

Light and Electron Microscopically Study of *Babesia cameli* (n. sp.) Infecting Camels (*Camelus dromedarius*)

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Abstract

Fifty one were found to be infected (26.1%) with *B. cameli* (n. sp.) from one hundred and ninety five (195) from blood samples of *Camelus dromedarius* examined. The light microscopy was showed that, many different stages as, ray body.

Transmission and Scanning electron microscopy were revealed that, different developmental stages of *B. sp.* were found and four different forms from the new parasite were distinguished according to the presence of abundant cytoplasmic organelles and osmiophilic bodies. In experimental infection, the new parasite of *Babesia cameli* was found to be transmissible to laboratory white mice.

Keywords: *Babesia cameli*; Ray body; Cytoplasmic organelles; Osmiophilic bodies.

1. Introduction

Babesiosis is vectored to humans by ticks that are ecto-parasites of rodents [1, 2]. *Babesia microti*, a species of rodent origin, has been recognized as an agent of human babesiosis in the United States [3]. 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 [4, 5] on classical methods. The recent description of *Theileria youngi* from rodents in California [6], 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.

Diseases transmitted by ticks are of major importance to human and animals, particularly to wildlife animals. A wide variety of tick-borne diseases caused by rickettsias, arboviruses, protozoan and bacteria has been described in domestic animals [7]. Therefore, the identification of pathogens in ticks is the first step to understand the epidemiology and transmission of tick-borne diseases. *Babesia cameli*(n. sp.) was infected *Camelus dromedarius* for the first time in Egypt through the present study so that; the present work aims to describe the different forms by both light and electron microscopy and to elucidate the zoonotic importance of the new parasite on the experimental animals (white rats).

2. Material & 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.

2.1 TEM

Few drops from blood which is highly infected with *Babesia* 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 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).

2.2 SEM

For scanning electron microscope of blood; few drops were fixed in 3 % Glutaraldehyde in buffer for 24

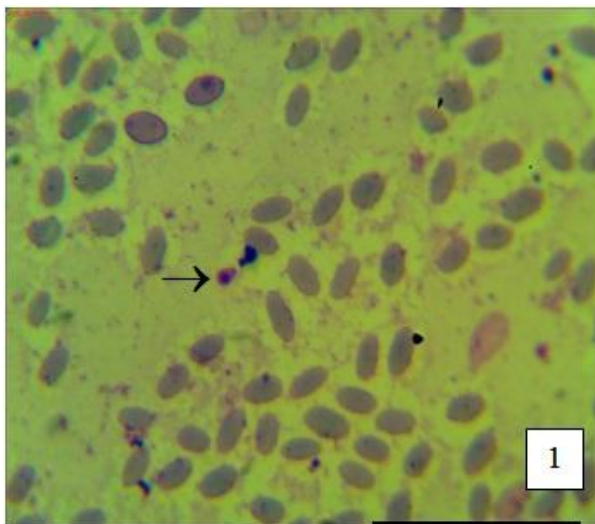
hours. Specimens were washed three times in Phosphate buffer and post fixed in 1% Osmium tetroxide for 2 hours and then washed in the same buffer. They were Dehydrated in different grades of ethyl alcohol and then mounted on special holders and coated with gold. Then they were examined in a JSM-T 200 L.V. 5400 Scanning Electron Microscopy (SEM).

2.3 Experimental Infection

One group of laboratory animals representing in five white rates were injected with freshly infected blood camels by doses 3 ml blood which was infected with the new parasite of *Babesia cameli* to examine the zoonotic importance for this parasites. Blood examination was performed daily for determine the infection of these laboratory animals.

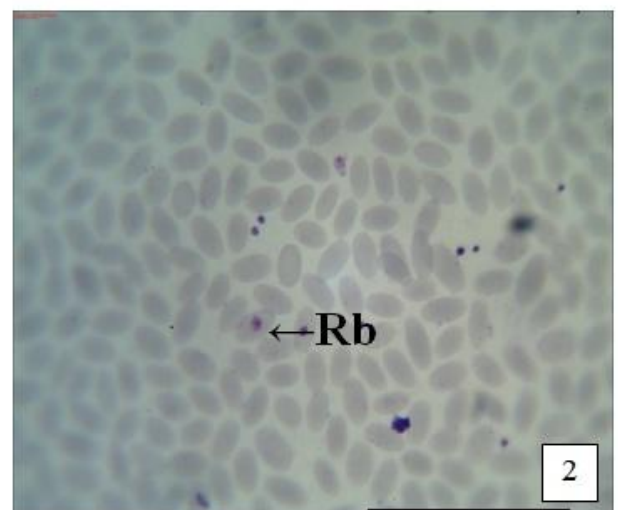
3. Results

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 (Figure 1), ray body in the red blood corpuscles (Figure 2) and other stages (Figures 3 & 4).The infection with this parasite sometimes was accompanied by *Theileria sp.*



20µm

Figure 1: Photomicrograph showing trophozoite of *Babesia cameli*. (B) Outside of the red blood cells of *Camelus dromedarius* stain with Geimsa stain.



20µm

Figure 2: Photomicrograph showing ray body (Rb) of *B. cameli*. In the red blood cells of *Camelus dromedarius* stain with Geimsa stain.

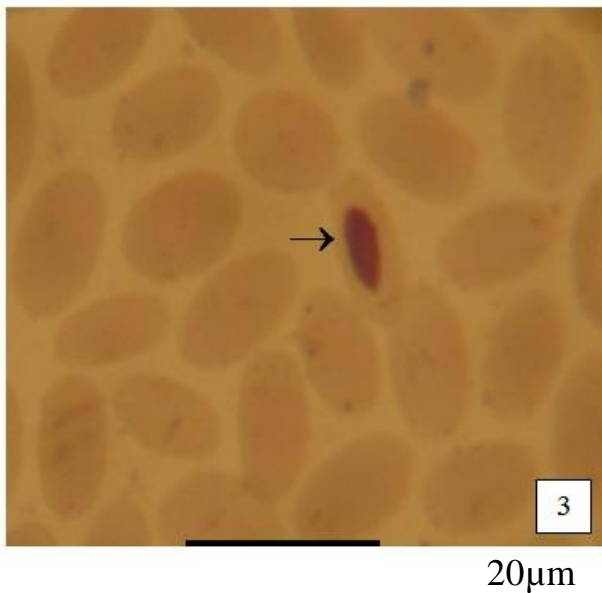


Figure 3: Photomicrograph showing kinete of *B. cameli*. In the red blood cells of *Camelus dromedarius* stained with Geimsa stain.

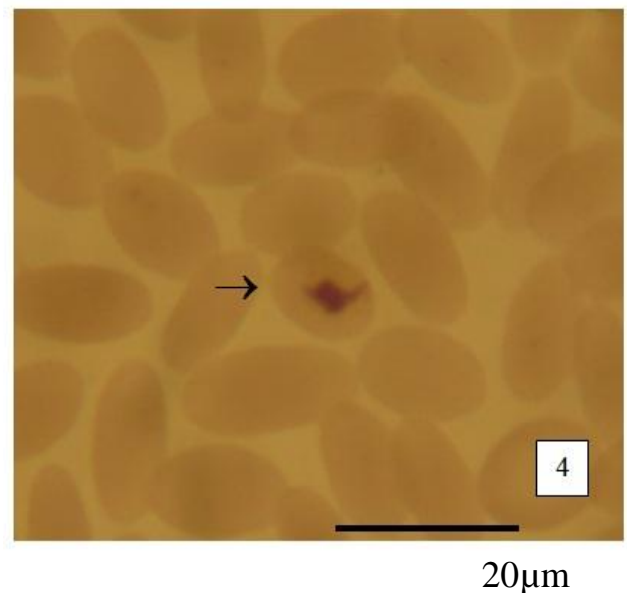


Figure 4: Photomicrograph showing gametogenesis of *B. cameli*. In the red blood cells of *Camelus dromedarius* stained with Geimsa stain.

Transmission electron microscopy revealed that, different developmental stages of *B. sp.* were found, rounded or oval shaped trophozoites measured ($1.58 \times 1.25 \mu\text{m}$) in diameter. The body was covered by a single membrane; one prominent nucleus was observed and measured ($1 \times 0.41 \mu\text{m}$) in diameter, well developed rough endoplasmic reticulum, some vacuoles and numerous free ribosomes (Figure 5)

Some trophozoites were presented with a single cytostome composed of a single electron - dense concentric ring, with an internal diameter of ($0.3 \times 0.076 \mu\text{m}$) and

located on the periphery of the parasite. Small intracytoplasmic food vacuoles were observed next to the cytostome. One of these vacuoles was contained material of similar density to hemoglobin.

Among these parasites was located outside the erythrocytes, two morphologically distinct types were observed. Some were presented with abundant cytoplasmic organelles, especially osmiophilic bodies which are oval or elongated electron- dense structures (Figure 6). In the other, the amount of organelles in the cytoplasm was significantly reduced (Figure 7).

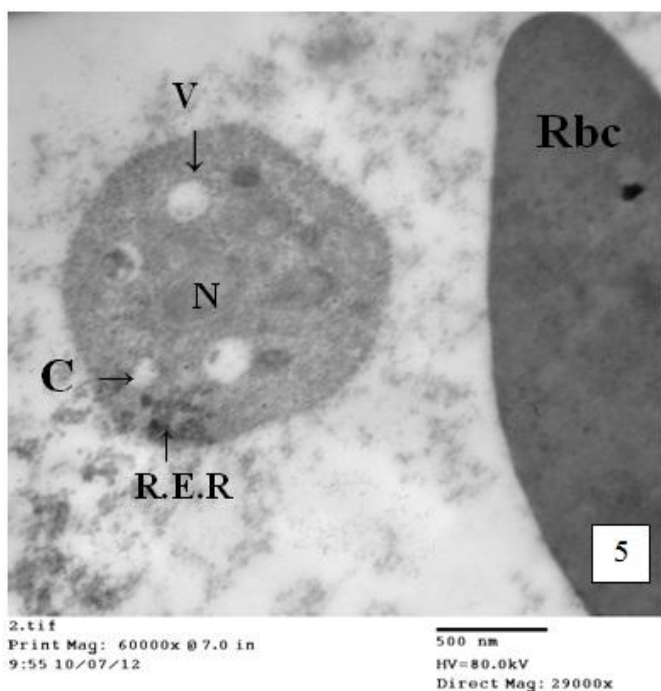


Figure 5: Transmission electron micrograph of the first form of *Babesia cameli*. Showing cytostome (c), rough endoplasmic reticulum (RER) and vacuoles (V).

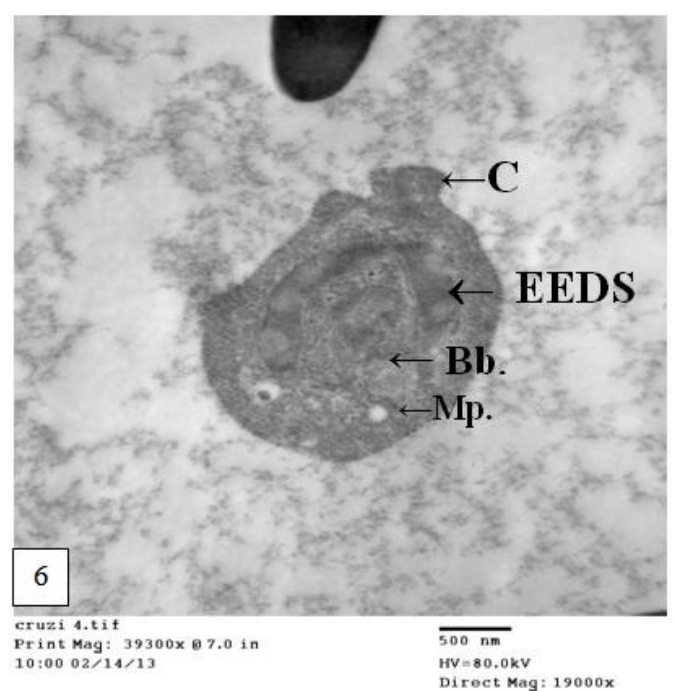


Figure 6: Transmission electron micrograph of the second form *Babesia sp*, showing cytostome (c), bright body (Bb), elongated electron dense structure (EEDS) and micropore (Mp).

Both parasite forms were surrounded by a single membrane and an electron-lucent, circular region and sometimes limited by a membrane. This vacuolated area in the parasite cytoplasm was contained a large amount of microtubules denoted as bright bodies. Microtubules running parallel to the cytoplasmic membrane or extending in cytoplasmic projections came out of this area. The only visible areas in the rays were varying numbers of microtubules parallel to the entire structure which is delimited by a membrane.

Also TEM revealed that, presence of another type *B. sp.* which was elongated or oval in shape and measured

($3.2 \times 1.7 \mu\text{m}$) (Figure 8). It had thicker and more electron-dense walls due to deposition of granules on its surface and presence of pores on the surface of erythrocytes. No nuclear fusion was observed. Small numbers of micro pores were observed (Figure 9).

Scanning electron microscopy showed that, *B. sp.* with a tubular structure which was contact with the erythrocyte (Figure 10). Supporting the first form of *B. sp.* Also presence of *B. sp.* and more clefts in the infected blood corpuscles (Figure 11) due to it's infected by *B. sp.*

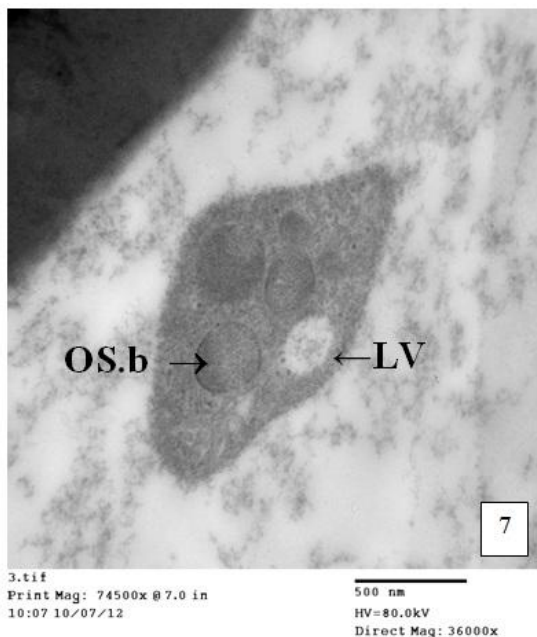


Figure 7: Transmission electron micrograph of the third form *Babesia cameli*. Showing, an osmiophilic body (OS. b) and large vacuole (LV).

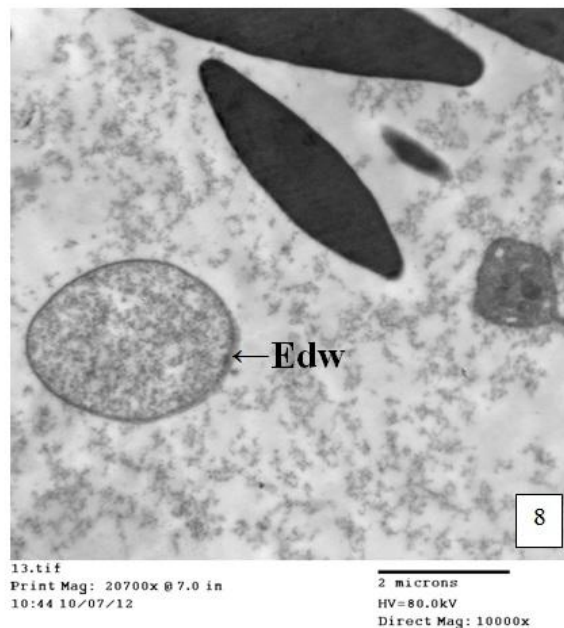


Figure 8: Transmission electron micrograph of the fourth form *Babesia cameli*. Showing more electron dense walls (Edw) in *Camelus dromedarius*.

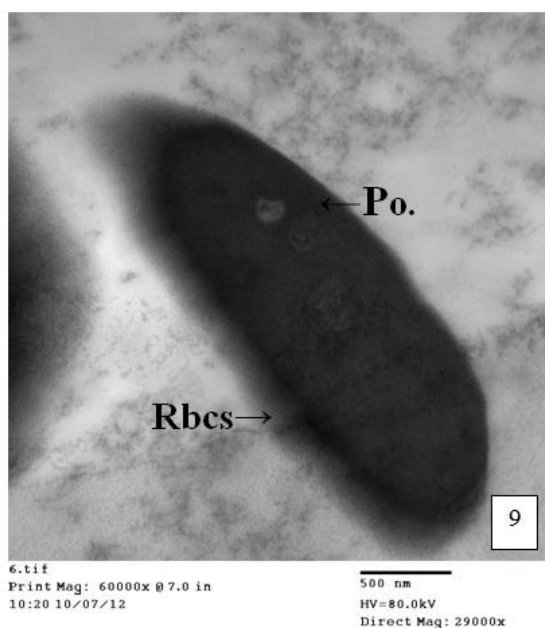


Figure 9: Transmission electron micrograph of pores (Po) in the surface of red blood cells of *Camelus dromedarius* due to its infected with *Babesia cameli*.

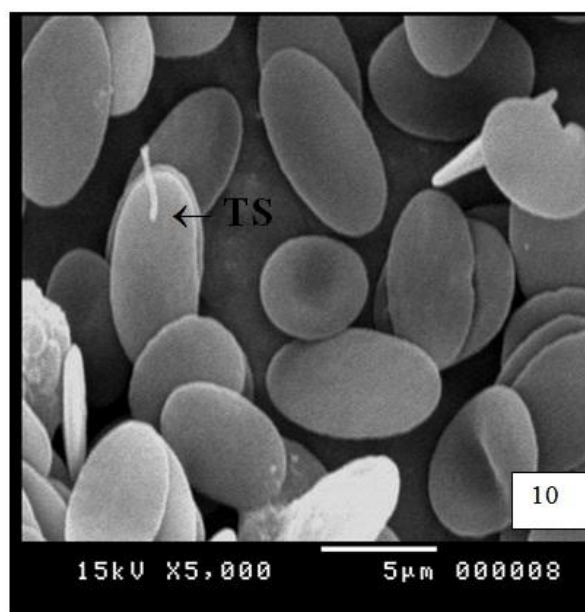


Figure 10: Scanning electron micrograph of red blood cells of *Camelus dromedarius* showing tubular structure (TS) of *B. cameli*.

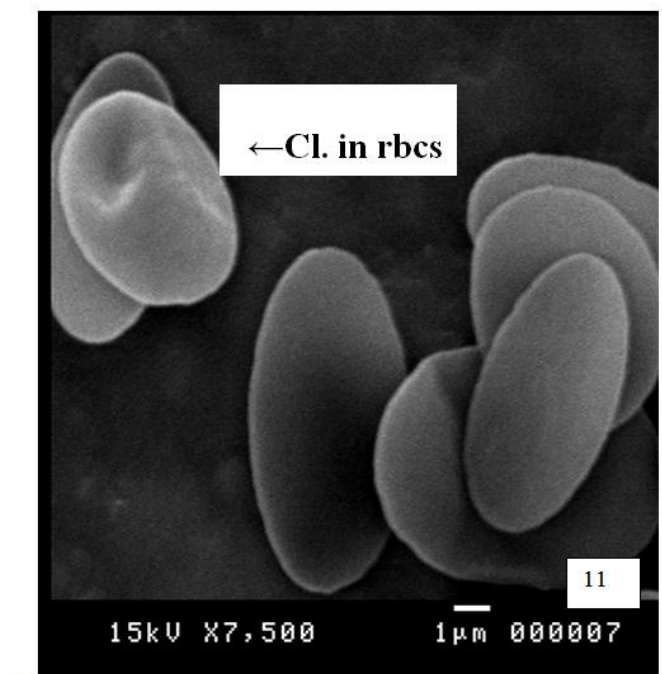


Figure 11: Scanning electron micrograph of red blood cells of *Camelus dromedarius* showing clefts in rbc due to its infected with *B. cameli*.

4. Discussion

By compared the ultrastructure of *B. equi* trophozoites which was isolated in Minas Gerais, Brazil [8] with the first form of *B. sp.* infected *Camelus dromedarius* in the present study was found that, presence of food vacuoles, endoplasmic reticulum and the tubular structure as feeding mechanism in the two species supported with transmission and scanning electron microscopy in the present study.

Also mitochondrial organelles were not observed in the intra-erythrocytic stages of *B. equi* and typical mitochondria have not been recorded in any previous studies [9 - 11].

A singular aspect of the morphology of the *B. equi* trophozoites in the presence of a cytostome and the tubular feeding structure involved in the feeding mechanism [12], suggesting that the first form in the present study was *B. equi*.

Presence of bright bodies and electron- dense organelles in the cytoplasm of the parasite among them the

osmiophilic bodies in two forms of *B. sp.* in the present study was similar to a large extent with description of the ultrastructure of gametes of *B. bigemina* was obtained "in vitro" red cell culture [13]. The structure observed were similar to these were described by [14-17].

At the same time when was compared the last form of *B. sp.* in the present study with its thick membrane electron- dense agree with that, *B. caballi* which its infection originated from horses [18] where the forms found in gut cells of *Anocentor nitens* ticks.

5. Conclusion

Babesia sp. for the first time was infected *Camelus dromedarius* at Assuit locality, Upper Egypt. The new parasite can infect rats experimentally. At the same time it was affected on the tissues of the infected rats and their hematological parameters as (RBCS, WBCS, Hb, Ht, MCH, MCHC and MCV).

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