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 dromedarius) 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 dromedarius; 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, Lypoeria and Haematobia [2]. Animal African trypanosomosis is a serious constraint to livestock sector development in sub-Saharan Africa. The disease, mainly caused by Trypanosoma congolesense, 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 equids 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 serologically purposes. Babesiosis is vectored to humans by ticks that are ectoparasites 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 Theileriaparvav, 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, Eperythrozoan, 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 dromedarius 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 Slaughter 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 at operating 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...
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 mice 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-atamma and Dairout). They revealed three genera of parasites including *Trypanosoma*, *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 *Camelus 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.

| 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 | Vacuolated 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.
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 multiplying 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 macrocytes 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**

**Trypanosoma evansi**

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
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 ultrastructure 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 indicated a regenerative response consistent with a hemolytic anemia. 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 hypoproteinemien. 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 cameli.

**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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