Some studies on prevalence and effect of Thieleria 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 from125 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 11out of 100 camelswere 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)*. *Thieleria 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 theleriosis.

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 scare 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 albendazoal 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 flouresent antibody technique (IFAT) according to *(14)*.

Fecal examination

Fecal samples were collected from camels positive for thieleriosis (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 parasitima (2%) and put on the different slide wells and fixed by acetone 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 concenteration.

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 theleriosis (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 3years 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 MCHC ($P<0.01$) of infected group than control group where that reflecting macrocytic norm chromic anemia (Table 3).

Table 1. Number of camels under study from different localities in New-Valley Governorate

Camel group	Group 1			Group 2		
Localities	EL-Karga	El-Dakla	El-Farafra	El-Karga	El-Dakla	El-Farafra
Number of animals	35	35	30	10	10	5

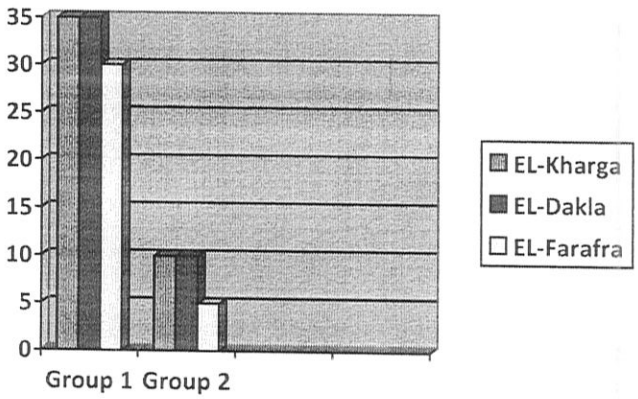

Fig. legend 1.Showing the animal localities under the study.

Table 2. the result of both direct smear and indirect antibody flouresence technique

Method \ Camel	No of examined camels	No of positive camels	False positive	False negative	Ratio (%)
Direct smear	100	9	------	--------	9%
Indirect flouresent antibody technique (IFAT)	100	11	3	1	11%

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

Parameters \ Animals	Infected camels	Control camels
PCV (%)	17.5±6.0 *↓	28.4±9.31
HB(g/dl)	7.27±2.06*↓	10.25±2.09
RBCs x10^6/µl	8.46±5.62*↓	14.38±5.67
WBCs x 10^3/µl	19.28±7.56	19.78±7.61
MCV (fl)	68.6±0.2*↑	60.1±0.4
MCH (pg)	20.3±0.1*↑	11.9±0.0
MCHC(g/dl)	44.3±0.2*↑	33.2±0.1
Neutrophil (%)	41.1±0.9	43.50±1.3
Band cell (%)	1.8±0.1	0.6±0.01
Lymphocytes (%)	54.5± 0.9	54.6±1.3
Monocytes (%)	0.7±0.1	0.7±0.1
Eosinophil (%)	0.8±0.1*↑	0.6±0.1
Basophile (%)	0.0±0.0	0.01±1.0

* Significant $P \leq 0.05$.

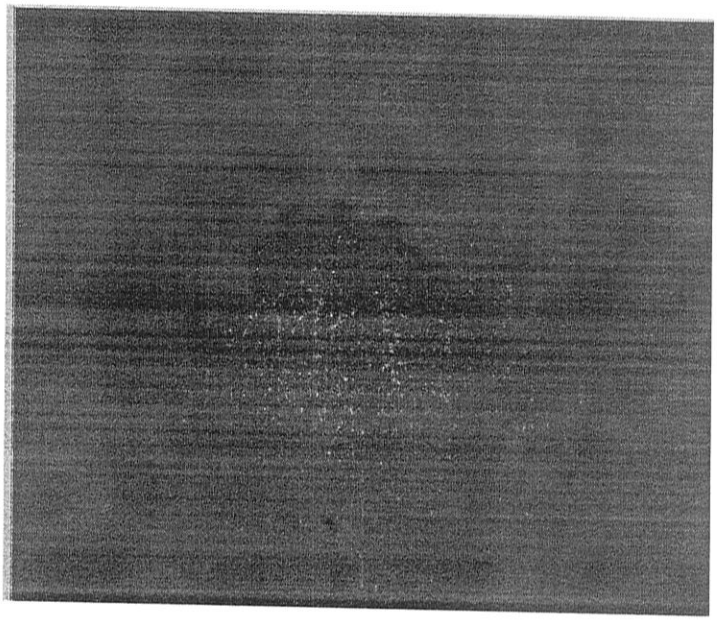

Fig. 2.Blood film from infested camels stained by Giemsa stain showing schizont of Theileria camelensis in lymphocytes. (X1000).

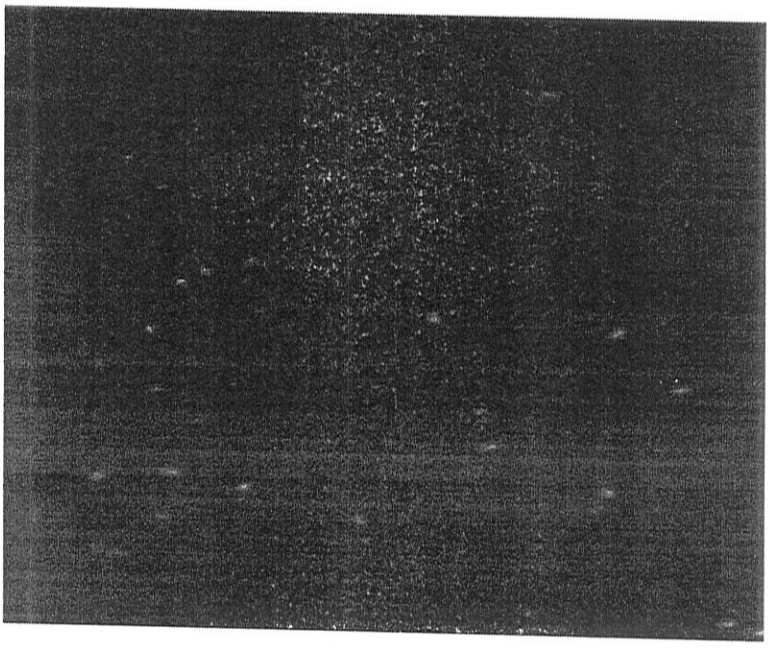

Fig.3.Greenish yellow fluorescence indicating intra-erythrocytic stages of *Theileria spp* using IFAT (×1000).

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. *Theileria camelensis* 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 mor 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 agred 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 theleriosis 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 seroepidemilogical studies of thelieriosis in camels. Our results may be attributed to misdiagnosis by microscopic examination because it is difficult to differentiate morphological structure of *theleria spp.* Also it may be explained by cross-reactivity among haemoparasites.spp, and these antibodies might be derived from immunization against theleriosis.

Macrocytic anemia was found in camel suffering from thieleriosis 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 thielieriosis 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 erythrophagocytosis in the reticulendothelial 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 considrablle 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 seroepidemilogical studies of thielieriosis in camel.

REFERENCES

1.Wikipedia (2011): Camel Retrieved 11.06.2011, 2011, from http: // en .wikipedia .org/wiki/Camel.

2.Wernery U and Kaaden OR (2002): Infectious Diseases in Camelids. Blackwell, Vienna, p. 403.

3.Uilenberg G (2006): Babesia –a historical overview. Vet. Parasitol .138, 3-10.

4.Farah A W (1995): Some immunological studies on *Theileria annulata* infection in cattle in Egypt. PhD.Thesis, Cairo University.

5.Handemir E and Dick B (1998): Prevalence of theileriosis in cattle and its vector ticks in Konya. Province. Veterinarian, 9: 1, 32-38.

6.Lawal IA, Folaranmi DO, Asselbergs M, Perie N, Okoro JE, Bale JS and Musa B (1998): Studies on prevalence Of bovine theileriosis in Nigeria Using the immune-fluorescent antibody (IFA) test and microscopic detection technique. Nigerian. Veterinary J, 19: 53-60.

7.Nasser A M (1992): Theileria infection in camels (Camelus dromedarius) in Egypt. Vet. Parasitol. 43 (1-2), 147-149.

8.El-Fayoumy M M, Abou Elnga TR, Abd El-Baky S M M and Abdou T A (2005): Prevalence of camel theileriosis and its vector tick in North Coast of Egypt. Journal of the Egyptian Veterinary Medical Association, 65: 291-302.

9.Boid R, Jones TW, Luclins A G (1985): Protozoa Diseases of Camels. Brit. Vet. J., 141 (1), 87-105.

10.Kaufmann J (1996): Parasitic Infections of Domestic Animals – A diagnostic Manual. Basel, Birkhauser Verlag. P. 274

11.Singh A P, Tanwar R, Chahar A, Wadhwa A and Shukla Y (2006): Mixed infection of Brucella and Ehrlichia in a dog-a case report. Veterinary Practitioner, 7(1): 63-64.

12.Tanwar R K, Wadhwa A, Chahar A, Singh AP and Bihani DK (2009): Seroprevalence of brucellosis and infectious bovine rhinotracheitis in buffaloes in and around Bikaner, Rajasthan, Veterinary Practitioner, 10(1): 32-33

13.Levine N D (1985): Veterinary Protozoology. Iowa State Univ. Press. pp. 365.

14.World Organization for Animal Health [OIE] (2008): Manual of diagnostic tests and vaccines for terrestrial animals [online]. Paris: OIE.

15.Soulsby E (1982): Helminthes, Arthropods and Protozoa of Domestic Animals. 7th edition, 790p.BalliereTindall,1st Annes Road, East bourne, East Sussex BN21.

16.El-Refaii MAH, Wahba AA and Shehab GJ (1998): Studies of theileria infection among slaughtered camels in Egypt. The Egyptian Journal of Medical Science 19,1-17.

17.Maha IH, Ahmed M A, Zaitoun Taha AA, El-Allawy and Mourad I M.(2011): Investigation of *Theileria camelensis* in camels infested by *Hyalomma dromedarii* ticks in Upper Egypt .Journal of Advanced Veterinary Research. 1; 4-7.

18.Mahran OM (2004): Some studies on blood parasite in camels (Camelus dromedaries) at shalatin city, Red sea Governorate. Assuit .Vet .Med .J ., 50 (102):172-18.

19.Jalailudeen LR, Hussein AK, Jashua k, Grema H, Usman A T and Harrison K D (2011): Effect of parasitic infection on erythrocytes of camels in Nigeria.Vet. Res. Forum J., 2 (1):59-63.

الملخص العربى

بعض الدراسات على معدل انتشار وتاثير الاصابة بالثايليريا على صورة الدم فى بعض المناطق فى محافظة الوادى الجديد- مصر

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الثايليريا طفيل أولى يصيب الحيوانات المستأنسة والبرية على مستوى العالم ويوثر على صحة الحيوانات المصابة ولهذا تم إجراء هذة الدراسة لتقيم تأثير إصابة الجمال بهذا الطفيل على صورة الدم وكذلك كفاءة اختبار الأجسام المضادة الفلورسينى المضادات الحيوية المشع الغير مباشر فى تشخيص هذة الإصابة فقد تم اخذ عينات دم من ١٢٥ جمل باختيار عشوائي فى أعمار بين ١-٩ سنوات من بعض المناطق فى محافظة الوادي الجديد وتم تقسيمها حسب الإصابة من عدمه الى مجموعتين ,مجموعة مصابة وعددها ١٠٠ جمل وهى مشتبه بإصابتها سريريا ومجموعة ضابطة وعددها ٢٥ وهى سليمة سريريا ومعمليا. وأضحت الدراسة إن ٩ جمال ايجابية للإصابة وان ٨ فقط منهم ايجابية باختبار السيرولوجى (الأجسام المضادة الفلورسينى المشع الغير مباشر) واعتبر الآخر أنة سلبي كاذب لهذا الاختبار ووجدت ٣ جمال ايجابية لاختبار الأجسام المضادة المشع الغير مباشر وتم اعتبارها ايجابي كاذب . وبينت الدراسة ان طفيل الثايليريا يوثر بشكل كبير على صورة الدم تمثلت فى حدوث انخفاض معنوي فى العدد الكلى لكرات الدم الحمراء وهيموجلوبين الدم وحجم الخلايا المرصوصة بينما لوحظ ارتفاعا معنويا فى متوسط الحجم الكروي ومتوسط خضاب الدم وتركيز خضاب الدم .

فى النهاية يمكن القول بان معدل إصابة الثايليريا فى الجمال ضعيف وأنة لا يسبب اى إعراض سريرية للجمال ولكن الفحص المعملي يوضح انخفاضا معنويا فى المعايير الدموية التي تعنى الأنيميا.