



Original Research Article

Histopathological and Hematological Changes in Rat Tissue after Injected with Babesia and Theileria parasites

Barakat Shehata Abd-Elmaleck^{1*}, Gamal Hassan Abed¹ and Ahmad Mohamad Mandour²

¹Zoology Department, Faculty of Science, Faculty of Medicine,
Assiut University, Assiut, 71516, Egypt

²Parasitology Department, Faculty of Medicine, Assiut University, Assiut, 71516, Egypt

*Corresponding author

A B S T R A C T

Babesia and *Theileria* are intraerythrocytic parasites which are capable of infecting a wide range of vertebrates causing huge economic losses. Histopathological and hematological changes during *Babesia* and *Theileria* inoculation in rat they were reported. Objective of the study is to determine the presence of *Babesia* & *Theileria* infected *Camelus dromedarius* at Assiut locality, Upper Egypt and their effects on hematological and tissues (liver, lung and kidney) of rat by injection in the blood. Blood samples were collected from *Camelus dromedarius* from different localities of Slaughter houses at Assiut city, Egypt (Dairout, Beni ady and Elethamna). Thick and thin blood smears were made for morphological examination of some protozoan blood parasites with electron microscopic studies. Out of (195) one hundred and ninety five *Camelus dromedarius* were examined and only twelve (6.1 %) were infected with *Th. assiutis* (n. sp.) and fifty one were found to be infected (26.1 %) with *B. Cameli* (n. sp.). For the first time the parasites were infected camels at Assiut. These parasites were injected in the blood of rat and the changes occurred on the organs were seen. Also Hemoglobin concentration, WBCs, RBCs, hematocrite, MCV, MCH and MCHC were measured for detection the hematological effects of these parasites. This study has reported for the first time the presence of *Th. assiutis* (n. sp.) & *B. Cameli* (n. sp.). in *Camelus dromedarius* and the results can lead to the prevention of babesiosis and theileriosis in the region to increase the livestock output.

Keywords

Babesia,
Theileria,
Slaughter houses,
Theileriosis,
Livestock,
Morphological,
Histopathological

Introduction

Babesiosis is vectored to humans by ticks that are ecto-parasites of rodents (Levine, 1971; Telford *et al.*, 1993). *Babesia microti*, a species of rodent origin, has been recognized as an agent of human babesiosis in the United States (Dammin *et al.*, 1981). Symptoms of the disease appear between 1 to 4 weeks after a person is bitten by infected

ticks. The patient suffers from a gradual onset of malaise, anorexia, fatigue, mild to moderate fever, sweats, and myalgia (Ruebush *et al.*, 1977a,b). On classical methods the recent description of *Theileria youngi* from rodents in California (Kjemtrup *et al.*, 2001), which previously might have been assigned to *B. microti* based on

morphology, serves as an example of the utility of DNA-based methods as a complement to microscopy and life cycle information.

Theileriosis is a protozoan disease in ruminants caused by *Theileria* species transferred from ticks belonging to the family Hyalomma. The disease caused by *T. annulata* is also called tropical theileriosis or Mediterranean coast fever (Gül *et al.*, 2006; Radostits *et al.*, 2006). Theileriosis causes major losses due to high mortality, decreased production, and reproductive problems. An increased risk of secondary infection can occur. While it appears in summer in subtropical regions, it can occur throughout the year in tropical regions (Dumanli *et al.*, 2005; Keleş *et al.*, 2001). The main reason for pathological change in theileriosis is progressive anemia and related disorders (Çiçek *et al.*, 2009; Shiono *et al.*, 2001; Singh *et al.*, 2001; Başbuğ *et al.*, 2011).

The study aims to estimate the histological, hematological and pathological changes induced in organs of rats due to infection with *Babesia cameli* and *Theileria assiutis*.

Materials and Methods

Out of 195 blood samples of camels (*Camelus dromedarius*) examined for blood protozoan parasites collected from different localities of Slaughter houses at Assiut city, Egypt (Dairout, Beni ady, Elethamna). These freshly collected blood samples were divided in two groups one in a tube coated with EDTA, and the other in a test tube for Centrifugation to obtain sera. Thick and thin blood smears were made for morphological examination of *Theileria* parasites. Electron microscopic studies.

TEM

Few drops from blood which is highly infected with *Babesia cameli* and *Theileria assiutis* immediately fixed in 3 ml. of 3% glutaraldehyde solution in phosphate buffer (pH 7.2), for 24 hours and Kept at 4°C in refrigerator. The samples were post fixed in 1% Osmium tetra-oxide in phosphate buffer (pH 7.2, 300 mom), for 30 minutes.

They were washed several times with phosphate buffer solution. The samples were then embedded in Epon which can preserve in structure from distortion during processing then ultra-thin sections were cut by an Ultra microtome and examined by JEOL, 100 CXII operating at 80 KV (TEM).

SEM

For scanning electron microscope of blood; few drops were fixed in 3 % glutaraldehyde in buffer for 24 hours. Specimens were washed three times in Phosphate buffer and post fixed in 1% Osmium tetra-oxide for 2 hours and then washed in the same buffer. They were Dehydrated in different grades of ethyl alcohol and then mounted on special holders and coated with gold. Then they were examined in a JSM-T 200 L.V. 5400 Scanning Electron Microscopy (SEM).

Experimental infection

Group of laboratory animals of twenty white rates were injected with freshly infected blood camels with *Babesia cameli* and *Theileria assiutis* by doses 3 ml blood to estimate the histological, hematological and pathological changes induced in tissue of rats due to infection with *Babesia cameli* and *Theileria assiutis*. Blood examination was performed daily for determine the infection of these laboratory animals.

Histopathology

Small pieces of liver, kidney and lung of the infected rats were fixed in 10% neutral buffered formalin, processed for light microscopic examination, and then 5–7 μm paraffin sections were cut and stained with Hematoxylin and Eosin for histological study.

Results and Discussion

Characteristics of *Babesia cameli* & *Theileria assuitis*

Babesia cameli (n. sp.) was infected the camels in a heavy infection in and outside the red blood corpuscles. The light microscopy was showed that, many different stages in and outside of the red blood corpuscles as, free trophozoite outside of the red blood corpuscles (Fig. 1).

The infection with this parasite sometimes was accompanied by *Theileria assuitis*. *Th. assuitis* was a cigarette or elongated in shaped and measured (6.11 \times 1.6 μm). The parasite was infected the camels (*Camelus dromedarius*) for the first time in Assiut locality in and outside the red blood corpuscles.

Light microscopy showed that, defferent developmental stages of these parasites (Figs 2–4) and erythrocytes contained theilerial piroplasms in different shapes including cocci and rods (Fig. 5 & 6), respectively.

Scanning electron microscopy showed that, *B. cameli*. with a tubular structure which in was contact with the erythrocyte (Fig. 7) and clefts in the infected blood corpuscles (Fig. 8) due to its infected by *B. cameli*. Presence of *Th. assuitis* outside and in attached with the red blood corpuscles (Fig. 9).

Transmission electron microscopy revealed that, different developmental stages of *B. cameli* were found, rounded or oval shaped trophozoites measured (1.58 \times 1.25 μm) in diameter. The body was covered by a single membrane; one prominent nucleus was observed and measured (1 \times 0.41 μm) in diameter, well developed rough endoplasmic reticulum, some vacuoles and numerous free ribosomes (Fig. 10). TEM also, revealed that, piroplasms and veils inside the red blood corpuscles (Figs 11 & 12), respectively.

Animal injection

Twenty (20) rats were injected with *Babesia cameli* and *Theileria assuitis* in the blood with three doses at different times and observed for hundred (100) days. They were checked daily for clinical symptoms which refer to babesiosis and theileriosis infection. Out of the twenty rats, only seven showed loss of weight, eye reddish colour and diarrhea and died in the fourth day of the infection of the third dose and the others continued alive at the end of the test.

Histopathological changes in rat organs

The liver, kidney and lung of infected rat undergo alterations in response to blood-stage *B. cameli* & *Th. assuitis*. At maximum parasitemia, the liver, kidney and lung became dark-brown and extremely friable, and it was highly edematous with largely dilated. Sinusoids were enriched with macrophages and parasite-containing erythrocytes (Figs 13–14). The Kupffer cells are enlarged, and occasionally appear to be in the process of phagocytosis of *B. cameli* & *Th. assuitis* infected erythrocytes (Fig. 15). Moreover, there is always a strong inflammation in the liver. The lobular inflammation is characterized by predominant infiltrations of lymphocytes,

plasma cells, and histiocytes, which are localized in perivascular and parenchymal areas (Fig. 16). Besides that, the hematological effects of these parasites such as Also Hemoglobin concentration, WBCs, RBCs, hematocrite, MCV, MCH and MCHC were encountered and showed clear effects on the infected animals as in table 1.

From the table 1 showed that, RBCs, Hb and Hct were increased in the healthy animals and decreased with the presence of blood parasites. An increase of WBCs in diseased camels and experimental rats indicates that, these WBCs from the first against infection and their number increases in the early phases of infectious diseases. MCV and MCH were increased in normal (healthy)

animals and decreased in infected one but MCHC don't change which was represented the similar in both infected and healthy animals.

Rats infected with blood-stage parasites are characterized by parasitemia sometimes exceeding 35% of infected erythrocytes and an acute inflammatory response. Animals induce immune complex-mediated hepatic tissue lesions similar to those in *Falciparum malaria* (Krucken *et al.*, 2009; Wozniak *et al.*, 1999). *Babesia* & *Theileria* parasites, like malaria parasites, invade erythrocytes of infected animals, resulting in the destruction of parasitized erythrocytes (Otsuka *et al.*, 2002).

Table.1 Comparative some of the hematological parameters in healthy and hemoparasitized camels (*Camelus dromedarius*) and experimental infected rats.

Parameter	Healthy camels	Diseased camels	Healthy rats	Diseased rats
Hb (g/dl)	15.7 - 18.45	8.31 - 15.59	8.50 - 9.54	6.20 - 7.97
RBCs (10 ⁶ /ml)	6.99 - 9.66	4.57 - 6.87	4.50 - 5.50	3.50 - 4.03
WBCs (10 ³ /ml)	3.10 – 4.85	5.90 – 18.85	2.5 – 2.85	2.95 – 3.30
Hct (%)	46.1 - 54.35	23.93 - 45.77	23 - 24.50	18.5 - 19.77
MCH (PG)	25.29-35.14	11.9 - 23.77	20 - 22.5	33.5 - 34.78
MCHC (%)	33.94 -34.72	33.8 - 34.5	33.5 - 34.5	30.7 – 56.8
MCV (Fl)	74.45-103.28	33.64 – 69.81	60.3 – 70.73	30.70 – 56.80

Fig.1 Photomicrograph showing trophozoite of *Babesia sp.* (B) outside of the red blood cells of *Camelus dromedarius* stain with Geimsa stain. X bar = 20 μ m

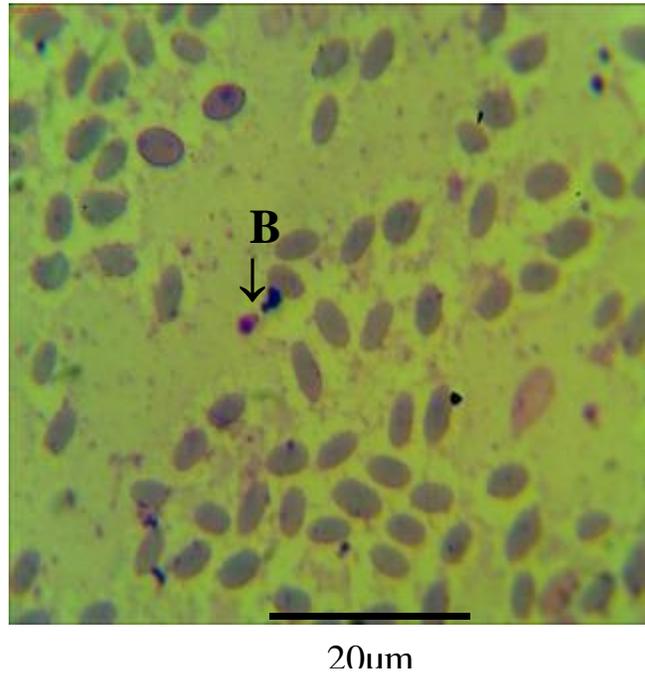


Fig.2 Photomicrograph showing ray body (Rb) of *B. sp.* In the red blood cells of *Camelus dromedarius* stain with Geimsa stain. X bar = 20 μ m

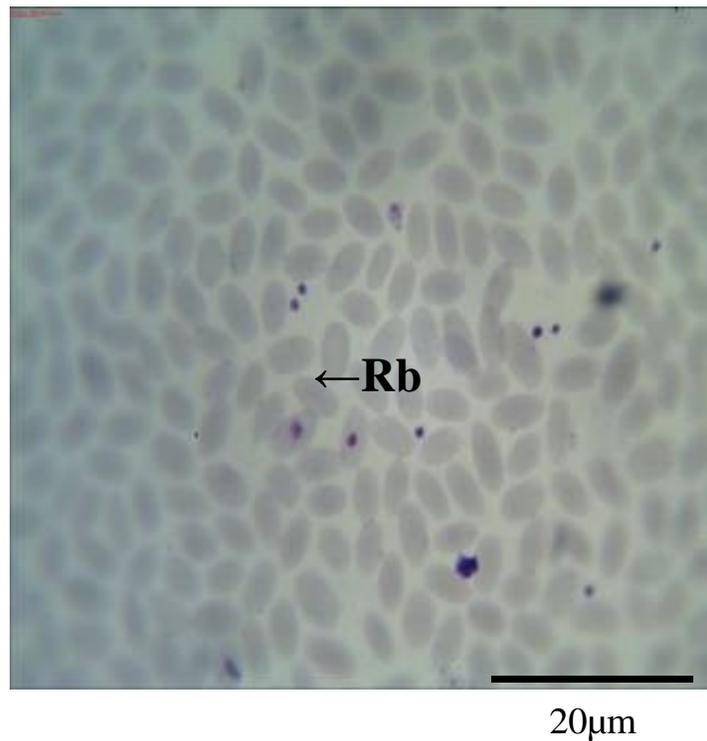


Fig.3 Photomicrograph showing kinete of *B. sp.* in the red blood cells of *Camelus dromedarius* stained with Geimsa stain. X bar = 20 μ m

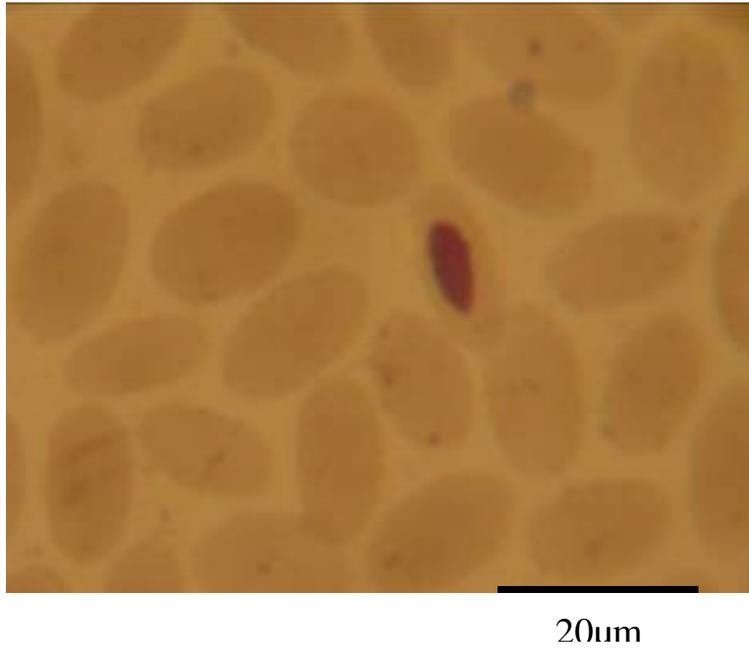


Fig.4 Photomicrograph showing many trophozoites (arrows) of *Th. sp.* (T) in the blood of *Camelus dromedarius* stained with Geimsa stain. X bar = 20 μ m

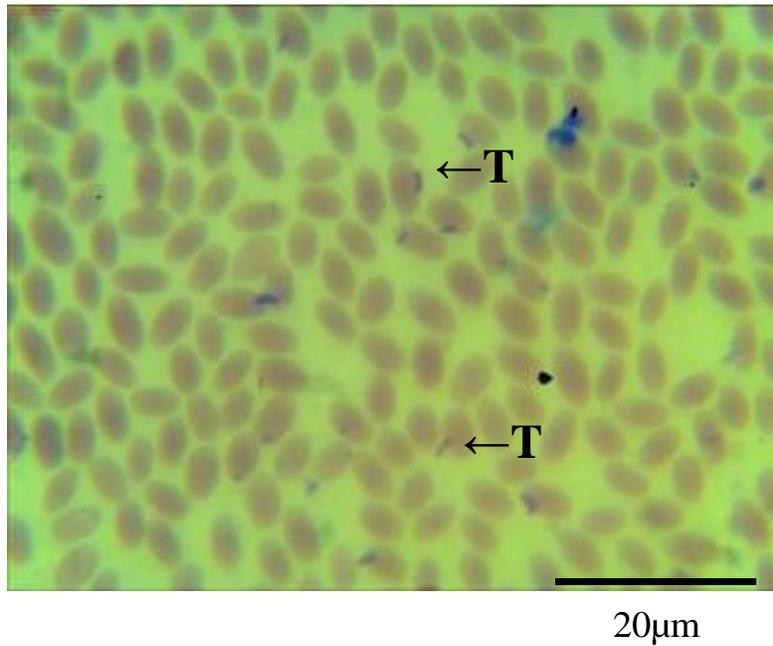
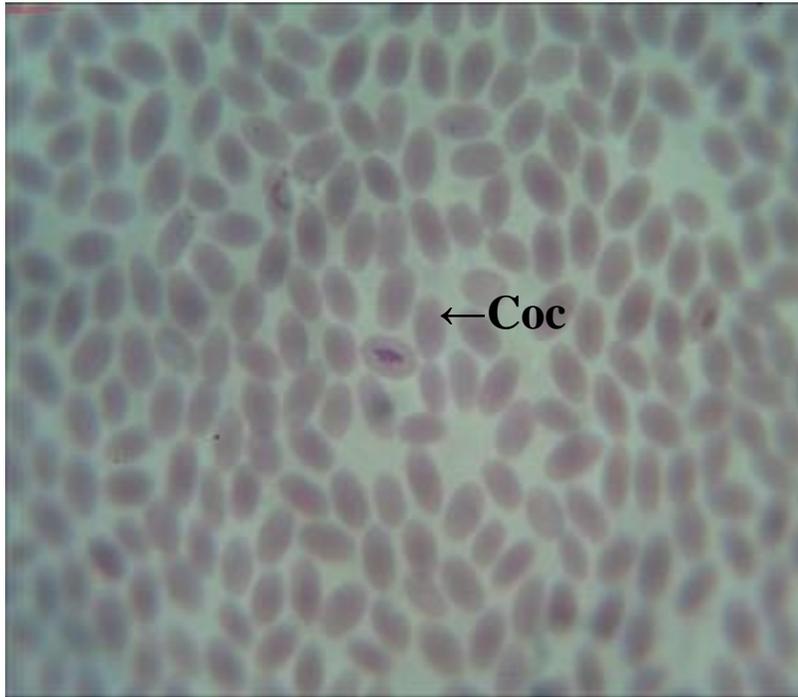
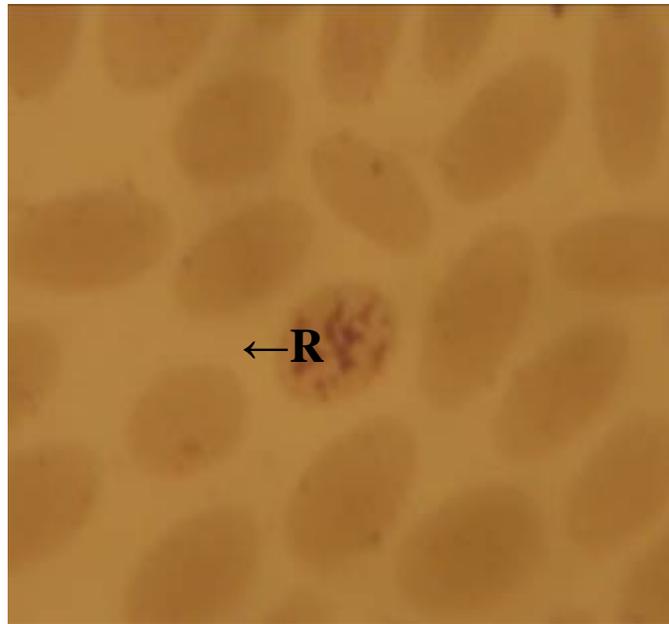


Fig.5 Photomicrograph showing cocci piroplasms of *Th. cameli* in the blood of *Camelus dromedarius* stained with Geimsa stain. X bar = 20 μ m



20 μ m

Fig.6 Photomicrograph showing rod (R) piroplasm of *Th. cameli*. in the blood of *Camelus dromedarius* stained with Geimsa stain. X bar = 20 μ m



20 μ m

Fig.7 Scanning electron micrograph of red blood cells of *Camelus dromedarius* showing tubular structure (TS) of *B. Sp*

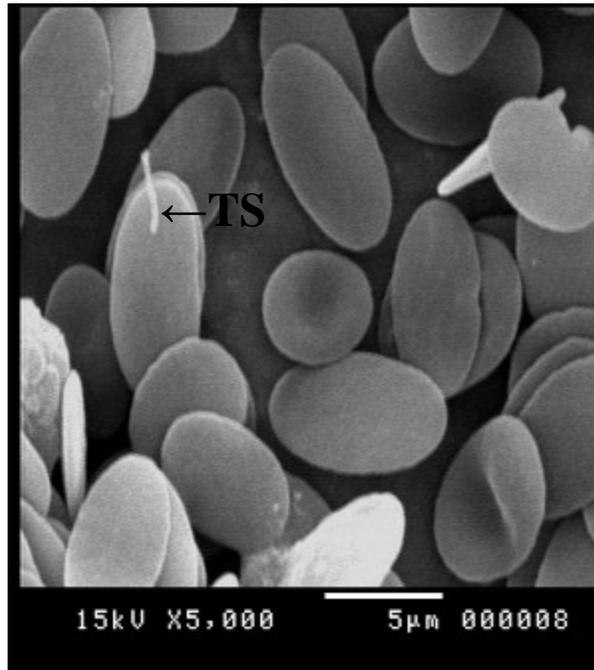


Fig.8 Scanning electron micrograph of red blood cells of *Camelus dromedarius* showing clefts in RBCs due to its infected with *B. Sp*

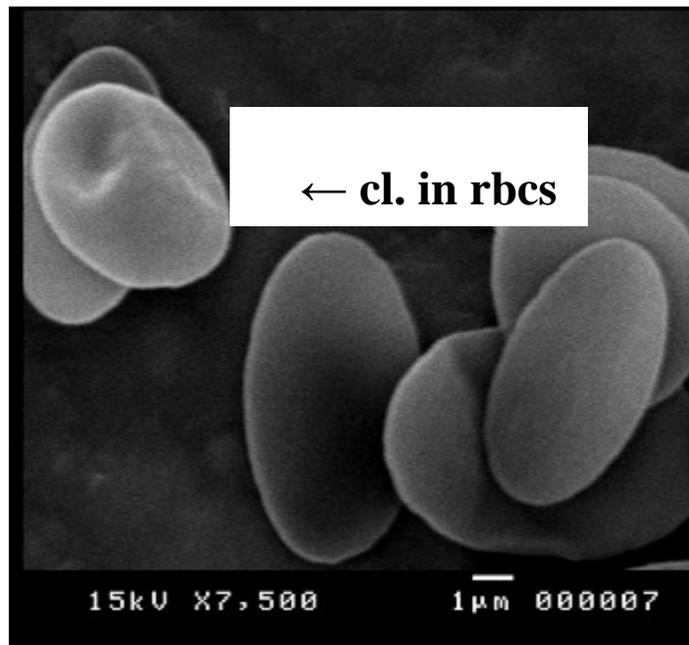


Fig.9 Scanning electron micrograph of cigarette shape *Theileria cameli* in the blood of *Camelus dromedaries*

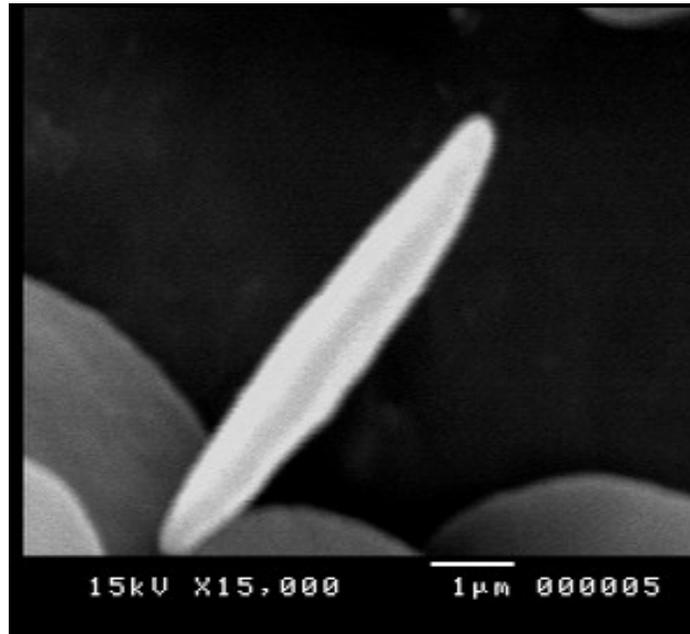


Fig.10 Transmission electron micrograph of *Babesia sp.* showing cytosome (c), rough endoplasmic reticulum (RER) and vacuoles (V)

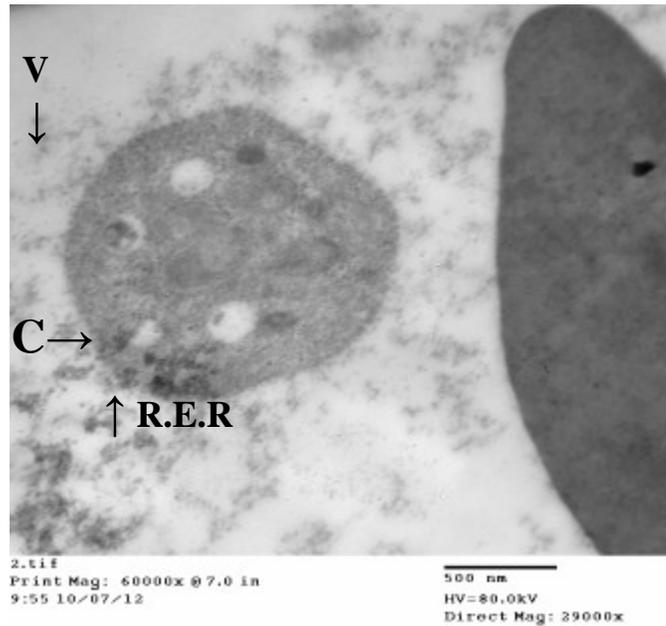


Fig.11 Transmission electron micrograph of piroplasm (P) in the red blood cells of *Camelus dromedarius* due to its infected with *Th. Cameli*.

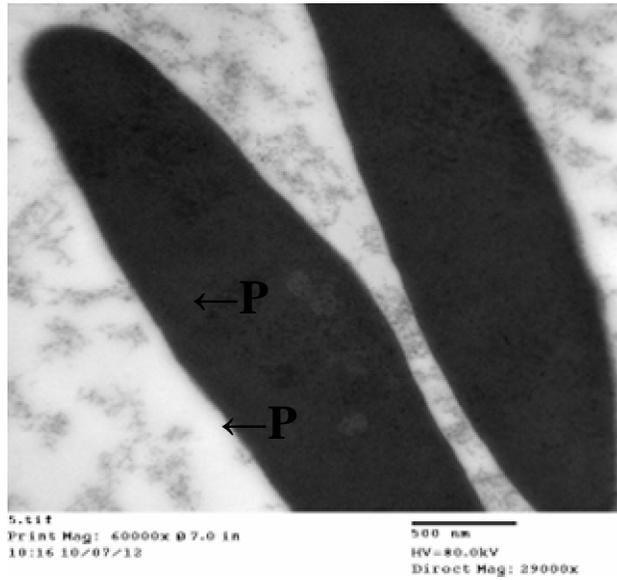


Fig.12 Transmission electron micrograph of veils (arrows) in the red blood cells of *Camelus dromedarius* due to its infection with *Th. cameli*

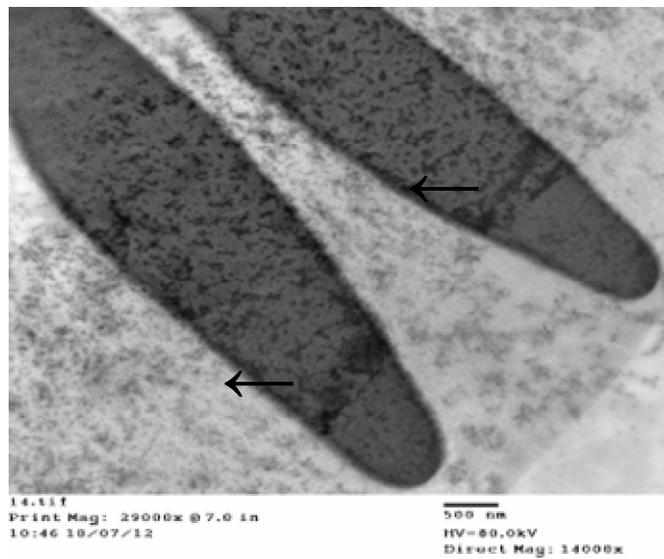
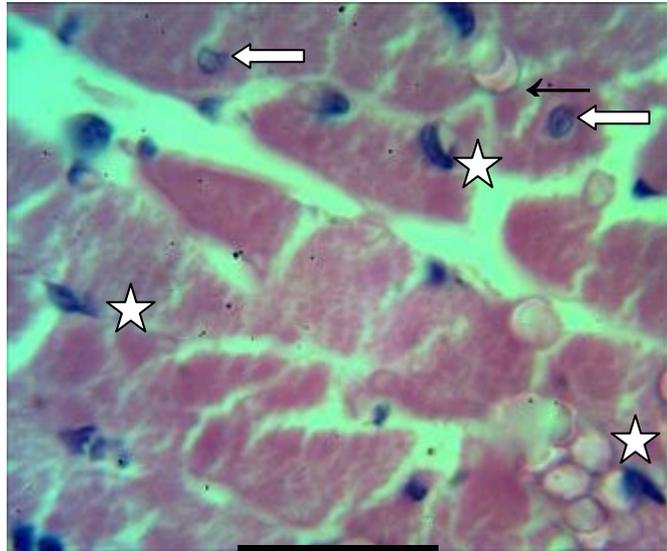
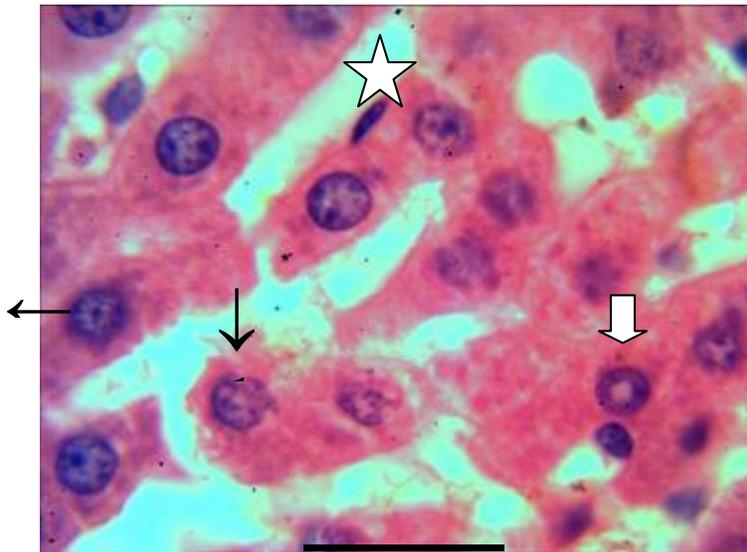


Fig.13 Photomicrograph showing changes in the kidney histology of the infected rats stained with Eosin & Hematoxylin, Erythrocytes (black arrows), *Babesia cameli*-parasitized erythrocyte (stars) and Kupffer cells (White arrows) X bar = 20 μ m



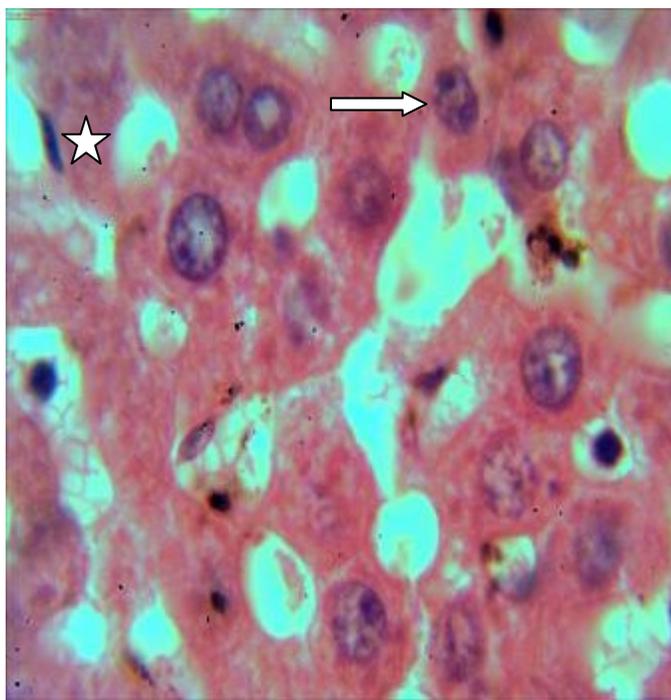
20um

Fig.14 Photomicrograph showing changes in the liver histology of the infected rats stained with Eosin & Hematoxylin, *Theileria assuitis* (star), Vacuolation in the liver cells of the infected rat (white arrow) and ruptured in the liver cells (black arrows). X bar = 20 μ m



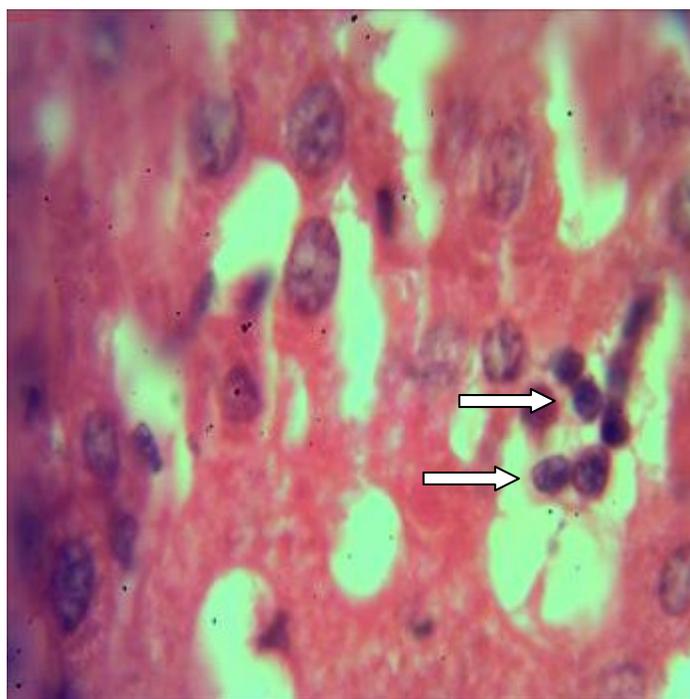
20um

Fig.15 Photomicrograph showing changes in the liver histology of the infected rats stained with Eosin & Hematoxylin, the process of phagocytosis of *B. cameli* (white arrow) and *Theileria assuitis* (star). X bar = 20 μ m



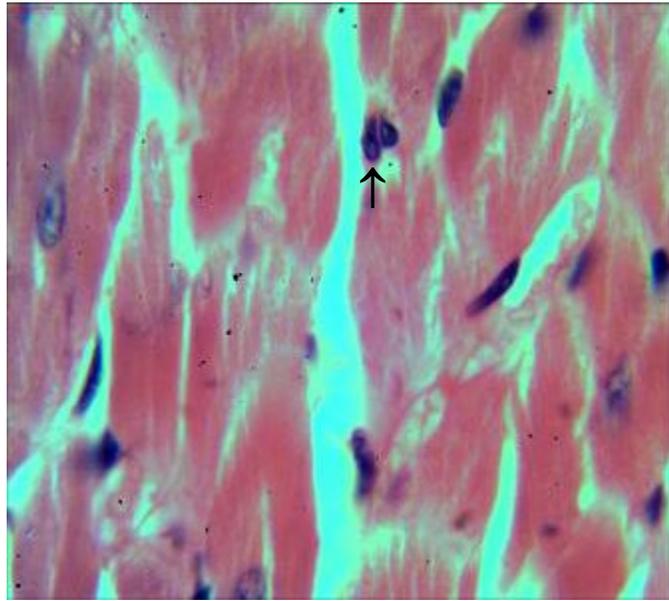
20 μ m

Fig.16 Photomicrograph showing changes in the liver histology of the infected rats stained with Eosin & Hematoxylin, the lobular inflammation which are localized in perivascular and parenchymal areas (white arrows). X bar = 20 μ m



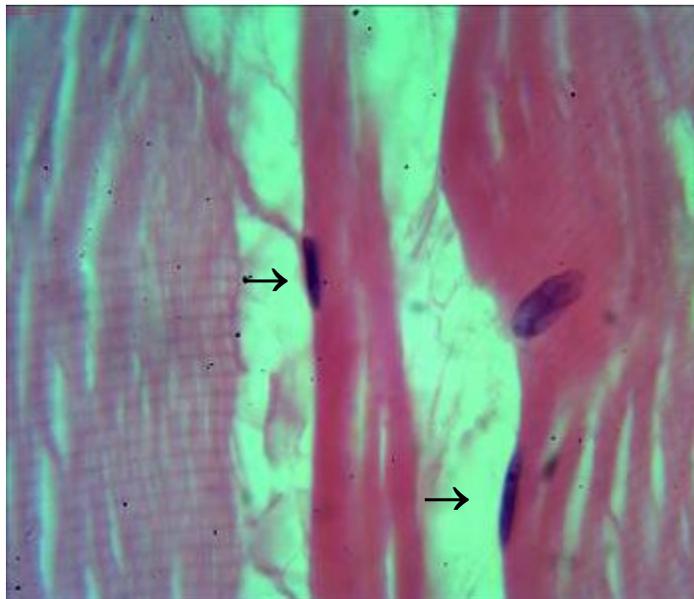
20 μ m

Fig.17 Photomicrograph showing two trophozoites of *Babesia Sp.* (arrow) in the kidney of the infected rats stained with Eosin & Hematoxylin. X bar = 20 μ m



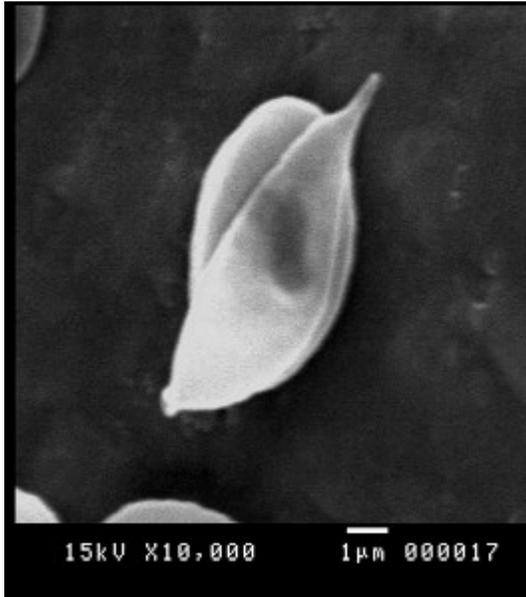
20um

Fig.18 Photomicrograph showing *Theileria assuitis* in the lung of the infected rats stained with Eosin & Hematoxylin. X bar = 20 μ m

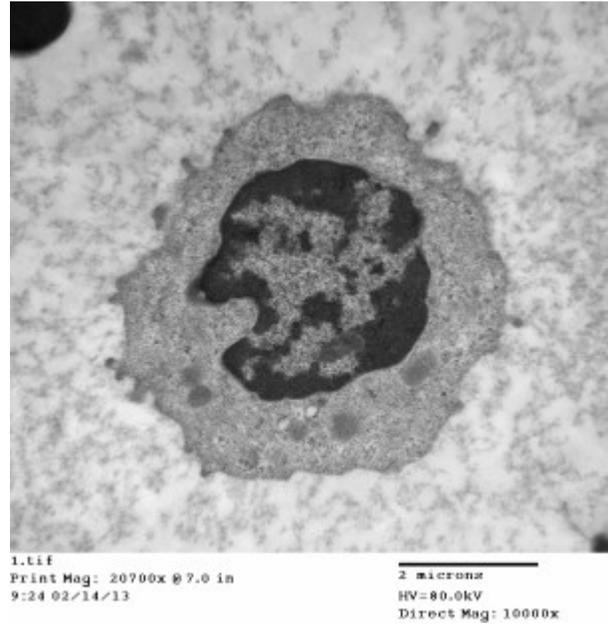


20um

Theileria sp.



Babesia sp



Camelus dromedarius infected with *Theileria* and *Babesia*



The liver plays a central role in babesiosis: it is known as the site where the pre-erythrocytic stages of *Babesia* & *Theileria* parasites asexually multiply and where host immune mechanisms develop to fight these pre-erythrocytic stages (Cohen and Lambert, 1982).

Hyperplasia of Kupffer cells were detected in liver and kidney sections of infected rats, this is due to the need of phagocytosis as a protective mechanism during the course of infection, and this result was in agreement with (Otsuka *et al.*, 2002) where they studied the immune response of gerbils due to *Babesia rodhaini* infection.

Hepatic tissue showed cytoplasmic vacuolation which is mainly a consequence of considerable disturbances in lipid inclusions and fat metabolism occurring under pathological cases (Zhang *et al.*, 1984; El-Banhawy *et al.*, 1993). The decrease in erythrocytic count, hemoglobin, hematocrit and increase in leukocyte counts might be due to severity of infection. Although anemia is the major symptom and cause of mortality in animals with *Babesia* & *Theileria* infection, the pathogenesis of the anemia remains unclear. In general, *Babesia* & *Theileria* parasites, like *malaria* parasites, invade erythrocytes of infected animals, resulting in the destruction of the parasitized erythrocytes (Kawamura *et al.*, 1987). The results of the present study indicated that the course of the infection induced by *B. cameli* & *Th. assuitis* might be determined by alterations in clinical, hematological and pathological findings.

It was concluded that body condition were very poor due to fever and anorexia that usually occur as a common clinical findings in animals infected with theileriosis and babesiosis. However enlargement of lymph nodes and corneal opacity are associated clinical findings with theileriosis, while pale mucous membranes are associated clinical findings with babesiosis. Normocytic hypochromic anemia is associated with theileriosis, while normocytic normochromic anemia is associated with babesiosis. Phagocytic cells lymphocytes are commonly increased in *Theileria* and *Babesia* infected animals. Theileriosis and babesiosis have harmful effect on the liver function in animals.

Acknowledgements

Thanks for Zoology Department – Faculty of Science – Unit of Electron Microscopy in Assiut University for supplying us by

systems and chemicals to complete the work.

References

- Başbuğ, O., Gül, Y. 2011. Tropikal tayleriyozisli sığırlarda hemoliz üzerine araştırmalar. *Kakas Univ. Vet. Fak. Derg.*, 17: 421–427 (article in Turkish with an English abstract).
- Çiçek, H., Eser, M., Tandoğan, M. 2009. Current status of ruminant theileriosis and its economical impact in Turkey. *Türkiye Parazitol. Derg.*, 33: 273–279.
- Cohen, S., Lambert, P.H. 1982. Immunology of parasitic infections, 2nd edn. Blackwell Scientific Publications, Oxford. Pp. 422–474.
- Dammin, G.J.A., Spielman, J.L., Benach, J., Piesman, J. 1981. The rising incidence of clinical *Babesia microti* infection. *Hum. Pathol.*, 12: 398–400.
- Dumanli, N., Aktas, M., Cetinkaya, B., Cakmak, A., Koroglu, E., Saki, C., Erdogmus, Z., Nalbantoglu, S., Ongor, E., Simşek, S. *et al.* 2005. Prevalence and distribution of tropical theileriosis in eastern Turkey. *Vet. Parasitol.*, 127: 9–15.
- El-Banhawy, M.A., Sanad, S.M., Sakr, S.A., El-Elaimy, I.A., Mahran, H.A. 1993. Histopathological studies on the effect of the anticoagulant rodenticide “Brodifacoum” on the liver of rat. *J. Egypt Ger. Soc. Zool.*, 12: 185–227.
- Gül, Y. Dolaşım sistemi ve kan hastalıkları. In: Gül, Y. (Ed). Geviş Getiren Hayvanların İç Hastalıkları, 2nd edn. Medipres Ltd. Şti., Malatya, Turkey. Pp. 241–299 (in Turkish).
- Kawamura, M., Maede, Y., Namioka, S. 1987. Mitogenic responsiveness of lymphocytes in canine babesiosis and

- the effects of splenectomy on it. *Jpn. Vet. Res.*, 35: 1–10.
- Keleş, İ., Değer, S., Altuğ, N., Karaca, M., Akdemir, C. 2001. Tick-borne diseases in cattle: clinical and haematological findings, diagnosis, treatment, seasonal distribution, breed, sex and age factors and the transmitters of the diseases. *Y.Y.U. Vet. Fak. Derg.*, 12: 26–32.
- Kjemtrup, A.M., Robinson, T., Conrad, P.A. 2001. Description and epidemiology of *Theileria youngi* n. sp from a northern Californian dusky-footed woodrat (*Neotoma fuscipes*) population. *J. Parasitol.*, 87: 373–378.
- Krucken, J., Delic, D., Pauen, H., Woytalla, A., El-Khadragy, M., Dkhil, M.A., Mossmann, H., Wunderlich, F., 2009. Augmented particle trapping and attenuated inflammation in the liver by protective vaccination against *Plasmodium chabaudi malaria*. *Malar. J.*, 8: 54–67.
- Levine, N.D. 1971. Taxonomy of the piroplasms. *Trans. Am. Microsc. Soc.*, 90: 2–33.
- Otsuka, Y., Amasaki, M., Yamato, O., Maede, Y. 2002. The effect of macrophages on the erythrocyte oxidative damage and the pathogenesis of anemia in *Babesia gibsoni*-infected dogs with low parasitemia. *J. Vet. Med. Sci.*, 64: 221–226.
- Radostits, O., Gay, C., Hinchclif, K. 2006. Constable, PD. Veterinary medicine: A textbook of the diseases of cattle, sheep, goats, pigs and horses, 10th edn. WB Saunders Company, London, UK.
- Ruebush, T.K., Cassaday, P.B., Marsh, H.J., Lisker, S.A., Human, D.S., Voorhees, A.B., Mahoney, E.B., Healy, G.R. 1977b. Babesiosis on Nantucket Island. *Clin. Features. Ann. Intern. Med.*, 86: 6–9.
- Ruebush, T.K., Juranek, D.D., Chisholm, E.S., Snow, P.C., Healy, G.R., Sulzer, A.J. 1977a. Human babesiosis on Nantucket Island, evidence for self limited and subclinical infections. *N. Engl. J. Med.*, 297: 825–827
- Shiono, H., Yagi, Y., hongnoon, P., Kurabayashi, N., Chikayama, Y., Miyazaki, S., Nakamura, I. 2001. Acquired methemoglobinemia in anemic cattle infected with *Theileria sergenti*. *Vet. Parasitol.*, 102: 45–51.
- Singh, A., Singh, J., Grewal, A., Brar, S. 2001. Studies on some blood parameters of crossbred calves with experimental *Theileria annulata* infections. *Vet. Res. Commun.*, 25: 289–300.
- Telford, S.R., Gorenflot, A., Brasseur, P., Spielman, A. 1993. Babesial infections in humans and wildlife. In: J. P. Kreier (Ed.), Parasitic protozoa, 2nd Edn., Vol. 5. Academic Press, Inc., New York, N.Y.
- Wozniak, E.J., Barr, B.C., Thomford, J.W., Yamane, I., McDonough, S.P., Moore, P.F., Naydan, D., Robinson, T.W., Conrad, P.A. 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*Canis familiaris*). *J. Parasitol.*, 83: 692–699.
- Zhang, L.Y., Wang, C.X. 1984. Histopathological and histochemical studies on the toxic effect of brodifacoum in mouse liver. *Acta Acad. Med. Sci.*, 6: 386–388.