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Some Protozoan Parasites Infecting Blood of Camels (*Camelus dromedarius*) at Assiut Locality, Upper Egypt

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Abstract

Out of ninety eight of camels (*Camelus dormadarius*) examined, only forty eight (48.9 %) were found to be infected with blood protozoan parasites (*Trypanosoma evansi, Theileria sp. and Babesia sp.*). The higher incidence of infection were found in males (36.7%) whereas, (12.24%) in females. Microscopical examination revealed that longitudinal binary fission, the stumpy, slender forms of *Trypanosoma evansi*, trophozoites of both *Theileria sp*. And *Babesia sp*. Experimental infection revealed that both of *Babesia* and *Theileria* have a zoonotic importance for their transmissible to the experimental animals.

Keywords: *Camelus dormaderius; Surra; Babesia; Piroplasms; Theileria*

Introduction

Camel (*Camelus dromedarius*) is an important multipurpose animal in arid and semi-arid areas of the world [1]. Protozoal diseases particularly trypanosomosis, cause remarkable losses on animal production in all the tropical and subtropical area. Trypanosomosis in camels is caused by *Trypanosoma evansi* and is transmitted from camel to camel by a number of species of haematophagous biting flies including *Tabanus, Stomoxys, Lyperosiaand Haematobia* [2]. Animal African trypanosomosis is a serious constraint to livestock sector development in sub-Saharan Africa. The disease, mainly caused by *T. congolense,* has a limitation in its diagnosis and treatment [3]. Trypanosomosis caused by *T. evansi* is the most recognized protozoan disease of camels and causes a disease known as Surra [4]. Surra has been reported in Pakistan [5], Jordan [6], Kenya [7], and many African countries. An outbreak of abortion and neonatal mortality associated with *T. evansi* infection in dromedary camels has been reported in Canary Islands [8].

Equine babesiosis is an infectious disease of horses and other equips caused by the protozoan hemoparasites *B. equi* and *B. caballi* [9]. *B. equi* infects 90% of the world equine populations [10], and transmitted naturally by the ticks of the Genera *Hyalomma, Dermacentor* and *Rhipicephalus* [11], and experimentally by *Boophilus microplus* [12]. *B. equi* was first isolated in Brazil by Ribeiro, et al. [13] from naturally infected horses to produce an antigen for serologicaly purposes. Babesiosis is vectored to humans by ticks that are ecto-parasites of rodents [14]. Human babesiosis caused by *B. microti* was first described from sites along the North Eastern United States terminal moraine [15] and later from Minnesota and Wisconsin [16]. *B. bovis* and *B. bigemina,* exhibit a typical apicomplexan life cycle characterized by merogony, gametogony and sporogony [17].

The disease caused by the apicomplexan protozoan parasite *Theileriaparva*, known as East Coast fever or Corridor disease, is one of the most serious cattle diseases in Eastern, Central, and Southern Africa [18]. Hemoparasites known to infect bovine erythrocytes and cause anemia include organisms from the genera *Anaplasma, Eperythrozoon*, *B.*, and *Th.* [19].

Accordingly the aim of the present work is to differentiate between different forms of *T. evansi,* to describe *Th.* and *B. sp*. as new species Infecting *Camelus dromedaries* using light and electron microscopy and to examine the zoonotic importance for these parasites on the experimental animals (White rates and mices).

Materials and Methods

Out of 98 blood samples of camels (*Camelus dromedarius*) examined for blood protozoan parasites collected from different localities of Sloughter houses at Assiut city, Egypt (Dairout, Beni Ady, El ethamna). 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 *Trypanosoma, Babesia* and *Theileria* 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 fine 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 dropswere 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

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special holders and coated with gold. Then they were examined in a JSM-T 200 L.V. 5400 Scanning Electron Microscopy (SEM).

Experimental infection: Two groups of laboratory animals representing in five white from both rates and mices were dispensed with freshly infected blood camels by *Babesia* and *Theileria* in three doses each dose 3 ml blood to examine the zoonotic importance for these parasites. Blood examination was performed daily for determine the infection of these laboratory animals.

Results

Out of ninety eight camels, (*Camelus dromedaries*) collected from different parts of Assiut (Benny Adie, El-atamna and Dairout). They revealed three genera of parasites including T*rypanosoma, Theileria* and *Babesia.*

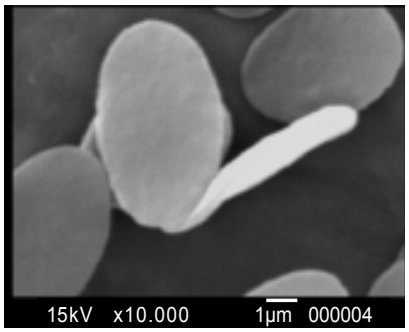

Figure 1: Presence of developmental stages Stumpy forms, Trypomastigote stage with free flagellum, undulating membrane and longitudinal binary fission.

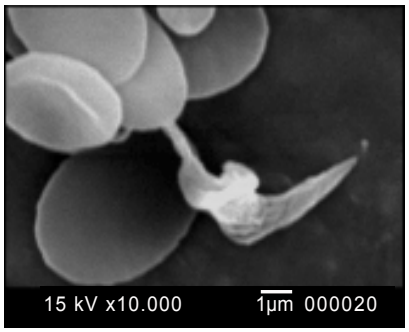

Figure 2: Presence of developmental stages Stumpy forms, Trypomastigote stage with free flagellum, undulating membrane and longitudinal binary fission.

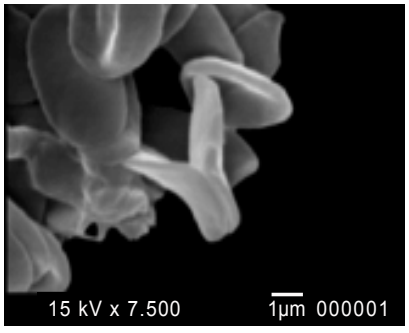

Figure 3: Presence of developmental stages Stumpy forms, Trypomastigote stage with free flagellum, undulating membrane and longitudinal binary fission.

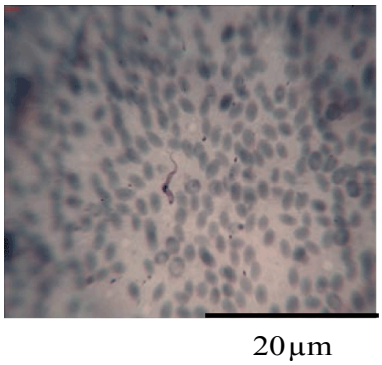

Figure 4: Free flagellum length 7 µm with two small folds of undulating membrane.

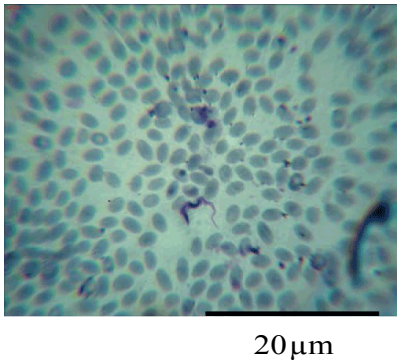

Figure 5: Free flagellum 8 µm with one to two small folds.

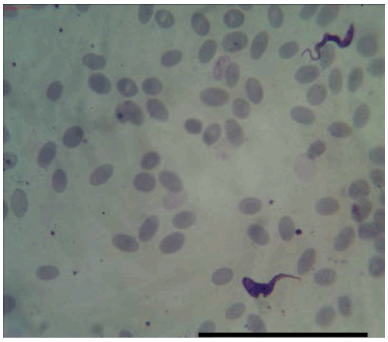

Figure 6: Eight with no folds.

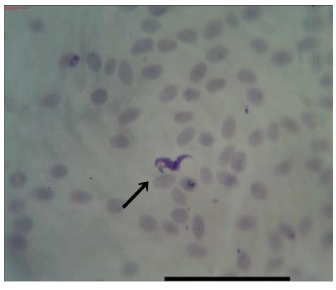

Figure 7: 17-20 with pulp body shape.

Trypanosoma sp.

Trypanosoma evansi is similar in shape with *T. evansi* in all mammals although it is different in size. Three camels from 98 (3.06 %) were infected. SEM revealed that presence of developmental stages Stumpy forms, Trypomastigote stage with free flagellum, undulating membrane and longitudinal binary fission as in Figures 1-3 respectively. They are five different forms according to the different morphological features (Total length, Total width, Nucleus index, the distance between (Kinetoplast to the posterior end, Nucleus to the anterior end, Nucleus to the posterior end),Presence of free flagellum, Presence of undulating membrane and the shape of posterior end) Figures 4-8 as in Table 1.

Babesia sp.

Forty six from ninety eight examined of *Camellus dromedaries* (46.9 %) were found infected with *Babesia sp*. It infects the camels by ticks with heavy infections as Figure 9 in the intra and extra of the red blood corpuscles (Figures 10 and 11) respectively and showing that different stages as ray body (Figures 12). The infection by the parasite sometimes accompanied by the infection with *Theileria sp*. SEM and TEM, reveal the sporokinetes of B. have elongate shape, 3.12 µm long and 1.56 µm wide, being wider at the anterior end and containing a single nucleus, large endoplasmic reticulum and abundant micronemes which was concentrated at the anterior end as in Figures 13 and 14.

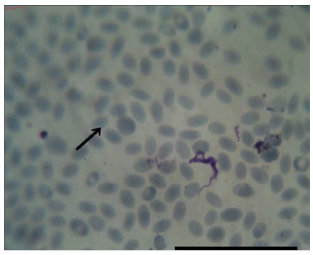

Figure 8: 10-12 with well-developed undulating membrane with two to four folds.

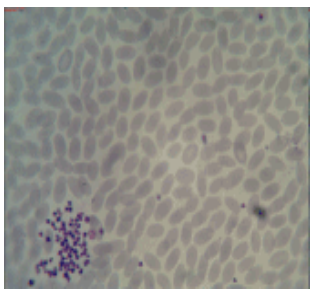

Figure 9: *Babesia sp* infects the camels by ticks with heavy infections in the intra and extra of the red blood corpuscles.

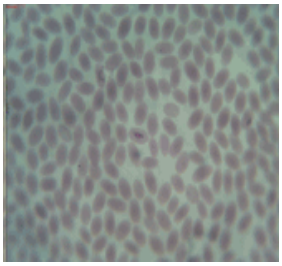

Figure 10: *Babesia sp* infects the camels by ticks with heavy infections in the intra and extra of the red blood corpuscles

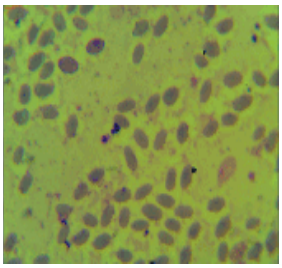

Figure 11: *Babesia sp* infects the camels by ticks with heavy infections in the intra and extra of the red blood corpuscles

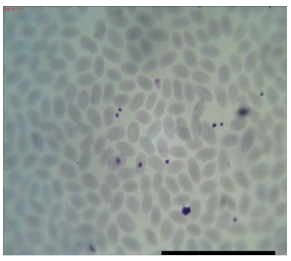

Figure 12: Different stages as ray body.

Type or form No	1	2	3	4	5
Total length	23-30	20-27	28-30	20-30	36-44
Total width	2.5-5	4-Mar	6-Apr	13.5-20	7-May
Nucleus index	6 x 2.5	2 x 5	2.5 x 4	4 x 4	3 x 6
Kinetoplast to the posterior end	2	4	Contact with it	13-Oct	5-Mar
Free flagellum and undulating membrane	Free flagellum length 7 µm with two small folds of undulating membrane	Free flagellum 8 µm with one to two small folds	8 with no folds	17-20 with pulp body shape	10-12 with well-developed undulating membrane with two to four folds
Shape of the posterior end	Pointed posterior end	Sharp posterior end	Truncated posterior end	Vacoulated like structure with granules	Normal posterior end
Nucleus to posterior end	15	15	8	3	15
Nucleus to anterior end	7	13	8	12	10
Figs. no.	Figure (4)	Figure (5)	Figure (6)	Figure (7)	Figure (8)

Table 1: Showing differences between different forms of T. evansi (measurements with µm).

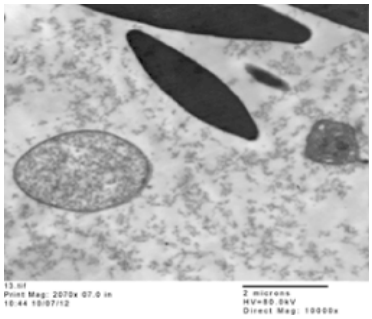

Figure 13: SEM and TEM, reveal the sporokinetes.

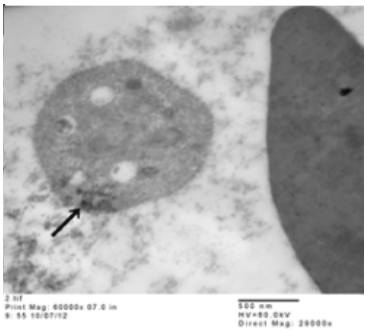

Figure 14: SEM and TEM, reveal the sporokinetes.

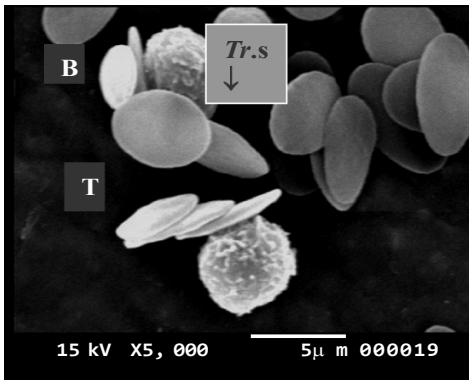

Figure 15: Sporokinetes having oval or elongate shapes.

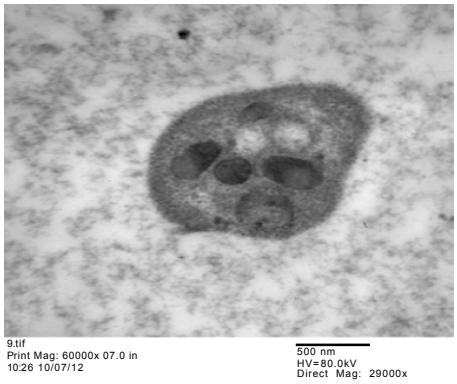

Figure 16: Transverse sections of sporokinetes.

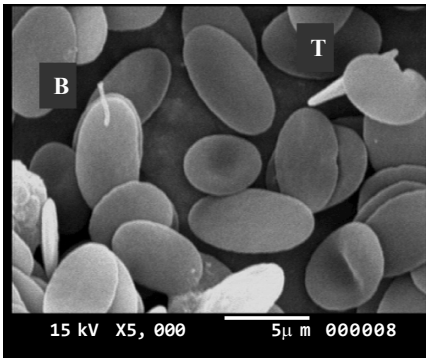

Figure 17: Concomitant invaginations of the plasma membranes of the erythrocyte and the parasite.

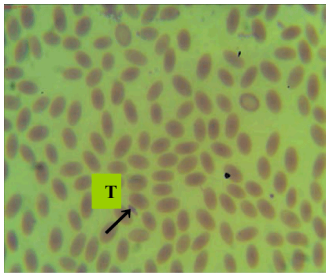

Figure 18: Trophozoite stages intra and extra of the red blood corpuscles.

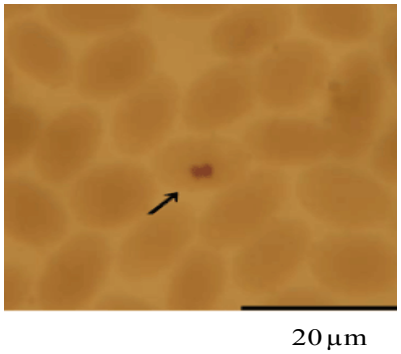

Figure 19: Blood films which stained with Giemsa contained Th. piroplasms including, cocci and comma shaped.

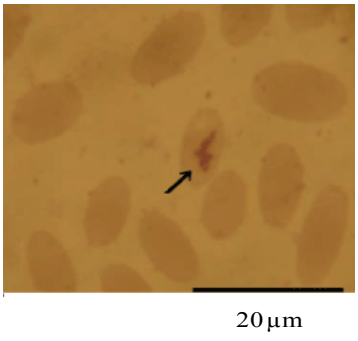

Figure 20: Blood films which stained with Giemsa contained Th. piroplasms including, cocci and comma shaped.

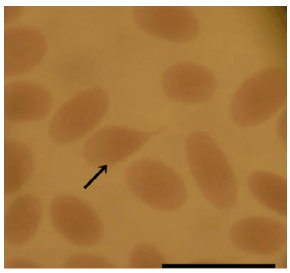

Figure 21: Abnormalities in erythrocyte structure included acanthocytosis.

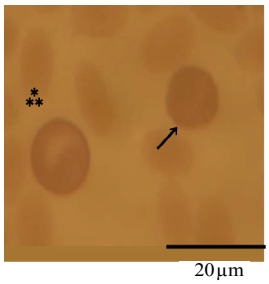

Figure 22: Spherocytosis and basophilic stippling.

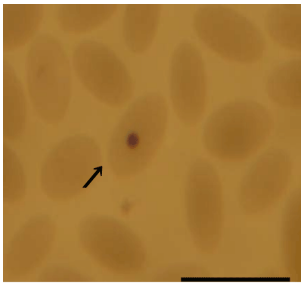

Figure 23: Howell-jolly body.

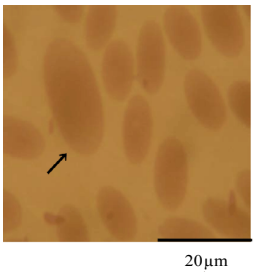

Figure 24: Macrocyte.

The *Encephalitozoon* – like microsporidia are present within the same specimen in which sporokinetes of *B. caballiare* found. They presented different morphological stages, suggesting a sequential phases of development. All stages contained a single nucleus. Sporonts have thicker and more electron-dense walls due to deposition of granules on the surface of the parasite. They have oval or elongate shapes (Figure 15). The sporonts multiplied producing the sporoblasts with polar filament. In the transverse sections, four coils of the polar tube and two food vacuoles were observed (Figure 16). Also, the scanning electron microscope show the trophozoites located very close to the erythrocyte membrane presented a tubular feeding structure, which emerged from the interior of the parasite and extended to the blood plasma, the tubular feeding structure was formed by the concomitant invaginations of the plasma membranes of the erythrocyte and the parasite Figure 17.

Theileria sp.

Only nine from ninety eight *Camelus dromedarius* (9.18%) are infected with the parasite. Trophozoite is a cigarette shaped and the light microscope shows some stages intra and extra of the red blood corpuscles as in Figure 18. Initial blood films examination revealed anemia, thrombocytosis and leukocytosis. Also, blood films which stained with Giemsa contained *Th.* piroplasms including, *cocci* Figure 19 and comma shaped Figure 20. Other abnormalities in erythrocyte structure included acanthocytosis Figure 21, spherocytosis and basophilic stippling, Figure 22, Howell-jolly body's Figure 23 and macrocyteas in Figure 24. Scanning electron microscopy also shows many different developmental stages.

Experimental infection

Theileria and *Babesia* were appeared in both of the white rates and mice after 24 days of infection but 3 mice died after 80 days of infection. Rates could tolerate the infection with appearing of some symptoms such as diarrhea and a very reddish color for rates eyes. Some different stages of both parasites at different times appeared after 44, 59 and 74 days of infection as in Figures 25 A-D. This experimental infection had been proved that both of *Theileria* and *Babesia* have zoonotic importance whatever for that, of a very economic importance in the life experimental field.

Discussion

Trypanosomaevansi

Trypanosomes of the section salivaria might or might not possess a free flagellum, their kinetoplast is terminal or subterminal in position and the posterior end of the parasite is usually rounded [20]. Several studies have observed a significant morphological difference in some *T. evansi* isolates [21,22]. The presence of vacuoles in *T. evansi* has been reported (ID and International Medicine Parasitology Volume 1 for web, 2008). In the present work *T. evansi* measured 20-44 µm in length, agreement with some species described by Hoare CA [23]. Although the main morphological parameters (total length with flagellum, total width, nuclear index, posterior end to the kinetoplast, free flagellum, shape of the posterior end, posterior end to the nucleus, and the nucleus to the anterior end) show some few differences with other data reported by authors Silva RAS, et al. and Sarataphan N, et al. [10,24]. The subgenus *Duttonella* trypanosomes have feebly developed undulating membranes and a large kinetoplast. The latter feature could resemble those of form 5 in the present study, but the undulating membrane contains two or three big fold sand its pointed posterior end is different from *T. vivax* [8,23,25]. By contrast, forms 3 and 4 showed rounded posterior extremity but the most important feature the *Duttonella* trypanosomes, the large kinetoplast, was absent [23]. The results obtained in the present study would indicate that biometrically distinct *T. evansi* could also be found in the same area and even in the same animal species.

Babesia sp.

Ticks are widespread in camel habitats. They cause serious adverse

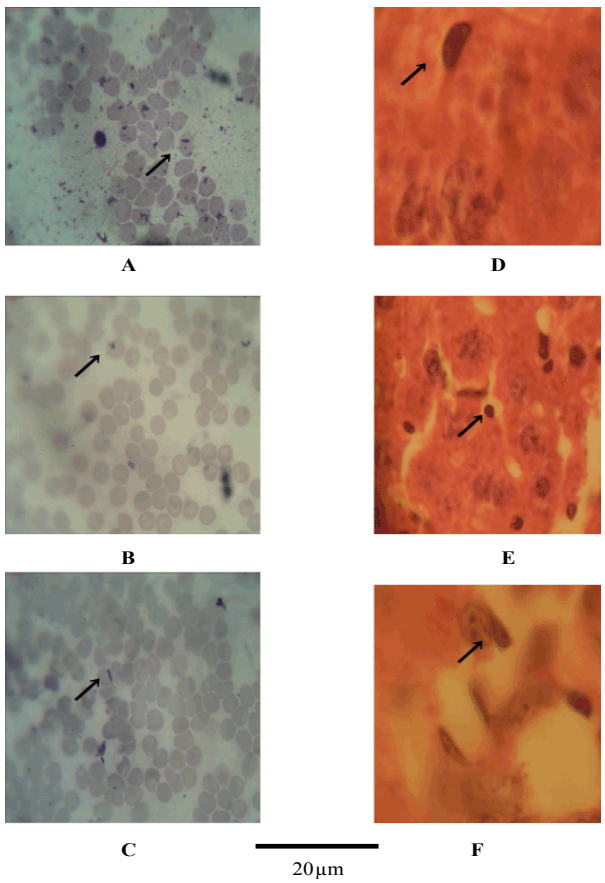

Figure 25: Different stages of both parasites at different times appeared after 44, 59 and 74 days of infection.

effects such as anemia, dermatitis, mastitis reduced meat and milk production and low quality hides [26]. Camels were infected with *Babesia caballi* for the first record in Sudan [26]. So, that the infection of *Camelus dromedaries* by *Babesia sp*. is the first record in Egypt. *Babesia caballi* is a hemoparasitic protozoan of the Phylum Apicomplexa that is transmitted naturally in New World by *Anocentor nitens* ticks [2]. Few papers have reported the multiplication of *B. caballi* in ticks and most of them were based on optic microscopy [27]. Only one paper on ultra-structure has used transmission electron microscopy to describe the development of *B. caballi* in salivary glands of *Hyalomma truncayum* [28]. The forms of *B. sp.* which are found in *Camelus dromedarius* in the present study are in agreement with those describe by Ribeiro, et al. [13] within the same cells infected with *B. caballi* sporokinetes or in other neighbor cells, it was observed that microsporidia organisms were undergoing different stages of development. Ultra-structural analysis showed that all stages of development of microsporidia with a single nucleus, while the stages of the sporoblast and spore had four coils of the polar tubes. Based on these findings, the microsporidia could be classified as *Encephalitozoon cuniculi* [29]. *Encephalitozoon cuniculi* infections were described in mammals, including horses, causing asymptomatic infections in immunocompetent hosts [4]. So that, according to the present work there are two species of B. one is *B. caballi* and the other *B. equi*. Which have two vacuoles.

Theileria sp.

Taxonomic classification of *Theileria sp*. was based on microscopic appearance of intraerythrocytic piroplasms, geographical location of the infected animal apparent pathogenicity of the organism and serologic testing [30,31] Most theilerial organisms of cattle that had low pathogenicity were called *Th. Mutans* [14]. As more *Th. sp.* were isolated and studied, some organisms were reclassified. For example, the US theilerial organism reported to be *T. mutans* was renamed *T. orientalis* in 1985 [31].These authors also considered some isolates of *Th. orientalis* to be the same stocks of *T. sergenti,* the name given to a more pathogenic species found in Southeastern Asia. So that, the presence of basophilic stippling indicateda regenerative response consistent with a hemolyticanemia. A combination of spherocytosis and acanthocytosis indicated intravascular erythrocyte fragmentation. Spherocytes can also be formed by immune-mediated processes. There is no evidence of blood loss, but there was a hypoprotienemia. For the first time theilerial infection in camels occurs in Egypt but intraerythrocytic piroplasms were highly pleomorphic, and rod forms were most common. Piroplasms shape may vary with stage of an infection and thus is not a reliable criterion of species differentiation [31]. Thus, the present study suggests that this Species is *Theileria camelli.*

Conclusions

The higher incidence of the infected camels with protozoan parasites in Egypt is (48.9%) due to some environmental conditions. It is the first time in Egypt to record infections of camels with both *B. sp* and *Th. sp.* with higher incidence (46.9% and 9.18%) respectively resulting from their habitat with other animals (cattle's and sheep's).

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