



## TRADITIONAL STUDY AND SEROPREVALENCE OF *THEILERIA* SPP. IN CAMELS IN MIDDLE OF IRAQ (WASIT, AL-QADISIYAH AND AL-NAJAF ALASHRAF)

Haider Hadi Alsaadi\* and Azhar Ali Faraj

Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq

\*Author for correspondence : bd.com77@gmail.com

### Abstract

The aim of this study was to identify and diagnosis of *Theileria* spp. in camels, based on microscopic examination and serological diagnosis by using indirect enzyme-linked immunosorbent assay (iELISA). The period of study was extended from 1<sup>st</sup> December, 2018 to 31<sup>th</sup> August, 2019, a total of 270 blood samples were collected from camels of both sexes, with different ages. Blood samples were collected from massacres and herds scattered in three provinces of Wasit, Al Qadissiyah and Al Najaf Al Ashraf in middle of Iraq. The microscopic examination revealed *Theileria* infection rate 28.88%. On the other hand the percentage of infection by *Theileria* spp. based on the serological diagnosis was recorded to be 59.70%. In addition statistically was recorded higher rates at >6 years (31.85%) than <6 years (21.95%) with significant differences (P<0.05). The prevalence was 15(22.38%) in male, whereas in female it elevated to 63(31.03%). The difference in prevalence due to sex was significant (P< 0.05). The results showed that the high prevalence in camel could increase the likelihood of the transmission of blood parasites diseases to other animals. Also it is very important to adopt new methods for camel prevention from protozoan blood infection as well as, the promotion and education to the risk of blood parasites for camel and others animals.

**Keywords:** Theileriosis, Seroprevalence, Microscopic Examination, ELISA and camels.

### Introduction

The breeding of camels in the world is growing expansionary due to increasing in consumption rate of its meat, milk and wool. Besides, The rise of profitability of this industry and importing of camels into the country through the South East borders make it important to recognize infectious diseases in this species. Theileriosis a fatal protozoan infection with worldwide distribution into the domestic animals. It has two important species, *Theileria camelensis*, and *Theileria annulata* (Moezi *et al.*, 2016).

*Theileria* is A tick-borne haemoprotzoal diseases also as known as Piroplasmosis that caused by several parasites which include *Babesia* spp. and *Theileria* spp. and Infects horses, mules, zebra, dogs and camels (Qablan *et al.*, 2012, 2013; Faraj *et al.*, 2019).

The diagnosis of tropical theileriosis is mainly base on clinical signs and confirmed by microscopic examination of Giemsa stained blood or lymph node smears for detect of piroplasms in erythrocyte and macroschizonts in lymphocytes (Nourollahi-Fard *et al.*, 2015).

In addition, serological tests can be utilized to detect circulating antibodies of *Theileria annulata* by using either piroplasms or schizonts as the antigen (Omer *et al.*, 2011).

Iraq is one of many countries complaining from Tropical Theileriosis and this disease is represented of the really challenge, many epidemiological studies was performed and indicated that the disease is endemic in many provinces such as, Kurdistan, Mosul, Diyala, Baghdad and Basra (Al-Robayi, 1999; Alkhaledi, 2008; Al-Saeed *et al.*, 2010; Al-khafaji and Al-Robayi, 2011; Al-Emarah *et al.*, 2012; Alsaad *et al.*, 2013). And because of unavailable of any epidemiological data about theileriosis in middle part of Iraq especially in Wasit, Al Qadissiyah and Al Najaf Al Ashraf provinces. The present study was aimed to investigate the prevalence of theileriosis infection in camels in middle of Iraq utilizing conventional and serological methods,

investigate effect of age, sex and regions on infection rates of Theileriosis.

### Materials and Methods

The study will be started from 1<sup>st</sup> December, 2018 to 31<sup>th</sup> August, 2019. A total number of 270 camel's blood samples of different ages and sexes were collected from the jugular vein evenly of middle provinces of Iraq. Blood samples will draw by 10 ml sterile syringe from camels.

### Blood samples collection

Each blood sample that will collecting is divided into two parts, the first one (5ml) without anticoagulant for serum collection, which will separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 °C till use for ELISA.

The second one (2 ml) with anticoagulant for microscopic examination. All blood collection tubes with EDTA will be inverted gently five times for directed mixing after collection (Chaudhri and Gupta, 2003).

### Blood smear examination

Giemsa stained thin blood smears (GSTBS) were prepared from the second part 2 ml of blood samples collected from all the animals (270) immediately after blood collection and examined microscopically according to (Faraj *et al.*, 2019)

### Indirect enzyme linked immunosorbent assay (iELISA)

Indirect-ELISA kit was used for serological surveillance of *Theileria* spp. IgG antibodies in serum samples of studied camels (Sunlong Biotech Co., Ltd., China).

### Statistical analysis

Statistical analysis for this study was achieved by using Chi Square tests ( $X^2$ ) at  $p \leq 0.05$  was used to analyze differences in *Theileria* spp. rate among samples, sex and different studied ages (Leech *et al.*, 2014).

## Results and Discussion

### Morphology of *Theileria* spp.

The microscopic examination revealed that only 78 from 270 examined camels were found infected with *Theileria* spp. at infection rate of 28.88% table (1). *Theileria* spp. were detected in RBCs with two forms include ;rod and comma shape as in figure (1).



**Fig. 1:** *Theileria* parasite inside a red blood cell.

**Table 1:** Total infection rate of *Theileria* spp. in camels microscopically.

Host	Total No. of animals examined	Traditional microscopy	
		+	%
Camels	270	78	28.88

Findings showed significant ( $P < 0.05$ ) impact according to the sex, a numerically, showed highest rate (31.03%) in female compare to lowest (22.38%) in males table (2). The study showed that all age groups were infected with *Theileria* parasite with variable rates, which was recorded between old and young age groups. The findings showed high (31.85%) numerically in the age group 6-12 years followed by age group >12years (30.95 %) compared to the lower rate (21.91%) in the age group <6 years. There were significant differences at  $p \leq 0.05$  between the age groups Table (3).

**Table 2:** Theileriosis in camels according to sex.

Sex	No .of samples	<i>Theileria</i> spp. infection	
		+	%
Males	67	15	22.38 <sup>B</sup>
Females	203	63	31.03 <sup>A</sup>
Total	270	78	28.88

Different letter = significant difference at  $p \leq 0.05$

**Table 3:** Theileriosis in camels according to the age groups.

Animals age	No.	<i>Theileria</i> infection	
		+	%
< 6	73	16	21.91 <sup>B</sup>
6-12	113	36	31.85 <sup>A</sup>
>12	84	26	30.95 <sup>A</sup>
Total	270	78	28.88

Similar letter = no significant difference at  $p \geq 0.05$

Different letter = significant difference at  $p \leq 0.05$

The results of the present study revealed that the local camels were infected with *Theileria* spp. and this fact confirms that the disease is widespread and affects all animal species including camels (Qablan *et al.*, 2012; Abd-Elmaleck *et al.*, 2014). In the current study, the microscopic examination confirmed that and *Theileria* spp. was infected

the camels with morbidity rate of 28.88%. Moreover the microscopic examination of the blood smear revealed that the *Theileria* spp. appear in many shapes inside the erythrocyte, These erythrocytic morphologic disorders are attributed to presence of the parasites in the erythrocytes, erythrocyte oxidation and immune-mediated processes (Luna *et al.*, 1968; YAGI *et al.*, 2002); these finding are agreement with other studies in Egypt, (Nassar, 1992) found (30%) and disagreement with recorded lower infection rate in Jordan (10%) by (Oncel *et al.*, 2007), Egypt (9.18%) by (Abd-Elmaleck *et al.*, 2014), (6.75%) in Upper Egypt by (Hamed *et al.*, 2011) and (5.8%) by (Jasim *et al.*, 2015) in the provinces of Al Muthanna and Basra, also the highest infection rate that recorded 27 (54%) by (Hassan *et al.*, 2012) and 59(39.33%) by (Kawan, 2019) with *Theileria* spp. based on the microscopic examination. Difference in results may be due to environmental conditions, geography, number of samplings, were examined, methods in those studies, vectors and poor animals health (Radostits *et al.*, 2007).

The prevalence of parasitic was found significantly ( $P < 0.05$ ) higher in female camels as compared with the male camels. This result is in disagreement with (Khamesipour *et al.*, 2015) who reported the higher infection was found in males (36.7%) whereas, (12.24%) in females. On the other hand, this result agreed with (Joshua *et al.*, 2008) who recorded the prevalence of 13.2 % in males and 15.6 % in females. (Wakil *et al.*, 2016) mentioned that the infections are more common among the female camels 73(36.1%) than in the males 49(24.3%). Also, (R Abou El Naga and M Barghash, 2016) found that the infection rate of male 32(35.6%) was significantly ( $P < 0.05$ ) less than female (67.2%). The differences could be due to the several effects such as the stress during gestation and milk production which rendering them more susceptible to blood parasites infection (Barghash, 2010; Zayed *et al.*, 2010).

The trend of infection increased along with the increasing of animal age. The high prevalence rate was detected at (>6) years age group. The results of this study are in agreement with (R Abou El Naga and M Barghash, 2016) who found that the highest prevalence rate at a group of  $12 \leq X < 6$  in all detected parasites; also (Al-Amery *et al.*, 2017) detected highest prevalence rate at (<7-8) years age group. This could be attributed to the owners and nomads who preferring to graze the animals of this age group in open fields' because in this age stage, they had a high performance and more activity. The open field increases the risk of infection because animals become more exposed to vector bites.

### Serological test

Out of 273camels sera examined by using indirect IgG ELISA, an overall prevalence of positive samples was 59.70 % Table (4).

**Table 4:** Total infection rate of Theileriosis in camels by indirect ELISA.

No. of samples examined	No. of + ve. samples	Percentage (%)
273	163	59.70

Many studies have been carried out in camels to investigate the prevalence of theileriosis, which in accordance with current results (59.70%), such as (He *et al.*, 2016) 63.75% in China. But a higher infection rate, such as 93.31% in China (Li *et al.*, 2016). Whereas a lower infection rate, such as 50.70% (Bilgic *et al.*, 2016) in India; 0% (Jesca *et al.*, 2017). Several factors account for this namely: disease management practices, tick vector abundance and climate. Climatic factors influence the habitat of tick vectors like rainfall, humidity and temperature (Oncel *et al.*, 2007).

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