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MICROBIOLOGY
Research Article

Theileria assiutis n. sp. Infecting *Camelus dromedarius* at Assiut locality, Upper Egypt

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Abstract

One hundred and ninety five (195) Camelus dromedarius were examined only twelve were infected with Th. assiutis (n. sp.). For the first time the parasite was infected camels at Assiut. Light microscopy was showed that different shapes of piroplasms in red blood cells of camelus dromedarius sush as, basophilic stippling, howell-jolly bodies and signet-ring. Transmission and Scanning electron microscopy were revealed that, many pores, clefts and veils inside the red blood corpuscles resulted in the infection with the new parasite.

Experimental infection showing that, Theileria assiutis (n. sp.) was found to be transmissible to laboratory white rates which were appeared in the serial sections of the rat infected organs which stained with eosin and hematoxylen stain.

Keywords: *Theileria Assiutis; Piroplasms; Basophilic; Howell-jolly; Signet-ring; Clefts and veils*

Introduction

Theileria sp. is considered to be benign or less pathogenic probably because of a long evolutionary relationship between the parasite and the host. Nevertheless, clinical disease may occur in stressful situations related to translocation of wildlife or/and when the host is debilitated by other parasitic organisms or malnutrition [1-3]. 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 [4-6].

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 [7,8]. The main reason for pathological change in theileriosis is progressive anemia and related disorders [9-12]. Increased fragility and oxidative damage in erythrocytes, damage to erythrocytes by parasites in the reticuloendothelial system, autoimmune reactions, and intra erythrocytic piroplasms contribute to the development of anemia [13].

The mortality observed in acute cases is directly related to the anemia process and the hematocrit (Hct) value can decrease below 10% within 4-5 days after the beginning of parasitemia in such cases [14]. The clinical and biochemical changes observed in this disease are related to degree of anemia, the degree of parasitemia, and the severity of hypoxia [15], so that the present work aims to study the new parasite with light and electron microscopy and to elucidate for its zoonotic importance through the infectious rats.

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 some protozoan blood parasites. Electron microscopic studies.

TEM

Few drops from blood which is highly infected with Theileria assiutis (n. sp.) 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 tetroxide in phosphate buffer (pH 7.2, 300 mm), 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 tetroxide 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

One group of laboratory animals representing in five white rates were injected with freshly infected blood camels with Theileria assiutis (n. sp.) by doses 3 ml blood to elucidate the zoonotic importance for this parasites. Blood examination was performed daily for determine the infection of these laboratory animals.

Results

Th. assiutis was a cigarette or elongated in shaped and measured (6.11 × 1.6 µm). The parasite was infected the camels (Camelus dromedarius) for the first time at Assiut locality in and outside the red blood corpuscles.

Light microscopy showed that, deferent developmental stages of the parasite Figures (1-3) and erythrocytes contained theilerial piroplasms in different shapes including cocci, rods, fusiform, signet-ring and pear shaped forms Figures (4-8) in respectively. Other erythrocytes abnormalities including spherocytosis Figure 9, acanthocytosis Figure 10, basophilic stippling Figure 11, Howell-jolly bodies Figure 12 and macrocytosis Figure 13 were also seen.

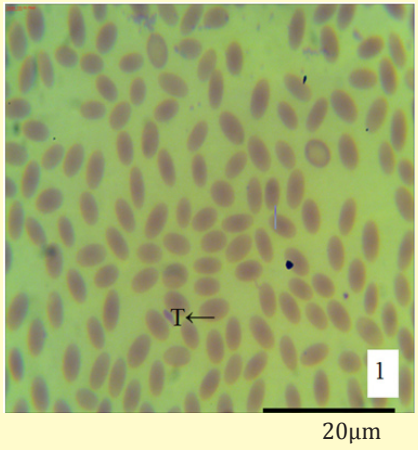


Figure 1: *Photomicrograph showing trophozoite of Th. assiutis. (T) in the blood of Camelus dromedarius stained with Geimsa stain.*

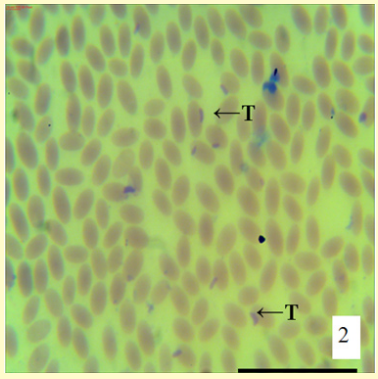


Figure 2: *Photomicrograph showing many trophozoites (arrows) of Th. assiutis. (T) in the blood of Camelus dromedarius stained with Geimsa stain.*

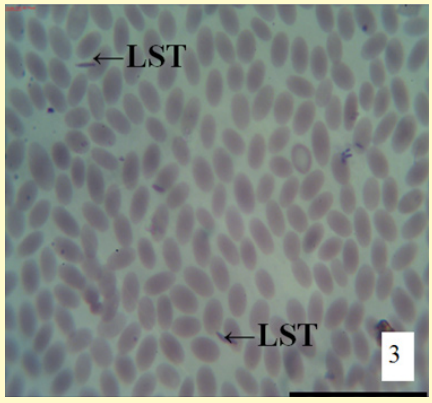


Figure 3: *Photomicrograph showing two large solitary trophozoites of Th. assiutis (LST) invades the RBCs of Camelus dromedarius stained with Geimsa stain.*

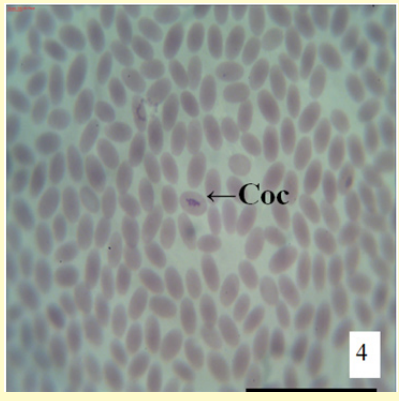
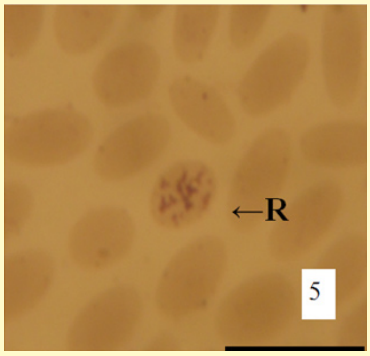
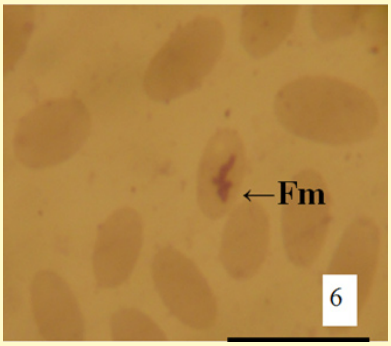


Figure 4: *Photomicrograph showing cocci piroplasms of Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*



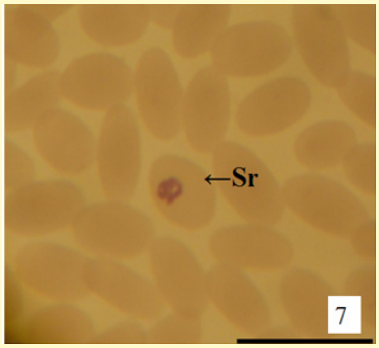
20µm

Figure 5: *Photomicrograph showing rod (R) piroplasm of Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*



20µm

Figure 6: *Photomicrograph showing fusiform (Fm) Piroplasm Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*



20µm

Figure 7: *Photomicrograph showing signet-ring (Sr) piroplasm of Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*

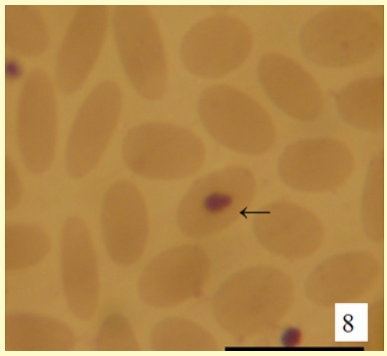


Figure 8: *Photomicrograph showing pear shaped piroplasm of Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*

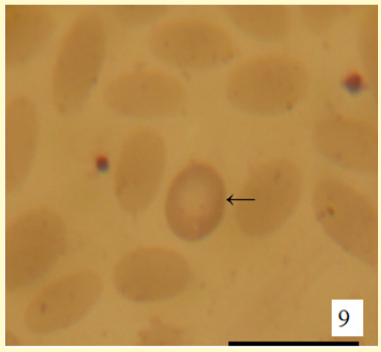


Figure 9: *Photomicrograph showing spherocytosis (S) red blood cell due to infection with Th. assiutis in the blood of Camelus dromedarius stained with Geimsa.*

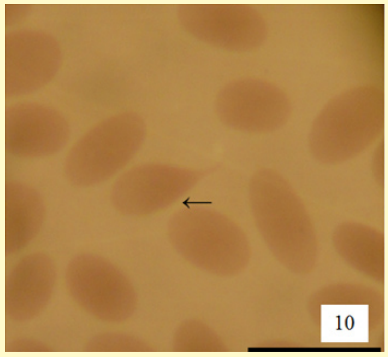
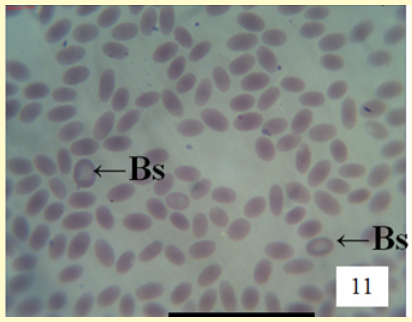
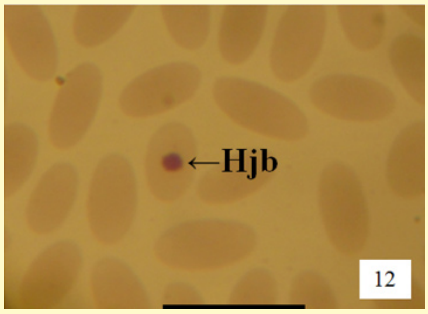


Figure 10: *Photomicrograph showing acanthocytosis (ac) red blood cell due to infection with Th. assiutis in the blood of Camelus dromedarius stained with Geimsa stain.*



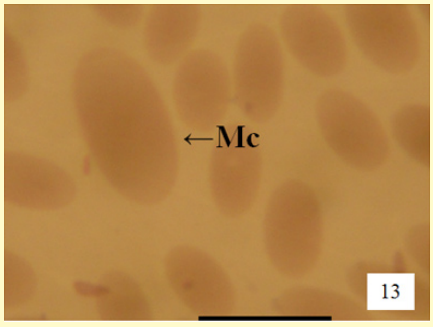
20µm

Figure 11: *Photomicrograph showing basophilic stippling (bs) red blood cell due to infection with Th. assiutis in the blood of Camelus dromedarius stained with Geimsa.*



20µm

Figure 12: *Photomicrograph showing Howell-jolly body (Hjb) due to infection with Th. assiutis in the blood of Camelus dromedarius stained with Geimsa.*



20µm

Figure 13: *Photomicrograph showing macrocytosis (Mc) in red blood cell due to infection with Th. assiutis in the blood of Camelus dromedarius stained with Geimsa.*

Transmission electron microscopy (TEM) revealed that, piroplasms and veils inside the red blood corpuscles Figures (14 and 15) respectively.

Scanning electron microscopy (SEM) was supported the previous stages and was revealed that, presence of Th. sp. outside and in attached with the red blood corpuscles (Figures 16 and 17).

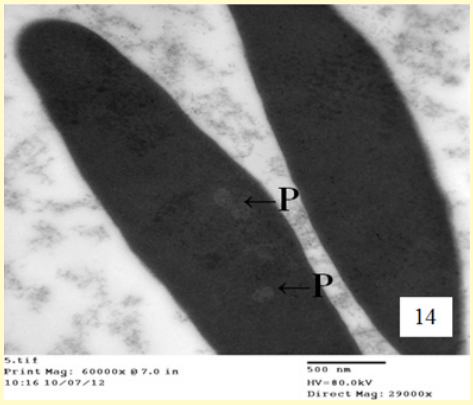


Figure 14: *Transmission electron micrograph of piroplasm (P) in the red blood cells of Camelus dromedarius due to its infected with Th. assiutis.*

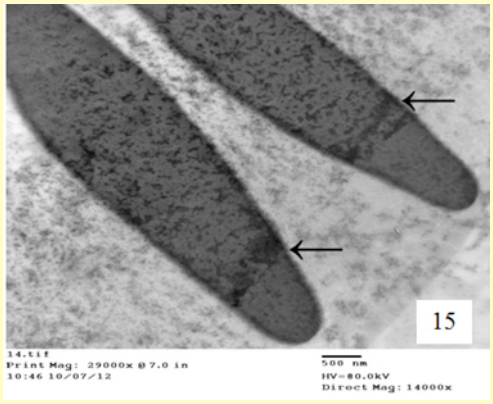


Figure 15: *Transmission electron micrograph of veils (arrows) in the red blood cells of Camelus dromedarius due to its infected with Th. assiutis.*

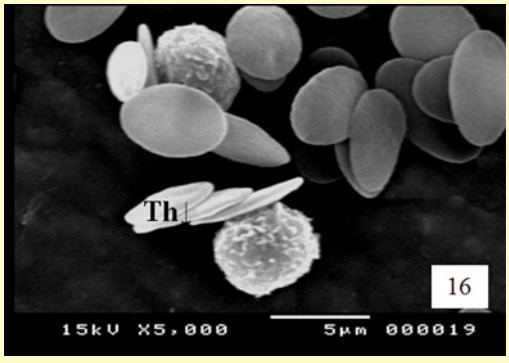


Figure 16: *Scanning electron micrograph of Theileria assiutis (Th) in the blood of Camelus dromedarius.*

In experimental infection, Th. assiutis (n. sp.) was found to be transmissible to laboratory white rats, cigarette shaped trophozoite after 74 days of the infection appears in the blood and in the liver of the infectious rat respectively as in Figure (18 and 19) and this elucidate that the zoonotic importance for the new parasite, in addition to that it's probably to infected other animals especially humans.

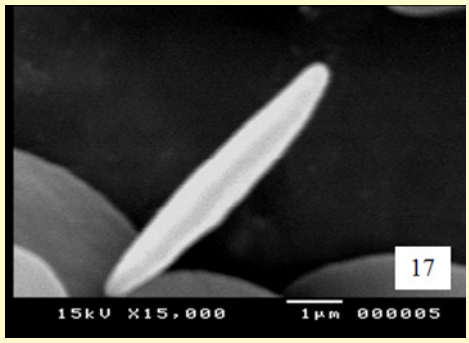


Figure 17: *Scanning electron micrograph of cigarette shape Theileria assiutis in the blood of Camelus dromedarius.*

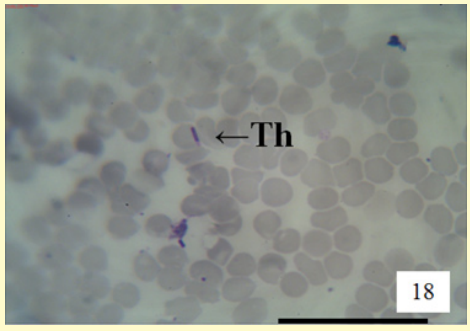


Figure 18: *Photomicrograph showing cigarette shaped Theileria assiutis (Th) in the blood of white rat after 74 day post- infection stained with Geimsa.*

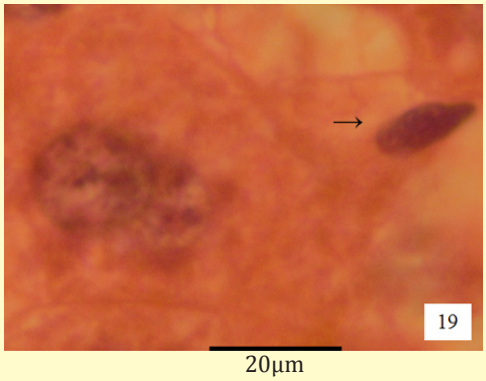


Figure 19: *Enlarged photomicrograph showing Theileria assiutis (Th) in the liver of white rat after 74 day post- infection stained with H & E.*

Discussion

Taxonomic classification of Theileria sp. was based on microscopic appearance of intra erythrocytic piroplasms, geographical location of the infected animal, apparent pathogenicity of the organism and serologic testing [16,17]. Most theilerial organisms of cattle that had low pathogenicity were called Th. mutans [18].

Th. assiutis in the present study was infected camels for the first time in Egypt. Some Th. sp when were isolated and studied, some of these organisms were reclassified by [19], to be Th. mutans which was renamed as Th. oriental is in 1985 [20]. This author also considered some isolates of Th. oriental is to be the same stocks of Th. sergenti. The name given to a more pathogenic species found in Southeastern Asia.

So that, the presence of basophilic stippling indicated a regenerative response consistent with a hemolytic anemia. At the same time, in the present species a combination between both of sphearocytosis and acanthocytosis was indicated that, intravascular erythrocyte fragmentation. Spherocytosis can also be formed by immune mediated processes. There is no evidence of blood loss, but there was a hypoprotienemia. For thus, for the first time in Egypt theilerial infection in camels (*Camelus dromedarius*) occurs. Also intra erythrocytic piroplasms were highly pleomorphic, and rod forms were most common. So that, throughout the present study suggesting that this species of Theileria which was infected Camelus dromedarius for the first time in Egypt called Theileria assiutis as a new species in camels at Assiut locality, Egypt.

Host:	*Camelus dromedarius*
Habitat:	Blood of camels.
Measurement:	(6.11 × 1.6 µm).
Locality:	Assiut, Egypt.
Parasite:	*Theileria assiutis* (n. sp).

Conclusion

Theileria assiutis (n. sp.) is a new parasite infecting camels (*Camelus dromedarius*) at Assiut locality for the first time. This parasite was infecting camels due to their harboring with different animals such as goats, sheep's and cattle's. The parasite has a zoonotic importance in which was an infectious to experimental animal.

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