

Effects of *Theileria Annulata* Infection on Hematology, Lipid Profile, and Milk Properties of Iraqi Dromedary Camels

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Abstract

Theileriosis has been reported in camels and other animals with variable severity and biological effects. However, these various effects have not been evaluated in camels as they have been in other animals. In this study, 109 apparently healthy camels of both sexes were tested for the presence of *Theileria spp.* using direct microscopic examination. Hematological values and lipid profile were examined for the participating camels in addition to the properties of the she camels' milk. From all tested camels, 25 harbored *Theileria annulata* (22.94%). Red and white blood cells, Hemoglobin, and Mean Corpuscular Hemoglobin were significantly lowered, and Eosinophil rate was significantly increased in the infected camels ($p < 0.05$). Levels of Cholesterol, Triglycerides, and Very Low-Density Lipoproteins (VLDL) were significantly reduced in infected camels, while Low-Density Lipoproteins (LDL) were significantly increased in infected camels ($p < 0.001$). Analysis of the milk properties of the participating she camels revealed no change in Fat, Lactose, or Protein content of the milk with the presence of infection; however, Total solids, Solid non-Fat, and Salt values decreased significantly in the milk of infected she camels ($p < 0.05$). In conclusion, regardless of presence of the clinical signs, infection with *Theileria annulata* can affect some hematological parameters, lipid profile, and milk properties of infected camels; highlighting the importance of specific diagnostic, therapeutic and preventive measures addressing this pathogen to promote this increasingly developing industry in Iraq.

Keywords: Dromedary Camels; Hematology; Lipid profile; Milk Properties; Theileriosis

Introduction

Camels with their magnificent adaptation to the harsh climate of the deserts are a great source for meat, milk, textile, and transportation in those areas. Tick borne diseases such as theileriosis are common in camels as they are in other farm animals such as cattle, water buffalos, sheep, and goats (Urquhart *et al.*, 2003; El-Naga and Barghash, 2016; Al-Naily and Jasim, 2018). Theileriosis has been reported as the most common tick-borne disease in camels in Egypt according to El-Naga and Barghash, 2016; while Babesiosis was more common in camels of Al-Qadisiya, Iraq (Al-Naily and Jasim, 2018). *Theileria equi*, *Theileria annulata*, *Theileria mutans*, and *Theileria ovis* have been reported in camels' blood so far (Qablan *et al.*, 2012; Tomassone *et al.*, 2012; Youssef *et al.*, 2015; Lorusso *et al.*, 2016; Sazmand *et al.*, 2016).

While *Theileria annulata* in cattle is common and causes a severe disease (Nazifi *et al.*, 2010), most infections of camels with *T. annulata* have been reported to be silent (Youssef *et al.*, 2015; Farhan and Hameed, 2017). Some researchers believe that the role and pathogenicity of *Theileria* pathogens in camels are still not clear (Sazmand and Joachim, 2017). Camels are hosts for *Theileria camelensis* and there are clinical outbreaks reported in Saudi Arabia with this pathogen (Ismael *et al.*, 2014). It is not uncommon for animals to get infected with other *Theileria spp* (Youssef *et al.*, 2015); however, studies are needed to evaluate the effect of these pathogens in camels. In dairy cattle *Theileria annulata* causes dramatic milk losses (Ayadi *et al.*, 2016) and affects some of those cows' hematological parameters (Nazifi *et al.*, 2009) and milk compositions (Perera *et al.*, 2014). Other animals' hematological and biochemical parameters have also been reported to be altered by this infection (El-Deeb and Younis, 2009; Al-Fetly, 2012; Ismael *et al.*, 2014).

To more evaluate the significance of *Theileria annulata* in camels which are of great economical value in the region, studies are needed to shed light on various biological effects of this pathogen on these animals. Since significant variations between hematological values have been reported in different breeds of camels local and breed specific assessments are necessary to fill this gap (Abdalmula *et al.*, 2018). In addition, to the best of the author's knowledge, the effect of theileriosis on camel milk properties or their lipid profile have not been assessed yet. Therefore, this study was designed to assess alterations of hematological parameters and lipid profile in

Almashael (local Iraqi breed) camels infected with Theileriosis and to investigate the changes in their milk properties.

Materials and methods

Study animals

A total of 120 apparently healthy Camels were included in this study which belonged to several herds distributed in an area near Iran-Iraq border in Wasit province. All participating camels belonged to the red-coated local breed, known as “Almashael”.

Samples collection

A total of 120 blood samples were taken from jugular veins of participating camels which were divided each into two separate tubes; one with anticoagulant (EDTA) and one without it. Thin blood smears were prepared from anticoagulated samples and were then Giemsa stained according to Chaudhri *et al.*, (2003); and examined microscopically. In addition, Complete blood count was performed using the automatic blood cell counter of Nehon system (Japan). Total red and white blood cells (RBC and WBC), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and leucocyte differential count were determined by the device. Out of all tested camels, 11 were excluded for having other hemo-parasitic infections and the remaining 109 were included in the assessment. Sera were extracted from blood samples without anticoagulant and were used for assessment of lipid profile. The lipid profile analysis was conducted using the automatic system of Fujiilm –Dri Chem (Japan). Values for Cholesterol, Triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL) were tested.

Milk samples

Of the total number of participating camels 53 she camels were producing milk and were included in the milk assessment of the study. All she camels had calved 3-7 weeks earlier. At the early morning round of milking, 10 ml of fresh milk samples were collected and kept aseptically and cold. They were then analyzed using the Ultrasonic milk analyzer (Germany). Fat, protein, Lactose, Solid non-fat, Total solids, salts, and pH of milk samples were recorded.

Statistical analysis

All data were recorded and categorized using Microsoft Excel. It was then analyzed using the SPSS software (version 20; SPSS, Chicago, IL, USA). The Chi-square test was used to find the possible correlation between age and the infection rate. Two-sample T-test was used to find significant difference in means of hematological values, lipid profile and milk components in healthy and infected camels. The same test was used to find out if there is a significant effect for age regarding the infection rate in camels. A *p* value of less than 0.05 was considered significant.

Results

The mean age of the participating camels was 6.53 years \pm 2.74 (standard deviation) and they included both sexes (91 female and 18 male). Twenty-five samples out of 109 were positive for *Theileria annulata* (22.94%) in the microscopic examination. The infection rate of camels distributed according to their sex is shown in Table 1.

Table 1- Rate of infection with *T. annulata* in participating camels' according to their sex

Sex	Uninfected (%)	Infected (%)	Total
Male	13 (72.2%)	5 (27.7%)	18
Female	71 (78.02%)	20 (21.98%)	91

Chi-square test did not show significant correlation between sex of camels and their infection rate ($p>0.05$). Moreover, results of the two sample T-test indicated no statistically significant correlations between age and presence of infection in camels ($p>0.05$). According to the results of the two sample T-test, RBC, WBC, Hb and MCH were significantly lowered in infected camels compared to those not infected alongside an increase in Eosinophils ($p<0.05$). Means of hematological parameters of infected and uninfected camels are shown in table 2. According to the results means for Cholesterol, Triglyceride, and VLDL were significantly lower in infected camels compared to the other group; while means of HDL was unaffected by the presence of infection. On the other hand, means for LDL was significantly higher in infected camels. Detailed values for Cholesterol, Triglyceride, HDL, LDL, and VLDL in healthy and infected camels are shown in table 3. Of the 53 she camels producing the milk samples, 14 were infected with *Theileria annulata*. Protein, Fat and Lactose of camels' milk were not affected by the

presence of infection ($p>0.05$); while, Solid non-fat, total solids, and salt values were significantly lowered in the milk of infected she camels ($P<0.05$). Values for components of milk samples obtained from 53 she camels are shown in table 4 according to the presence of infection.

Table 2 – Means of hematological parameters in infected and uninfected camels participating in this study

Hematological Parameters	Camel's Sex	Uninfected camels (n=84)		Infected camels (n=25)	
		Mean \pm SD	Min-Max	Mean \pm SD	Min-Max
RBC ^c (10^6 /ml)	Male	9.31 \pm 1.63	7.87-11.9	7.69 \pm 1.78	6.5-8.14
	Female	8.84 \pm 0.98	7.45-12	7.55 \pm 0.82	6.3-9.9
Hb ^c (g/dl)	Male	11.38 \pm 0.56	10.2-13	8.45 \pm 0.77	6.5-9.8
	Female	11.4 \pm 1.07	9-13.7	8.45 \pm 0.69	7.3-9.9
PCV (%)	Male	27.53 \pm 1.44	25.4-31	26.32 \pm 2.63	21.1-32
	Female	27.52 \pm 1.83	24-32.1	27.15 \pm 2.44	19-31
MCV (fl)	Male	33.38 \pm 1.32	31-35.6	31.73 \pm 1.99	25-36.19
	Female	32.98 \pm 1.84	30-37.3	32.26 \pm 2.55	26.1-37.17
MCH ^a (pg)	Male	13.51 \pm 0.91	12.35-15.3	12.44 \pm 0.29	11.1-13.1
	Female	13.5 \pm 0.79	12-15.3	13.04 \pm 0.99	10.89-15.2
MCHC (g/dl)	Male	42.93 \pm 1.43	40.1-45.7	41.32 \pm 1.74	35.3-45.2
	Female	43.29 \pm 1.95	39.9-47.4	42.91 \pm 2.03	38.42-47.4
WBC ^c (10^3 /ml)	Male	14.29 \pm 0.59	12.9-15.9	12.62 \pm 0.0	11.5-13.4
	Female	13.85 \pm 0.82	12-15.7	12.04 \pm 1.03	10.2-13.9
Neutrophil (%)	Male	54.92 \pm 5.48	47-67	55.6 \pm 6.14	45-69
	Female	56.69 \pm 6.89	46-73	56.65 \pm 6.28	45-70
Lymphocyte (%)	Male	34.54 \pm 6	20-42	30.4 \pm 6.75	20-40
	Female	33.27 \pm 6.8	20-42	29.15 \pm 5.42	20-41
Eosinophil ^c (%)	Male	2.15 \pm 0.69	1-3	8.8 \pm 1.22	3-9
	Female	2.07 \pm 0.83	1-5	7.85 \pm 2.18	3-12
Monocyte (%)	Male	7 \pm 1.8	4-10	4.8 \pm 2.28	3-9
	Female	6.31 \pm 1.78	2-10	5.95 \pm 1.9	3-10
Basophil (%)	Male	0.62 \pm 0.64	0-2	0.4 \pm 0.5	0-1
	Female	0.62 \pm 0.57	0-2	0.65 \pm 0.67	0-2

^a $P\leq 0.05$, significant; ^b $P\leq 0.01$, highly significant; ^c $P\leq 0.001$, very highly significant; Statistical analysis was conducted on means of infected and uninfected camels regardless of their sex

Table 3- Means of Lipid profile in infected and uninfected camels of both sexes participating in this study

Lipid Profile	Camel's sex	Uninfected camels (n=84)		Infected camels (n=25)	
		Mean \pm SD	Min-Max	Mean \pm SD	Min-Max
Cholesterol ^c mg/dl	Male	76 \pm 13.87	55-95	68.8 \pm 5.22	62-76
	Female	75.53 \pm 12.63	50-107	62 \pm 15.63	31-86
Triglyceride ^c mg/dl	Male	69.3 \pm 12.89	50-86	29.4 \pm 3.11	23-36
	Female	69.72 \pm 11.19	49-88	30.55 \pm 5.13	22-40
HDL mg/dl	Male	33 \pm 2.53	30-36	32.8 \pm 2.29	29-38
	Female	33.24 \pm 4.12	20-45	32.45 \pm 6.98	20-46
LDL ^c mg/dl	Male	28.82 \pm 8.92	15-40.4	45.6 \pm 17.84	20-65
	Female	28.3 \pm 7.66	10.8-44.4	43.2 \pm 19.22	8-80
VLDL ^c mg/dl	Male	13.98 \pm 2.75	10-17.6	7.8 \pm 2.05	6-11
	Female	13.9 \pm 2.21	9.8-17.6	9.5 \pm 2.64	4-14

Table 4- Means of milk properties in healthy and infected she camels participating in this study

Milk property	Uninfected she camels (n=39)		Infected she camels (n=14)	
	Mean \pm SD	Min-Max	Mean \pm SD	Min-Max
Protein%	3.56 \pm 1.24	2-7.3	3.45 \pm 0.62	2.79-4
Lactose%	3.97 \pm 0.77	2.5-4.87	4.0 \pm 0.55	3.19-4.68
Fat%	3.26 \pm 0.64	2.14-4.8	3.08 \pm 0.41	2.36-3.9
Solid Non-Fat ^c %	8.52 \pm 0.59	7.5-10.4	6.93 \pm 1.28	4.99-8.75
Salt Values ^b %	0.74 \pm 0.08	0.64-0.91	0.69 \pm 0.05	0.56-0.77
Total solids ^b %	8.89 \pm 0.54	7.98-9.99	7.5 \pm 1.46	5-9.77
pH	6.49 \pm 0.2	6.18-6.88	6.56 \pm 0.19	6.1-6.82

^a $P \leq 0.05$, significant; ^b $P \leq 0.01$, highly significant; ^c $P \leq 0.001$, very highly significant; Statistical analysis was conducted on means of infected and uninfected camels regardless of their sex

Discussion

According to the results of this study 22.94% of the camels harbored *Theileria annulata* without having clinical signs using direct microscopic examination (DME). Although, serological, and molecular methods can detect infected animals with more precision (El-Naga and Barghash, 2016; Abaker *et al.*, 2017; Alsaadi and Faraj, 2020a), but DME still remains the most popular and affordable method certified by many veterinarians in the region. Many studies have shown various infection rates of *T. annulata* in healthy camels in other countries using the same method (El-refaai *et al.*, 1998; Hamed *et al.*, 2011; Al-Naily and Jasim, 2018; Alsaadi and Faraj, 2020a). The rate of infection with *T. annulata* in the current study was lower than the rates reported in Al-Qadisiya, Iraq (46.5%) in 2018 and Egypt (50.8%) in 2016 using DME. This is while in another study also conducted on various Iraqi camels from three different provinces including Wasit (the province in which our study was conducted) a rate (28.88%) close to ours (22.9%) was reported (Alsaadi and Faraj, 2020a). It seems that geographical distribution of camels and presence of other domestic animals nearby in addition to the ticks responsible for its transmission could be a major factor in spreading various species of *Theileria* among camels and other domestic animals.

Sex and age of camels did not seem to affect the infection rate in our study (Table 1). Our findings agree with other studies that found both sexes equally susceptible to the disease (El-Naga and Barghash, 2016; Ibrahim *et al.*, 2017). However, other studies suggested that older camels could be at higher risk of infection than the younger ones (Farhan and Hameed, 2017; Alsaadi and Faraj, 2020a). Reports of higher incidence in females also do exist (Alsaadi and Faraj, 2020b). It seems that the fact that usually female camels outnumber the males in herds might have something to do with finding.

Most studies agreed with our findings in that RBC count and Hb concentration decreased in *Theileria* infected camels (Ismael *et al.*, 2014; Youssef *et al.*, 2015). Similar effects were observed in calves and cattle (Nazifi *et al.*, 2009; Alsaad *et al.*, 2013; Ayadi *et al.*, 2017; Abubakar *et al.*, 2019), sheep (Al-Fetly *et al.*, 2012), and water buffalos (Hasanpour *et al.*, 2008; El-Deeb and Younis, 2009). Moreover, most of these studies found a decrease in the PCV as well, a finding that was not present in ours (Hasanpour *et al.*, 2008; Al-Fetly *et al.*, 2012; Ayadi *et al.*, 2017). There was no significant alteration in the MCV of our infected camels suggesting

probably a milder anemia in our cases. MCV was another parameter decreased in infected camels according to Youssef *et al.* (2015); however, contradictory results were published in sheep (Al-Fetly *et al.*, 2012) and cattle (Nazifi *et al.*, 2009). The decrease in MCH that we found was not detected in the Egyptian camels infected with *T. annulata* (Youssef *et al.*, 2015). Most of our participating camels were female (91/109) and this might have affected some of the primary hematological parameters in our camels, since some studies suggest that male camels have higher values in PCV, Hb, MCV, and MCH (Abdalmula *et al.*, 2019). Anyhow, it seems that some level of anemia occurs in infected animals because of destruction of affected erythrocytes by macrophages in the spleen that can fluctuate in type with the level and severity of infection (Singh *et al.*, 2001).

Alterations of WBC counts were also variable; in that some studies on cattle and water buffalos backed our finding of Leukopenia (Hasanpour *et al.*, 2008; Ayadi *et al.*, 2017; Abubakar *et al.*, 2019; Al-Abedi *et al.*, 2020); while, other studies suggested otherwise (Youssef *et al.*, 2015). In a study on cattle, Leukocytosis was reported in some cases while leukopenia was reported in others (Ugalmugle *et al.*, 2010). Sandhu *et al.* (1998) mentioned that there was an immediate increase of WBC counts in the first stage of the disease followed by a significant decrease later in the disease process. Eosinophilia was also another finding that other studies agreed on, since eosinophils are known to fight parasites and increase subsequently in parasitic diseases (Youssef *et al.*, 2015).

Our findings revealed a significant decrease in levels of Cholesterol, Triglyceride, and VLDL with significant increase in the LDL levels of infected camels ($p < 0.001$). Youssef *et al.*, (2015), also reported a decrease in the levels of Cholesterol and Triglyceride in *Theileria* infected camels. Camels infected with *Trypanosoma evansi* were reported to have lower values of Cholesterol, Triglyceride and HDL and higher values of LDL (Asal and Al-Samarai, 2020). While we could not find significant effect on HDL levels of infected camels the beforementioned studies reported contradictory results one suggesting its increase (Yousef *et al.*, 2015) while the other suggested otherwise (Asal and Al-Samarai, 2020). More detailed studies are necessary to find out the exact effect of each parasite on the camels' lipid profile.

No significant changes in fat, protein, and lactose components of the camels' milk were found in the current study with similar results reported in cows with silent Theileriosis (Perera *et al.*,

2014). Solid non-fat, total solids, and salt values, on the other hand, were significantly lowered in the milk of infected she camels in our study. In infected cows and water buffalos' milk total solids and fat were increased while ash, protein, and lactose were reduced accompanied by lower milk yields (Memon *et al.*, 2017). Total milk yield was not evaluated in this study because of specific limitations and therefore we cannot conclude whether a change in total milk production in these camels could have affected these components or not. This variation in changes in milk properties of infected animals in various species and areas may be cleared by extensive large-scale studies evaluating the total milk yield as well.

Conclusion

According to the results of this study apparently healthy Iraqi local camels infected with *T. annulata* showed significant changes in values of some hematological and lipid profile parameters, in addition to the changes observed in their milk properties. Therefore, although Theileriosis may not be considered one of the most important pathogenic diseases in camels, still it can affect these animals' production and biological parameters silently and regardless of presence of the clinical illness. And for this reason, therapeutic and preventive measures regarding this pathogen are highly recommended to improve these animals' health conditions and to possibly increase their production potential.

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