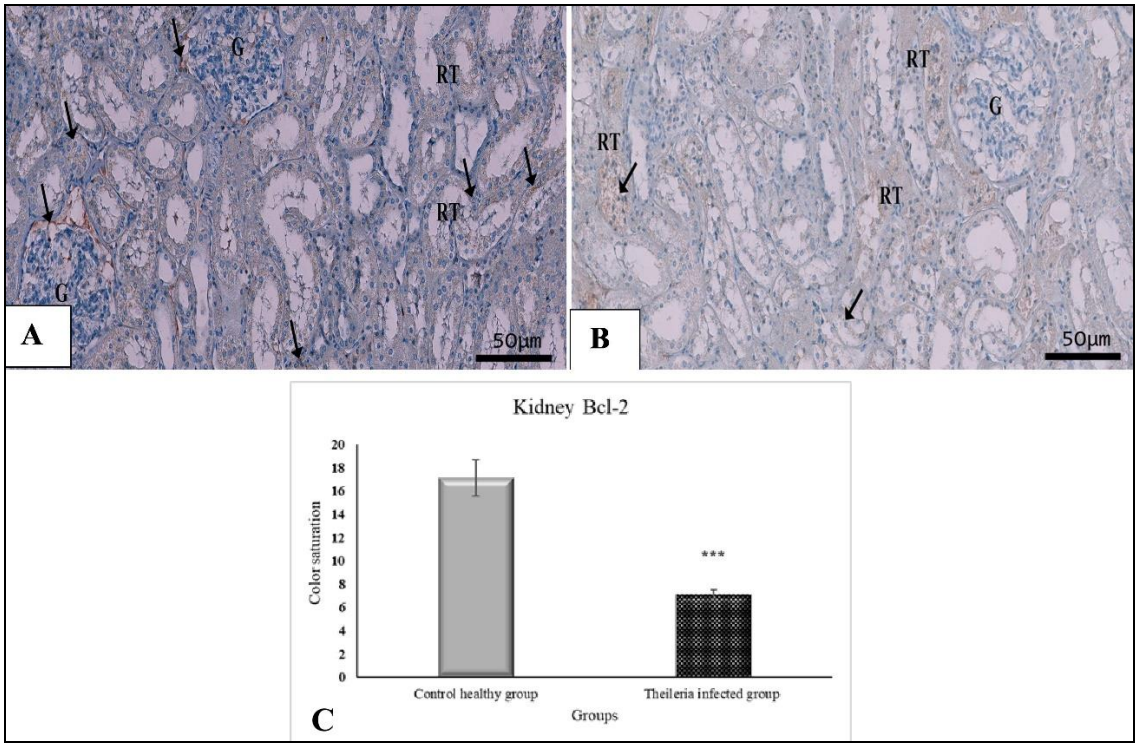

Figure 8. *Photomicrographs of kidney sections showing in control group (A) moderate positive cytoplasmic brownish reaction to Bcl-2 antibodies (↑) in the renal glomeruli (G) and renal tubules (RT). In Theileria infected group (B) sections showing a weak positive cytoplasmic brownish reaction to Bcl-2 antibodies. (Bcl-2 IHC, X 10;100 µm). (C) Comparison between kidney sections of Theileria infected group and control healthy group in the brownish reaction to Bcl-2 stain density. Data were mean ± SD. ⁎p < 0.0001 vs. control*

Discussion

One of the major challenges to livestock production in tropical and subtropical countries is the prevalence of parasitic infections, especially those caused by tick-borne hemoprotozoans, which pose a significant risk to the health of ruminants (Nasirian, 2024).

The *Hyalomma dromedarii* tick, known for feeding on camel blood, is frequently found in the Kingdom of Saudi Arabia. The region's climatic conditions provide an ideal environment for tick species that thrive in dry conditions. This tick has a high prevalence in Saudi Arabia and is known to transmit diseases like *T. camelensis*, which affects camels, as an important and highly valued livestock species in Saudi Arabia (Perveen et al., 2021).

Theileriosis pathogenesis typically hinges on lymphocyte schizont formation and erythrocyte piroplasm synthesis. Our investigation found that 24.56% of the investigated camel's Giemsa-stained blood smears had *T. camelensis*. Infected red blood cells were identified by a chromatin dot on one side. The infected lymphocytes showed signs of Schizont stages, which were characterized by a proliferation of light-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

bluish bodies. These results were confirmed by later studies like El-Seify et al. (2011) that detected *Theileria camelensis* infection in camels by (48.58%), in Shalatin city, Red Sea governorate, Egypt. The study found that out of 551 camels, 154 (27.94%) were infested with *H. dromedarii* tick species specially in pregnant female camels due to their lower resistance, as a result of pregnancy, so susceptible to infestation. Another study (Hamed et al., 2011), confirmed *H. dromedarii* infestation in 224 camels and 15 Giemsa-stained camels' blood smears of them recorded rod, rounded and ring shaped erythrocytic forms of *T. camelensis* and recorded its schizont in lymphocytes. The study identified *T. camelensis* Piroplasms and schizonts from 67 infected camels out of 173 (38.73%) investigated camels from the Riyadh region of Saudi Arabia (Ismael et al., 2014). A more recent study conducted in Aswan Province, Egypt revealed that 15% of *Thieleria* spp. infestation out of one hundred investigated camels and predicted the erythrocytic forms and schizont in lymphocytes (Ismaeil et al., 2023). A survey study on camels, cows, sheep, and goats from the Middle East and North regions of the United Arab Emirates predicted that 60.6% of the diagnosed Giemsa-stained blood films of the investigated animals (536) were infected by *Theileria* spp (Ismaeil et al., 2023). Therefore, the presence of infected camels indicates high pathogenicity and suggests the initial introduction of Theileriosis into this herd.

In the present study, the healthy control group recorded hematological and biochemical parameters with mean values within the normal ranges as previously described (Faye and Bengoumi, 2018). While the *Theileria* infected camel's blood, showed a significant decrease in the RBCs count, Hb content, and the HCT value as compared to the healthy camel's mean values which are considered as anemia indicators. In the meantime, the infected camel's blood showed a significant increase in WBCs count. Results reflected the *Theileria* infection in camel's pathogenicity. Where *Theileria* infects the host's mononuclear WBCs through tick transmission as sporozoites which mature into macroschizonts, and stimulate the proliferation of the host's cells and then develop into microschizonts and eventually into merozoites, which are released from the WBCs. Microseizont proliferation significantly impacts on lymphocytes, leading to inflammatory reactions of the lymphoid tissue, also stimulating it and stem cells in the bone marrow to increase the production of WBCs as a defensive mechanism to the parasite. *Theileria* infected leukocytes exhibit increased expression of metabolic regulators, enhanced glucose uptake, and elevated lactate production. The parasites within the host cells disrupt the redox balance and increase reactive oxygen species (ROS), which is linked to the chronic stabilization of hypoxia-inducible factor 1 alpha. As a result, the parasite can induce transformed phenotypes in infected cells by reprogramming glucose metabolism and redox signaling (Medjkane et al., 2014; Metheni et al., 2014). Meanwhile, the released merozoites surpass RBCs and transform into piroplasms, causing hemolysis with the proposed involvement of oxidative stress due to oxygen radicals. Hence, the evolved anemia and leukocytosis due to *T. camelensis* infection in camels as previously discussed (Ali et al., 2024; Al-Saad et al., 2006; Ismael et al., 2014).

As regard to the detected blood biochemical parameters, the *Theileria* infected group showed a significant increase in the liver enzymes activities AST and ALT and the blood urea nitrogen and creatinine levels compared to the control healthy values. Theileriosis diagnosis relies heavily on enzyme activity measurements of blood AST and ALT, which indicate the liver's physiological activity (Al-Fetly, 2012). Because most of the body's tissues can be thought of as a large reservoir of enzymes that could

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

be released and detected during a pathological condition, damage to the hepatic tissue could explain the rise in the liver function enzymes. Previously, in *T. camelensis* infested camel's blood was evaluated compared to the control values a significant increase in AST, ALT and GGT (γ–glutamyltransferase) enzymes' activity and the kidney function parameters creatinine and blood urea nitrogen levels. In addition to the highly significant increase in the LDH blood content in affected camels (Al-Saad et al., 2006; Ismael et al., 2014). In the same line, another study predicted in *Theileria* infected camels a significantly elevated AST enzyme activity and urea level in their serum as compared to the normal non-infected corresponding blood values (Abouzaid et al., 2022). In addition, not only the liver was affected by *Theileria* infection, but the kidney function was also disturbed as confirmed by the rise in serum BUN and creatinine levels. Mahmoud et al. (2019) explained an elevated urea level due to indirect damage to the kidneys and the presence of globin catabolites released from hemoglobin lysis by the reticuloendothelial system through erythrophagocytosis. In the present study, based on the significant increase of renal function results in infected group where it caused severe degenerative histological changes in the kidney of the naturally infected camels as previously described. These findings are in agreement with later studies declared that *Theileria* caused inflammation, tubular atrophy and lumen dilatation, reduction of Bowman's space, congestion, and necrosis in the renal tissue (Clift et al., 2020; Ma et al., 2020).

The spleen is the largest secondary lymphoid organ in the body and as such hosts a wide range of immunological functions alongside its roles in hematopoiesis and red blood cell clearance (Maina et al., 2014). The camel's spleen type is sinusal that is capable of storing blood due to its thick muscular capsule and trabeculae which pump the stored blood according to the body's needs. The camel's spleen acquired closed and open circulatory systems. where the venous return in the camel's spleen is unique, by blood moving from the venous sinusoids in the red pulp to the peritrabecular sinuses, then to the subcapsular sinuses, and ultimately to the splenic vein. The presence of a closed circulation in the camel's spleen and the lack of a marginal sinus may lessen the blood filtration role of the marginal zone which may clarify the primary problematic health effect of the blood parasites in camels (Zidan et al., 2000). The present study showed that tissue damage enzyme indicator LDH in the spleen tissue of camels infected with *T. camelensis* was significantly higher in activity compared to its activity in the spleen of healthy control camels. The recorded elevated spleen LDH activity in line with the previously recorded serum elevated enzyme (Ismael et al., 2014), and in line with the direct relationship between the serum LDH activity and that of the spleen tissue as previously confirmed by proposing the spleen tissue damage due to *T. camelensis* infection in camels (Shaker et al., 1989). The spleen plays a crucial role in regulating the number and circulation of erythrocytes, and under conditions of stress or dehydration, its function can influence hematological parameters. The activity of LDH was recorded to be elevated in case of insufficient oxygen, during stress, inflammation and anemia in camels (Abu Damir et al., 2018; Ali et al., 2023; El Khasmi, 2024).

Theileria infection stimulates the immune system to keep out the parasitic invaders via the proinflammatory cytokines as an immune response which are produced by various immune cells in response to the presence and proliferation of protozoa in an animal's body. Also, lipid peroxidation and oxidative reactions play a role in the pathophysiology of anemia as a result of infection. Where, in order to inflict harm to the host's tissues and cells, parasites boost lipid peroxidation and free radical production in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

the host's cells, organs, and tissues. The present study recorded in the infected group a significant decrease in GSH level which is included in the antioxidant system and a significant elevation in MDA level that was commonly applied for the assessment of lipoperoxidation and their both levels were used as oxidative stress markers in camels (Tharwat and El-Deeb, 2021). Infection with *Theileria* causes oxidative stress in the liver and kidneys as a result of parasites, which in turn increases lipid peroxidation. Cell membrane breakdown and necrotic deaths are the end results of lipid peroxidation, which is mediated by ROS (Abd Ellah, 2013; Gopalakrishnan et al., 2015). Also, a recent study evidenced the oxidative stress and the involved immune response in *T. annulata* naturally infected camels through the significant elevated blood oxidative stress and proinflammatory cytokines markers (Clift et al., 2020; Ramadan et al., 2024).

As regard to the present study, histological findings for the infected group, Theileriosis is pathogenic characterized histologically by the induction of gross lesions in different organs like liver, kidney and spleen as previously recorded in wild artiodactyls and domestic livestock (Clift et al., 2020). It was previously discussed that *Theileria* spp. in renal tissue leads to focal or diffuse coagulative necrosis and/or heightened protein catabolism due to reduced appetite during infection. Also, congestion in some areas of spleen with severe lymphocytic necrosis. White pulp has deteriorated without lymphoproliferation in *Theileria* infected sheep (Eliwa et al., 2021). Focal hemorrhage and necrosis in the renal cortex, glomerular edema, hyperplasia of endothelial and endometrial cells, contraction or loss of the renal capsule, occasional protein exudates or foreign bodies, loss of glomerular integrity, accumulation of renal tubular and lymphatic cells, infiltration of some glomeruli and vessels, and protein patterns in renal tubules of *Theileria* infected castles (Ma et al., 2020). Regarding splenic capsules and trabeculae, they were thickened in the *Theileria* naturally infected camels. This finding may be part of connective tissue expansion as approved by Cheeseman and Weitzman (Cheeseman and Weitzman, 2015) and Tretina et al. (2015). In the present study, the red pulps were expanded with dilated congested splenic sinuses. These findings are consistent with a study used experimentally infected cattle with *T. annulata* where the red pulp region enlargement was produced by dilated and crowded splenic sinuses (Ma et al., 2020). In cirrhosis, the congestion was due to portal hypertension, but in the current study the congestion might be due to cellular degeneration and splenic fibrosis caused by *Theileria* infection. Mechanical compression due to fibers deposition in the sinusoidal wall induced the observed congestion and dilatation. Also, numerous studies have demonstrated that a variety of inflammatory cells are induced or activated by various oxidant-generating enzymes to kill intracellular and extracellular parasites (Dkhil et al., 2014; Tolosano et al., 2002).

In the current study, the collagen distribution in the spleen and kidney was detected using Masson trichrome staining. *Theileria* infected camels' spleen and kidney displayed evident massive collagen accumulation, which is a common characteristic of spleenic and renal fibrosis. Consistent with findings in induced cirrhosis models, prior research has shown substantial collagen buildup in the spleen. Red pulp region enlargement was produced by dilated and crowded splenic sinuses (Aoyama et al., 2017). Although portal hypertension was the known cause of congestion in cirrhosis, this study suggests that diabetes-related cellular deterioration and splenic fibrosis may be to blame for the same symptoms. They found congestion and dilatation as a result of mechanical compression caused by fiber deposition in the sinusoidal wall (Iwakiri and Trebicka, 2021).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

A member of the Bcl-2 family has been found to play a role in apoptosis regulation. Apoptosis is inhibited by Bcl-2. They belong to the Bcl-2 protein family, which regulates the outer mitochondrial membrane integrity during apoptosis. Intrinsic transmembrane switch inhibition has been demonstrated for Bcl-2. Also, Bcl-2 may prevent apoptosis by sequestering the caspase precursor (Refaay et al., 2023). In the present work, it was observed that Bcl-2 level was reduced in renal, and splenic tissues of *Theileria* infected group. That supports the hypothesis about the oxidative stress contribution to apoptosis (Florentino et al., 2021). At the border between the spleen's red and white pulps is the marginal zone, an area rich in macrophages. B cells in the marginal zone coexpress Bcl-2 and are positive for B-cell markers (CD19, CD20, CD22) (Borch et al., 2019). In the current study, a weak positive cytoplasmic positive brownish reaction of Bcl-2 cells in the marginal zone of the white pulp was noticed in the *T. camelensis* naturally infected camels.

The analysis of mononuclear leukocyte populations in various tissues was carried out using immunohistochemistry. Macrophages and CD3 + T lymphocytes made up the bulk of the cell population in the spleen (Medina-Colorado et al., 2017). It appeared that the immunopositively reactivity of CD3 T-lymphocytes increased in both the red and white pulps. The current results may be explained by oxidative stress caused by *Theileria* infection. Lymphoid cells, the so-called periarteriolar lymphoid sheath (PALS), and neighboring outpouchings of nodular lymphoid tissue surround the arterioles that make up the splenic white pulp. T cells and flattened reticular cells make up most of the PALS. While a small percentage of the T cells stain for CD8, the vast majority express the standard set of T-lymphocyte antigens (CD2, CD3, CD5, and CD7) (Borch et al., 2019). In the current study, there was an apparent increase of CD3 positive cells in the splenic tissue of the *T. camelensis* infected animals as compared to control healthy animals. The current study's findings corroborated those of Branco et al. (2010). They found T cells and macrophages in infected calves with fetal bovine theileriosis in Portugal, but only a small number of B lymphocytes in various tissues.

Conclusion

This study demonstrates that dromedary camels are vulnerable to infection by *T. camelensis*, which can cause damage to various organs. The findings further indicate that theileriosis adversely affects camel health by altering their hematological and biochemical parameters, as well as the histological structure of their kidneys and spleen. Notably, this is the first study to describe the immunological response and oxidative stress in camels naturally infected with *T. camelensis*.

REFERENCES

[1] Abd Ellah, M. R. (2013): Involvement of free radicals in parasitic infestations. – Journal of Applied Animal Research 41(1): 69-76.

[2] Abouzaid, A. A. (2022): Clinicopathological studies on camels (Camelus dromedaries) infected with Thieleriosis in Aswan Governorate. – Aswan University Journal of Environmental Studies 3(3): 337-345.

[3] Abouzaid, A. A., Khaleel Abd Elghaffar, S., Karmi Husseinc, M., Ibrahim, E. (2022): Clinicopathological investigations on babesiosis among camels (Camels dromedaries) in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

Aswan Province, Egypt. – Aswan University Journal of Environmental Studies 3(3): 284-292.

[4] Abu Damir, H., Ali, M. A., Khalafalla, A. E. (2018): Investigation of different enzyme markers in some pathological disorders of the dromedary camels in comparison with other species. – CABI Reviews. https://doi.org/10.1079/PAVSNNR20171204.

[5] Alanazi, A. D., Nguyen, V. L., Alyousif, M. S., Manoj, R. R. S., Alouffi, A. S., Donato, R., Sazmand, A., Mendoza-Roldan, J. A., Dantas-Torres, F., Otranto, D. (2020): Ticks and associated pathogens in camels (Camelus dromedarius) from Riyadh Province, Saudi Arabia. – Parasites & Vectors 13: 1-9.

[6] Al-Fetly, D. R. H. (2012): Detection of Theileria spp. in blood samples and estimation of haematological and biochemical changes in sheep in Al-Diwaniya province. – Kufa Journal for Veterinary Medical Sciences 3(2).

[7] Ali, M. A., Abu Damir, H., Adem, M. A., Ali, O. M., Amir, N., Shah, A. A. M., Al Muhairi, S. S. M., Al Abdouli, K. O. S., Khawaja, J. R., Fagieri, T. A. (2023): Effects of long-term dehydration on stress markers, blood parameters, and tissue morphology in the dromedary camel (Camelus dromedarius). – Frontiers in Veterinary Science 10: 1236425.

[8] Ali, R. K., Rahawi, A. M., Al-hbiti, Y. T. (2024): Lesion of theileriosis in camel: a review article. – Egyptian Journal of Veterinary Sciences 55(7): 2089-2094. https://doi.org/10.21608/ejvs.2024.267224.1819.

[9] Al-Saad, K. M., Al-Obaidi, Q. T., Al-Obaidi, W. A. (2006): Clinical, haematological and biochemical study of theileriosis in one-humped Arabian camels (Camelus dromedarius). – Iraqi Journal of Veterinary Sciences 20(2): 211-218.

[10] Aoyama, T., Kuwahara-Arai, K., Uchiyama, A., Kon, K., Okubo, H., Yamashina, S., Ikejima, K., Kokubu, S., Miyazaki, A., Watanabe, S. (2017): Spleen-derived lipocalin-2 in the portal vein regulates Kupffer cells activation and attenuates the development of liver fibrosis in mice. – Laboratory Investigation 97(8): 890-902.

[11] Bancroft, J. D., Gamble, M. (2008): Theory and Practice of Histological Techniques, 7th Ed. – Elsevier Health Sciences, Amsterdam.

[12] Borch, W. R., Aguilera, N. S., Brissette, M. D., O'Malley, D. P., Auerbach, A. (2019): Practical applications in immunohistochemistry: an immunophenotypic approach to the spleen. – Archives of Pathology & Laboratory Medicine 143(9): 1093-1105.

[13] Branco, S., Orvalho, J., Leitão, A., Pereira, I., Malta, M., Mariano, I., Carvalho, T., Baptista, R., Shiels, B. R., Peleteiro, M. C. (2010): Fatal cases of Theileria annulata infection in calves in Portugal associated with neoplastic-like lymphoid cell proliferation. – Journal of Veterinary Science 11(1): 27-34.

[14] Cheeseman, K., Weitzman, J. B. (2015): Host–parasite interactions: an intimate epigenetic relationship. – Cellular Microbiology 17(8): 1121-1132.

[15] Clift, S. J., Collins, N. E., Oosthuizen, M. C., Steyl, J. C. A., Lawrence, J. A., Mitchell, E. P. (2020): The pathology of pathogenic theileriosis in African Wild Artiodactyls. – Veterinary Pathology 57(1): 24-48. https://doi.org/10.1177/0300985819879443.

[16] Dkhil, M. A., Al-Quraishy, S., Al-Khalifa, M. S. (2014): The effect of Babesia divergens infection on the spleen of Mongolian gerbils. – BioMed Research International 2014: 483854. https://doi.org/10.1155/2014/483854.

[17] El Khasmi, M. (2024): Stress Transport in the Dromedary Camel. – In: Padalino, B., Faye, B. (eds.) Dromedary Camel Behavior and Welfare: Camel Friendly Management Practices. Springer, Cham, pp. 207-229.

[18] El-Deeb, W. M., Younis, E. E. (2009): Clinical and biochemical studies on Theileria annulata in Egyptian buffaloes (Bubalus bubalis) with particular orientation to oxidative stress and ketosis relationship. – Veterinary Parasitology 164(2-4): 301-305.

[19] Eliwa, M., Mahran, K. M. A., Mousa, W. A., Hagag, N., Shaalan, M. I., Bashandy, M. M. (2021): Ovine theileriosis: clinical, pathological and molecular investigations. – Adv. Anim. Vet. Sci 9(4): 462-472.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

[20] El-Seify, M. A., Mahran, O. M., Abdelaal, A. M. I. (2011): Epidemiological studies on hard ticks and tick borne parasites, in Shalatin City, red sea governorate, Egypt. – Assiut Veterinary Medical Journal 57(130): 1-28.
[21] Faye, B. (2020): How many large camelids in the world? A synthetic analysis of the world camel demographic changes. – Pastoralism 10(1): 1-20.
[22] Faye, B., Bengoumi, M. (2018): Hematology. – In: Faye, B., Bengoumi, M. (eds.) Camel Clinical Biochemistry and Hematology. Springer International Publishing, Cham, pp. 13-45. https://doi.org/10.1007/978-3-319-95562-9_2.
[23] Florentino, P. T. V., Mendes, D., Vitorino, F. N. L., Martins, D. J., Cunha, J. P. C., Mortara, R. A., Menck, C. F. M. (2021): DNA damage and oxidative stress in human cells infected by Trypanosoma cruzi. – PLoS Pathogens 17(4): e1009502.
[24] Gopalakrishnan, A., Maji, C., Dahiya, R. K., Suthar, A., Kumar, R., Kumar, S. (2015): Oxidative damage inflicted by Theileria equi on horse erythrocytes when cultured in vitro by microaerophilous stationary phase technique. – Journal of Equine Veterinary Science 35(9): 763-767.
[25] Hamed, M. I., Zaitoun, A. M. A., El-Allawy, T. A. A., Mourad, M. I. (2011): Investigation of Theileria camelensis in camels infested by Hyalomma dromedarii ticks in Upper Egypt. – Journal of Advanced Veterinary Research 1(1): 4-7.
[26] Ismaeil, S. M. A., Parambil, R. K. T., Kerab, M. S., ElBakri, A. M. K., Abu-Abdoun, I. I. (2023): Parasitological detection of Theileria parasites in livestock in various regions of the Northern Emirates of the United Arab Emirates. – Veterinary World 16(5): 1071.
[27] Ismael, A. B., Swelum, A. A., Khalaf, A. F., Abouheif, M. A. (2014): Clinical, haematological and biochemical alterations associated with an outbreak of theileriosis in dromedaries (Camelus dromedarius) in Saudi Arabia. – Pakistan Veterinary Journal (Pakistan): 34(2): 209-213.
[28] Iwakiri, Y., Trebicka, J. (2021): Portal hypertension in cirrhosis: pathophysiological mechanisms and therapy. – JHEP Reports: Innovation in Hepatology 3(4): 100316. https://doi.org/10.1016/j.jhepr.2021.100316.
[29] Kendrick, S. L., Redd, L., Muranyi, A., Henricksen, L. A., Stanislaw, S., Smith, L. M., Perry, A. M., Fu, K., Weisenburger, D. D., Rosenwald, A. (2014): BCL2 antibodies targeted at different epitopes detect varying levels of protein expression and correlate with frequent gene amplification in diffuse large B-cell lymphoma. – Human Pathology 45(10): 2144-2153.
[30] Ma, Q., Liu, J., Li, Z., Xiang, Q., Wang, J., Liu, A., Li, Y., Yin, H., Guan, G., Luo, J. (2020): Clinical and pathological studies on cattle experimentally infected with Theileria annulata in China. – Pathogens 9(9): 727.
[31] Mahmoud, M., Al-Dhalimy, A., Al-Dujaily, A. (2019): Study of hematological and biochemical changes in sheep and goats infected with theileriosis AT-Najaf province, Iraq. – Biochemical and Cellular Archives 19(1): 1863-1867.
[32] Maina, M. M., Usende, I. L., Igwenagu, E., Onyiche, T. E., Yusuf, Z. M., Ntung, N. O. (2014): Gross, histological and histomorphometric studies on the spleen of one humped camel (Camelus dromedarius) found in the semi-arid region of North Eastern Nigeria. – J. Vet. Adv 4(10): 703-711.
[33] Medina-Colorado, A. A., Osorio, E. Y., Saldarriaga, O. A., Travi, B. L., Kong, F., Spratt, H., Soong, L., Melby, P. C. (2017): Splenic CD4+ T cells in progressive visceral leishmaniasis show a mixed effector-regulatory phenotype and impair macrophage effector function through inhibitory receptor expression. – PloS ONE 12(1): e0169496.
[34] Medjkane, S., Perichon, M., Marsolier, J., Dairou, J., Weitzman, J. B. (2014): Theileria induces oxidative stress and HIF1α activation that are essential for host leukocyte transformation. – Oncogene 33(14): 1809-1817.
[35] Metheni, M., Echebli, N., Chaussepied, M., Ransy, C., Chéreau, C., Jensen, K., Glass, E., Batteux, F., Bouillaud, F., Langsley, G. (2014): The level of H2O2 type oxidative stress

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

regulates virulence of T heileria-transformed leukocytes. – Cellular Microbiology 16(2): 269-279.

[36] Meyer, P. N., Fu, K., Greiner, T. C., Smith, L. M., Delabie, J., Gascoyne, R. D., Ott, G., Rosenwald, A., Braziel, R. M., Campo, E. (2011): Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. – Journal of Clinical Oncology 29(2): 200-207.

[37] Ministry of Municipalities and Rural Affairs in Saudi Arabia (2008): Veterinary Inspection Guide of Meat. – King Fahd National Library, Riyadh, pp. 3-18.

[38] Nasirian, H. (2024): Hard tick species parasitism levels in domestic ruminants with their distribution and role as vectors: a detailed global meta-analysis and systematic review. – Acta Parasitologica 69(1): 1-105.

[39] Nourollahi Fard, S. R., Fathi, S., Norouzi Asl, E., Asgary Nazhad, H., Salehzadeh Kazeroni, S. (2012): Hard ticks on one-humped camel (Camelus dromedarius) and their seasonal population dynamics in southeast, Iran. – Tropical Animal Health and Production 44: 197-200.

[40] Perry, A. M., Alvarado-Bernal, Y., Laurini, J. A., Smith, L. M., Slack, G. W., Tan, K. L., Sehn, L. H., Fu, K., Aoun, P., Greiner, T. C. (2014): MYC and BCL 2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. – British Journal of Haematology 165(3): 382-391.

[41] Perveen, N., Muzaffar, S., Bin, Al-Deeb, M. A. (2021): Ticks and tick-borne diseases of livestock in the Middle East and North Africa: a review. – Insects 12(1): 83.

[42] Ramadan, R. M., Bakr, A. F., Fouad, E., Mohammed, F. F., Abdel-Wahab, A. M., Abdel-Maogood, S. Z., El-Bahy, M. M., Salem, M. A. (2024): Novel insights into antioxidant status, gene expression, and immunohistochemistry in an animal model infected with camel-derived Trypanosoma evansi and Theileria annulata. – Parasites & Vectors 17(1): 474.

[43] Refaay, N., Halloull, N., Borai, E. (2023): DNA deterioration and biochemical changes induced by Sodium Fluoride on Rat kidney and the potential effect of Resveratrol. – Egyptian Journal of Histology 46(3): 1135-1148.

[44] Riaz, M., Nasreen, N., Khan, A., Said, M. B. (2023): Differential diagnosis of theileriosis through blood smear examination and polymerase chain reaction in small ruminants from Pakistan. – Open Veterinary Journal 13(6): 697-704. https://doi.org/10.5455/OVJ.2023.v13.i6.4.

[45] Salman, D., Sivakumar, T., Otgonsuren, D., Mahmoud, M. E., Elmahallawy, E. K., Khalphallah, A., Kounour, A. M. E. Y., Bayomi, S. A., Igarashi, M., Yokoyama, N. (2022): Molecular survey of Babesia, Theileria, Trypanosoma, and Anaplasma infections in camels (Camelus dromedaries) in Egypt. – Parasitology International 90: 102618.

[46] Shaker, M., Mostafa, M. A., Amer, H. A., Sabry, M. M. (1989): Some enzyme interrelationships in serum and organs of Egyptian camel. – Archiv Für Experimentelle Veterinarmedizin 43(2): 241-247.

[47] Soulsby, E. J. L. (1982): Helminths, arthropods and protozoa of domesticated animals. 7th Ed. – Bailliere Tindall and Cassel Ltd, London.

[48] Tharwat, M., El-Deeb, W. (2021): Biomarkers of stress in healthy and diseased dromedary camels: a mini review. – Journal of Camel Practice and Research 28(3): 297-302. https://doi.org/10.5958/2277-8934.2021.00046.1.

[49] Tjandra, A. D., Heywood, T., Chandrawati, R. (2023) Trigit: a free web application for rapid colorimetric analysis of images. – Biosensors and Bioelectronics X(14): 100361.

[50] Tolosano, E., Fagoonee, S., Hirsch, E., Berger, F. G., Baumann, H., Silengo, L., Altruda, F. (2002): Enhanced splenomegaly and severe liver inflammation in haptoglobin/hemopexin double-null mice after acute hemolysis. – Blood, The Journal of the American Society of Hematology 100(12): 4201-4208.

[51] Tretina, K., Gotia, H. T., Mann, D. J., Silva, J. C. (2015): Theileria-transformed bovine leukocytes have cancer hallmarks. – Trends in Parasitology 31(7): 306-314.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616

[52] Van De Vlekkert, D., Machado, E., d'Azzo, A. (2020): Analysis of generalized fibrosis in mouse tissue sections with Masson's trichrome staining. – Bio-Protocol 10(10): e3629–e3629.
[53] Youssef, S. Y., Yasien, S., Mousa, W. M. A., Nasr, S. M., El-Kelesh, E. A. M., Mahran, K. M., Abd-El-Rahman, A. H. (2015): Vector identification and clinical, hematological, biochemical, and parasitological characteristics of camel (Camelus dromedarius) theileriosis in Egypt. – Tropical Animal Health and Production 47: 649-656.
[54] Zidan, M., Kassem, A., Dougbag, A. L. I., El Ghazzawi, E., Abdelaziz, M., Past, R. (2000): The spleen of the one humped camel (Camelus dromedarius) has a unique histological structure. – The Journal of Anatomy 196(3): 425-432.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_55975616