Some studies on prevalence and effect of Theileria infection on erythrocytes profile in camel in some localities at New-Valley, Governorate, Egypt

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ABSTRACT

Theileria spp is protozoan parasite infecting wild and domestic animals throughout the world and affect the healthy state of the infected animals. Therefore this study was carried to evaluate the effects of natural infection of camels with Theileria spp on blood picture and efficacy of indirect fluorescent antibody technique in the diagnosis of this infection. The blood samples were collected from 125 apparently healthy dromedary camels aged 1-9 years, held in some localities in New-Valley Governorate and classified into two groups according to infection, the first group is suspected clinically infected and considered as infected group (100 camels) and the remaining number (25) was clinically and laboratory healthy and considered as a control group where all of them were examined by thin blood smear, fecal examination and indirect fluorescent antibody technique. Thin blood smears revealed that 9 out of 100 camels were positive for Theileria spp in ratio of 9% while indirect fluorescent antibody technique revealed that 11 out of 100 camels were positive (11%), with one sample as false negative and 3 samples as false positive. Therefore the indirect fluorescent technique remains the most convenient test for theileria spp diagnosis in camels.

Hematological analysis revealed a significant decrease in PCV, HB, RBCs count with a significant increase in MCV, MCH and MCHC in infected group when compared with the control one. The frequency of theileriosis in camels is low and Theileria spp do not seem to induce a significant alteration in clinical signs of naturally-infected dromedary camels but by laboratory means a significant decrease in hematological parameters which translated to anemia was resulted.

INTRODUCTION

A camel is an even-toed ungulate within the genus Camelus, bearing distinctive fatty deposits known as “humps” on its back. The two surviving species of camel are the dromedary, or one-humped camel (C. dromedarius), which inhabits the Middle East and the Horn of Africa; and the bactrian, or two-humped camel (C. bactrianus), which inhabits Central Asia. Both species have been domesticated: they provide milk, meat, hair for textiles or goods such as felted pouches, and are working animals with tasks ranging from human transport to bearing loads. It has been largely domesticated in the arid regions of western Asia and Northern Africa as the chief beast of burden and the “ship of the desert” (1). The one humped camel or Camels dromedaries is physiologically and anatomically adapted to survive harsh conditions. Also it is a widely distributed domestic animal in arid and semi-arid regions of Africa, Arabia and Western Asia up to India (2).
Piroplasmids belonging to the genera Babesia and Theileria are suspected of infecting dromedaries' camel. These tick-borne apicomplexans were generally considered as highly specific for a given host species (3).

Indirect fluorescent antibody technique (IFAT) has been effectively employed by many authors as a speedy and accurate serological test for detection of bovine theileriosis (4-6). *Theileria camelensis* is an intra-erythrocytic protozoan parasite infecting camels; its presumed vector is *Hyalomma dromedarii*. The parasite forms in the erythrocytes were predominantly rod shaped and no schizonts were detected in the prescapular lymph node impression smears as previously reported (7). The predominant clinical findings of camels infected with theileria are fever, ocular watery discharge, severe emaciation, diarrhea in the form of intermittent bouts, in addition to the systemic signs, enlargement of superficial lymph nodes were also noticed (8). On the other hand, camels may be apparently healthy in spite of theileria infection (9). *Theileria camelensis* has been reported from most of the regions which camels are raised in and transmitted by common camel tick, *Hyalomma dromedaries* (7), while the erythrocyte piroplasm stage of the parasites was present (10).

No microschizont stages have been yet described and the taxonomic status of these parasites remains unclear and *T. cameliness* is generally thought to be non-pathogenic and its economic impact appears to be small (9). Understanding the pathogenesis and immunology of infectious diseases helps policy makers define control strategies. These control plans become more important in developing nations where control measures are not properly designed and implemented (11,12).

The aim of the study was to investigate the effect of camel theileriosis on erythrocytes parameters in area of study, in addition to the efficacy of indirect fluorescent antibody technique (IFAT) in diagnosis of camel theileriosis.

**MATERIALS AND METHODS**

Study area and examined animals

A- Study area

This study was carried out in some localities in New-Valley Governorate (in the western Egyptian desert). This area is a depression that lies between the Nile, Sudan and Libya with its capital at the Kharga Oasis where the rainfall is almost scarce throughout the year and the ground water is the main source of water.

B- Animals

One hundred and Twenty five camels aged 1-9 years from both sexes were examined in this study. The animals were treated with albendazol twice one week interval to confirm these camels free from any internal parasite. Camels' were classified into two groups after last treatment by 15 days. Group 1 (diseased group) and include one hundred camels and group 2 (control group) which include twenty five camels. All of these camels were reared in different localities in New-Valley governorate as in Table 1 and figure legend 1.

Blood samples

a- 5 ml blood samples were collected from jugular vein of 125 apparently healthy dromedary camels aged 1-9 years into clean dry sterile tubes containing dipotassium salt of Ethylene Diamine Tetra-acetic Acid (EDTA) as an anticoagulant for used in hematological examination, the thin blood smears were prepared directly from ear vein were air dried, fixed in methanol and stained with Giemsa stain (13) and examined microscopically for presence of *Theileria camelensis*.

b- 5 ml blood were collected from jugular vein in centrifuge test tube, left to clot and clear serum was obtained for serological analysis by indirect fluorescent antibody technique (IFAT) according to (14).
Fecal examination

Fecal samples were collected from camels positive for thelerosis (treatment with an anti parasitic drug, Albendazol twice one with week interval) fifteen days after last treatment to confirm these camels free from internal parasites.

Seriological examination

a- Slid antigen

Slid antigen preparation were made from the blood of high parasitina (2%) and put on the different slide wells and fixed by aceton in goblin jar and washed three successive time with PBS and the slide was dried by Schwarz and kept in deep freeze until used as described previously (14).

b- IFA test procedure

50 μml of undiluted tested serum added for each slide well and incubated for half hours followed by three successive washing by PBS then added ant bovine conjugated with fluorescence dye (1; 80 dilution) and incubated for 3/4 hours, three successive washing by PBS and finally added the cover on the slide with glycerin and mounted by fluorescence microscope. The technique adopted for IFAT was described by previous author (14), using Rabbit-ant bovine IgG fluorescent isothiocyanate (FITC).

Hematological analysis

Hematological analysis between various sexes, and infection and parasite free camels were analyzed using long established techniques according to (15). The following parameters were determined: total red blood cell (RBC) counts, packed cell volume (PCV), hemoglobin concentration (Hb), total white blood cell (WBC) counts with differential cell count, while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration.

Statistical analysis

Data were analyzed by SPSS 16 software, using independent Student’s t-test. Non infected animals were considered as control for comparison of results.

RESULTS

Hundred camels were examined in the present study revealed that 9, out of 100 camels examined by thin blood smear were found to be positive for thelerosis (9%) as indicated in figure 2, but with indirect fluorescent antibody technique 11 out of 100 (11%) were found to be positive as illustrate in figure 3, where the sample number 7 positive by thin blood smear was negative by IFAT and considered as false negative. While 3 samples were negative by thin blood smear but positive by IFAT and considered as false positive, all of them being mature and over 3 years old, Table 2.

The hematological analysis showed a significant decrease in RBCs count, hemoglobin content and packed cell volume (PCV), with a significant increase in MCV, MCH and MCH (P<0.01) of infected group than control group where that reflecting macrocytic norm chonic anemia (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Number of camels under study from different localities in New-Valley Governorate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camel group</td>
</tr>
<tr>
<td>Localities</td>
</tr>
<tr>
<td>Number of animals</td>
</tr>
</tbody>
</table>
Fig. legend 1. Showing the animal localities under the study.

Table 2. The result of both direct smear and indirect antibody fluorescence technique

<table>
<thead>
<tr>
<th>Method</th>
<th>Camel</th>
<th>No of examined camels</th>
<th>No of positive camels</th>
<th>False positive</th>
<th>False negative</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct smear</td>
<td></td>
<td>100</td>
<td>9</td>
<td></td>
<td></td>
<td>9%</td>
</tr>
<tr>
<td>Indirect fluorescent antibody technique (IFAT)</td>
<td>100</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Erythrocytes parameters in both diseased and control camels (Mean values ±SE).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Infected camels</th>
<th>Control camels</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>17.5±6.0 *↓</td>
<td>28.4±9.31</td>
</tr>
<tr>
<td>HB(g/dl)</td>
<td>7.27±2.06*↓</td>
<td>10.25±2.09</td>
</tr>
<tr>
<td>RBCs x10⁶/μl</td>
<td>8.46±5.62*↓</td>
<td>14.38±5.67</td>
</tr>
<tr>
<td>WBCs x10³/μl</td>
<td>19.28±7.56</td>
<td>19.78±7.61</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>68.6±0.2*↑</td>
<td>60.1±0.4</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.3±0.1*↑</td>
<td>11.9±0.0</td>
</tr>
<tr>
<td>MCHC(g/dl)</td>
<td>44.3±0.2*↑</td>
<td>33.2±0.1</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>41.1±0.9</td>
<td>43.50±1.3</td>
</tr>
<tr>
<td>Band cell (%)</td>
<td>1.8±0.1</td>
<td>0.6±0.01</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>54.5±0.9</td>
<td>54.6±1.3</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0.8±0.1*↑</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Basophile (%)</td>
<td>0.0±0.0</td>
<td>0.01±1.0</td>
</tr>
</tbody>
</table>

* Significant P ≤ 0.05.
Fig. 2. Blood film from infested camels stained by Giemsa stain showing schizont of Theileria camelensis in lymphocytes. (X1000).

Fig. 3. Greenish yellow fluorescence indicating intra-erythrocytic stages of Theileria spp using IFAT (x1000).
DISCUSSION

Theileriosis is considered to be the second most important hemoprotozoal disease following trypanosomosis affecting dromedary camels in tropical and subtropical countries. There are different types of Theileria species implicated as etiologic agents of the disease. *Theileriacamelensis* appears to be the principal cause of camel theileriosis particularly in Egypt (7,8,16).

The current study indicated that 9% (9 out of 100) of the examined camels were harboring the erythrocytic forms of *Theileria camelensis* by blood smear and most of the positive cases had no apparent characteristic clinical signs. This may be attributed to the chronic nature of Theileria infection and/or to the investigated *Theileria camelensis* was probably a more pathogenic and that agree with previous study (7), who examined 200 apparently healthy camels under Egyptian field conditions and found that 30% of them were infected with *Theileria camelensis* and indicated that theileriosis in camels is asymptomatic infection. The prevalence rate of Theileria infection in one-humped camel varied in different studies as reported previously (7,8,16), which were 30% (60 of 200), 62.1% (46 of 74), 44.8% (56 of 125) respectively, where these result were higher than that reported in the present study but nearly agreed with the result reported by (17), who recorded 6.75% (15 of 224). These variations in the different results may be attributed to different localities, population density of camels, environment, hygienic measures and camel management.

Serological tests may not be sensitive enough to detect all infected camels with theileriosis due to cross-reaction occurs between different species and cannot detect antibodies in latent infection. However we can see that the indirect fluorescent antibody technique (IFAT) remains so far the most commonly used test for seroepidemiological studies of theileriosis in camels. Our results may be attributed to misdiagnosis by microscopic examination because it is difficult to differentiate morphological structure of *Theileria* spp. Also it may be explained by cross-reactivity among haemoparasites spp, and these antibodies might be derived from immunization against theileriosis.

Macrocytic anemia was found in camel suffering from theileriosis where RBCs count, PCV and Hb concentration for infected camels was significantly lower than the control group with a significant increase in the value of MCV. This type of anemia may be due to lyses of red blood cells that agreement with (18,19).

Finally, we can see that anemia in camels infected with thileriosis could be attributed to the direct effect of parasite on the infected erythrocytes (Incrimination of RBCs, decrease life span of RBCs and suppression of hemopiotic system), or due to extensive erythropagocytosis in the reticuloendothelial system initiated by parasite damage to erythrocytes.

CONCLUSION

Theileriosis in Egypt is economically one of the most serious tick borne protozoan parasitic diseases where in the present study Theileria spp identified in camels in considerable value without induce any effect on clinical signs but induced anemia. Also we can conclude that the indirect fluorescent antibody technique (IFAT) remains so far the most commonly used test for seroepidemiological studies of thileriosis in camel.
REFERENCES


الشخص العربي
بعض الدراسات على معدل انتشار وتاثير الإصابة بالثدييريا على صورة الدم في بعض المناطق في محافظة الوادي الجديد - مصر

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وحدة الطفيليات - معهد بحوث صحة الحيوان
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وحدة الكيمياء الحيوية - معهد بحوث صحة الحيوان

الثدييريا طفيل أولى يصيب الحيوانات المستأنسة والبرية على مستوى العالم ويثير على صحة الحيوانات المصابة. ولذا تم إجراء هذه الدراسة لتقييم تأثير إصابة الجمال بهذا الطفيل على صورة الدم وكذلك كفاءة اختبار الأجسام المضادة الفلوسني في تشخيص هذه الإصابة.

قد تم إجراء الدراسة على 120 جمالي بالثدييريا على عشوائي في أعمار بين 6-9 سنوات من بعض المناطق في محافظة الوادي الجديد وتم تقييمها حسب الإصابة من عمالقة إلى مجموعتين، مجموعة مصابة وعئدها 100 جمالي وعئدها 60 جمالي مشتبه بإصابته سريبا ومجموعتين ضابطة وعئدها 20 وعئدها 20 سليمة سريبا ومعمليا.

وأصبحت الدراسة إن 9 جمالي إيجابية للإصابة وإن 8 فقط منهم إيجابية باختبار السيرولوجي (الأنسج المضادة الفلورسني المشع الغير مباشر) واعتبر الآخر آنها سليماً كاذب لهذا الاختبار ووجدت 3 جمالي إيجابية لاختبار الأجسام المضادة المشع الغير مباشر وتم اعتبارها إيجابي كاذب. وبناء الدراسة أن طفيل الثدييريا يثير شكل كبير على صورة الدم تمثلت في حدوث اختلاف معنوي في العدد الكلي للكريات الدم الحمراء وهيموجلوبين الدم وحجم الخلايا المرصودة بينما لوحظ ارتفاعاً معيوناً في متوسط الحجم الكروي ونحو 5 حمض الدم وتركيز مضادات الدم.

في النهاية يمكن القول بأن معدل إصابة الثدييريا في الجمالي ضعيف وأنه لا يسبب أي إعراض سريري للجمالي ولكن الفحص المعملي يوضح اختلافاً معيناً في المعابير الدموية التي تتعلق الأنثيميا.