



RESEARCH ARTICLE

Clinical, Haematological and Biochemical Alterations Associated with an Outbreak of Theileriosis in Dromedaries (*Camelus dromedarius*) in Saudi Arabia

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ARTICLE HISTORY (13-380)

Received: August 22, 2013
Revised: September 29, 2013
Accepted: October 25, 2013

Key words:

Dromedary camel
Clinical
Haematological
Biochemical
Theileriosis

ABSTRACT

The aim of the study was to investigate and describe the clinical, parasitological haematological and biochemical findings induced by natural theileriosis in *Camelus dromedarius*. Sixty-seven out of 173 dromedary camels suffering clinically from fever, anorexia, swelling of the superficial lymph nodes, a rapid loss of condition, lacrimation, abortion and/or infertility were included in the study. Parasitological examinations of blood and faecal samples were performed for all camels using Giemsa-stained blood smears and standard flotation sedimentation techniques, respectively. The clinically affected camels were diagnosed with theileriosis (n=67) with a 38.73% overall morbidity and a 0% case fatality rate. Camels that suffered from theileriosis were subjected to haematological and biochemical analysis and matched with clinically healthy camels as controls (n=23). The haematological analysis revealed a highly significant reduction (P<0.001) in the total RBC count, HGB concentration, HCT and MCV in the affected camels. In addition, significant increases (P<0.01) in platelets and PCT were observed. The biochemical analysis revealed a highly significant reduction (P<0.001) in the iron level. Significant increases (P<0.01) in GGT, AST, ALT, total bilirubin, blood urea nitrogen and LDH blood levels were detected in affected camels when compared with the controls. In conclusion, theileriosis greatly affected the hematobio-chemical parameters of dromedary camels, including the liver, kidney and muscle functions. Our results can serve as the basis for subsequent studies in dromedaries under natural and experimental conditions.

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To Cite This Article: Ismael AB, AA Swelum, AF Khalaf and MA Abouheif, 2014. Clinical, haematological and biochemical alterations associated with an outbreak of theileriosis in dromedaries (*Camelus dromedarius*) in Saudi Arabia. Pak Vet J, 34(2): 209-213.

INTRODUCTION

Old World camels inhabit some of the most inhospitable areas of the world, including the Bactrian camel (*Camelus bactrianus*) in the high deserts of Asia and the Dromedary camel (*Camelus dromedarius*) in the hot, dry deserts of the Middle East. Camels are famous for their ability to subsist on harsh, dry and sparse vegetation, at least for a time (Fowler, 1996; Farooq *et al.*, 2012). Before 1970, very little was known about the infectious diseases of camels. However, during the last two decades, there has been a tremendous increase in the number of scientific papers published about camels. It is known that

infectious diseases cause more than 50% of fatalities in camelids.

Although ticks are often found on camels in large numbers (Hamed *et al.*, 2011; Nazifi *et al.*, 2011; Alim *et al.*, 2012), very few reports have been published concerning tick-borne pathogens in camels. In addition, some case reports are not considered reliable because they usually fail to give adequate taxonomic descriptions. Theilerioses are the tick-borne protozoan diseases associated with *Theileria* spp. (*Piroplasmida*, *Theileridae*) in cattle, sheep and goats as well as in wild and captive ungulates. The genus *Theileria* belongs to the Apicomplexa phylum, which includes Babesia,

Toxoplasma, Neospora, Plasmodium and others (Radostits *et al.*, 2007). Theileriosis is one of the most devastating blood parasites affecting cattle in Saudi Arabia, causing lethal infections in exotic cattle (El-Metenawy, 2000), but very little is known about this disease in camels. *Theileria* spp. that have been reported in camels include *Theileria camelensis* and *T. dromedarii*; the former has been recorded in Turkmenistan, Egypt and Somalia. However, no schizont stages of these species have been described. *T. dromedarii* has been reported in India and is thought to be non-pathogenic (Hekmatimoghaddam *et al.*, 2012). Whereas *Theileria equi* and *Babesia caballi* of equine were identified by PCR in Jordanian dromedaries (Qablan *et al.*, 2012). In addition, *T. ovis* of ovine and *H. anatolicum* ticks were detected in Egyptian dromedaries (Mazyad and Khalaf, 2002). *Hyalomma dromedarii* ticks are the principal vector in transmission of *T. camelensis* among camel population and *theileria* has various developmental stages of different shapes and forms inside this vector (Salimabadi *et al.*, 2010; Hamed *et al.*, 2011). Therefore, prevention of the disease by controlling ticks seems necessary and a prerequisite for improving camel meat and milk production (Nazifi *et al.*, 2011; Hekmatimo-ghaddam *et al.*, 2012). 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 especially superficial cervical lymph nodes were also noticed (El-Fayoumy *et al.*, 2005; Hamed *et al.*, 2011).

Haematology has been widely used in attempts to provide information about disease states, performance problems and fitness in animals. A deviation of certain blood parameters from their normal limits might serve a guide for diagnosis or for the differential diagnosis of a disease condition (Mal *et al.*, 2001).

The aim of this investigation was to describe the natural infection of an outbreak of theileriosis in dromedary camels and to study its effect on hematological and biochemical parameters.

MATERIALS AND METHODS

Animals: The present study was carried out on a dromedary camel herd composed by 173 camels aged 2-9 years from the Riyadh region, Saudi Arabia. Sixty-seven camels suffered clinical signs which were indicative of theileriosis and were infested with ticks. Camels from the same herd that were clinically healthy, not infested with ticks, not received anti-parasitic or antimicrobial therapy during the 30-day period before the beginning of the study, had no surgical interference, non-pregnant, not recently delivered or vaccinated were selected as control animals (n=23), and a complete analysis was performed to confirm that they were free from any disease. The selected animals had been reared under similar feeding systems, management and environmental conditions. All camels were subjected to careful clinical and laboratory investigations.

Sample collection and parasitological examination: Faecal and blood (EDTA tube and plain tube) samples

were collected from all camels. Giemsa stained blood films were examined microscopically for presence of *Theileria camelensis* and other blood protozoa. Additionally, ten millilitres of EDTA tube blood samples was centrifuged for 15 minutes in microhaematocrit tubes and analysed for *Trypanosoma evansi* in its buffy coat layer (Tejedor-Junco *et al.*, 2011). Moreover, a standard flotation sedimentation technique was carried out on the faecal samples for the detection of gastrointestinal parasites (Coles, 1986).

Haemato-biochemical analysis: A complete blood count was conducted using an Automatic Blood Cell Counter (BC-2800 Vet Analyzers – China) according to Feldman *et al.* (2000). The biochemical analysis including liver, kidney and muscle functions in addition to elements was performed in accordance with the Bio-system A-15 automated biochemistry analyser-Spain.

Statistical analysis: The Statistical Products and Service Solutions program (version 16, SPSS Inc., Chicago, IL, USA) was used for all analyses. Data are expressed as the mean±SE. Comparisons among groups were tested using an analysis of variance (ANOVA). Differences were considered to be significant at P<0.05.

RESULTS

Clinical picture: The clinically affected camels were diagnosed with theileriosis, with 38.73% overall morbidity and a zero percent case fatality. A sudden increase in the number of cases of *Theileria* was observed: 11 cases were observed at the beginning of the study, which increased to 37 cases and finally to 67 cases (38.73%) during 53 days of clinical period. The observed clinical signs included fever, anorexia, swelling of the superficial lymph nodes, a rapid loss of condition and lacrimation. However, not all animals showed the typical clinical picture and the majority (n=54) showed mild manifestation. Mixed infections of *Theileria* with other pathogens, including *Babesia*, *Anaplasma*, gastrointestinal nematodes and *Balantidium coli*, gave approximately the same picture as infection with *Theileria* alone, with no significant difference.

Parasitological findings: *T. camelensis* was detected in Giemsa-stained blood smears from 67/173 camels. The 67 camels with positive blood smears were the unique animals in the herd presenting clinical signs that might indicate the 1st introduction of theileriosis in this herd. *T. camelensis* trophozoites were observed within the infected RBCs and appeared as a light ring shaped with a red chromatin dot at one side and they did not appear as the typical form of other animal species. Schizont stages were observed within the infected lymphocytes and appeared as a mass of light-bluish bodies. Forty camels of 67 were infected with *Theileria* only, and the other 27 camels were infected with *Theileria* and other pathogens (7 cases showed mixed infection of *Theileria* and *Babesia*, one case showed mixed infection of *Theileria* and *Anaplasma*, 16 cases showed mixed infection of *Theileria* and gastrointestinal nematodes, and 3 cases showed mixed infection of *Theileria* and *Balantidium coli*). The degree

Table 1: Hematological parameters (Mean±SE) of clinically healthy and *Theileria*-infected camels

Parameters	Units	Clinically healthy control camels (n=23)	Camels infected with <i>Theileria camelensis</i> (n=40)	Camels infected with <i>Theileria camelensis</i> and other parasites ^a (n=27)
WBCs count	×10 ⁹ /L	11.45±0.88	12.62±0.51**	14.46±0.84***
RBCs count	×10 ¹² /L	10.69±0.51	8.85±0.29***	8.21±0.27***
HGB	g/dL	13.23±0.55	9.35±0.27***	9.31±0.34***
HCT	%	31.33±1.02	26.79±0.88*	27.90±0.97**
MCV	fL	32.33±0.88	28.70±0.42***	28.37±0.63***
MCH	pg	12.44±0.37	12.31±0.28	12.47±0.21
MCHC	g/dL	40.88±1.29	41.50±0.67	43.79±1.13 [†]
RDW	%	16.85±0.31	16.79±0.16	16.59±0.15
PLT	×10 ⁹ /L	118.23±1.63	130.20±1.88**	129.25±2.45**
MPV	fL	6.04±0.11	6.10±0.09	6.04±0.09
PCT	%	0.076±0.003	0.087±0.002***	0.082±0.002***
PDW	%	14.32±0.05	14.44±0.17	14.27±0.17

WBCs: White blood cells, RBCs: Red blood cells, HGB: haemoglobin concentration, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red distribution width, PLT: Platelets, MPV: mean platelet volume, PCT: plateletcrit, PDW: platelet distribution widths; ^aOther parasites: *Babesia*, *Anaplasma*, gastrointestinal nematodes and *Balantidium coli*; ***=significant (P<0.001), **=significant (P<0.01), *=significant (P<0.05)

Table 2: Biochemical parameters (Mean±SE) of clinically healthy and *Theileria*-infected camels

Parameters	Units	Clinically healthy control camels (n=23)	Camels infected with <i>Theileria camelensis</i> only (n = 40)	Camels infected with <i>Theileria camelensis</i> and other parasites ^a (n = 27)
Liver function				
Total protein	g/dL	5.78±0.17	6.11±0.12	6.02±0.14
Albumin	g/dL	3.42±0.15	3.34±0.12	3.11±0.13
Globulin	g/dL	2.64±0.13	2.97±0.12	2.83±0.15
GGT	μ/L	8.23±1.03	38.72±6.61*	39.82±9.53*
AST	μ/L	97.88±4.66	193.68±18.29**	195.53 ±19.81**
ALT	μ/L	9.19±0.83	27.29±2.65***	17.003±1.43***
Total bilirubin	mg/dL	0.35±0.05	0.60±0.05**	0.50±0.04**
kidney function				
Creatinine	mg/dL	1.15±0.12	2.38±0.33***	1.24±0.05*
BUN	mg/dL	26.82±2.09	52.03±5.97**	44.85±1.87**
Muscle function				
LDH	μ/L	807.54±84.65	1742.9±133.7***	1743.1±191.9***
Elements				
Iron	mg/dL	126.15±4.95	70.51±4.51***	72.92±5.76***

GGT: gamma-glutamyltransferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, LDH: lactate dehydrogenase; ^aOther parasites were *Babesia*, *Anaplasma*, gastrointestinal nematodes and *Balantidium coli*; ***=significant (P<0.001), **=significant (P<0.01), *=significant (P<0.05)

of *Theileria* infection varied from severe, moderate to mild infection depending on the percentages of infected red cells of the various camels, which ranged between 1 and 9%. No trypanosome was detected in the buffy coat.

Haematological findings: The mean values and standard error (SE) of the haematological parameters in clinically healthy camels and *Theileria*-infected individuals are presented in Table 1. Camels infected with *Theileria* alone showed a highly significant reduction (P<0.001) in the total RBC count, HGB concentration, HCT and MCV, indicating the microcytic hypochromic type of anaemia. Significant increases (P<0.01) in platelets and PCT were observed, whereas the other haematological parameters were close to normal values. Sex, age, lactation and seasonality slightly influenced the haematological

parameters and there were no significant differences (results not shown).

Biochemical findings: The mean values with the SE of biochemical parameters, including liver, kidney and muscle functions and elements, in clinically healthy and *Theileria*-infected camels are shown in Table 2. A highly significant reduction (P<0.001) in the mean values of iron was determined in camels infected with *Theileria* alone when compared with the mean values of controls. Highly significant increases (P<0.01) in the mean values of GGT, AST, ALT, total bilirubin, blood urea nitrogen and LDH were found in affected camels when compared with the mean values of control camels.

DISCUSSION

Most of the relevant previous studies described the clinical findings of theileriosis in cattle and other species, but few studies have described the clinical picture of this disease in camels. In Egypt, Nassar (1992) examined 200 apparently healthy camels and found that 30% were infected with *Theileria* spp. In contrast, only 6.75% of the examined camels were harboring the erythrocytic forms of *Theileria camelensis* in another study in Upper Egypt (Hamed *et al.*, 2011). In Iran, Hekmatimoghaddam *et al.* (2012) examined 114 apparently healthy camels and found that 15.79% were infected with *Theileria* spp, while Borji *et al.* (2009) did not find *Theileria* spp. organisms in their epidemiologic study on 262 camels in eastern Iran. In our study, 67 of 173 camels were infected with *T. camelensis* (38.73%). This variation in prevalence rate might be attributed to localities, methods in those studies, especially in selection of animals population, density of camels, hygienic measures, circumstances and environment. The enlargement of superficial lymph nodes could be explained by lymphoid hyperplasia in the early stage of the disease (Radostits *et al.*, 2007).

Parasitologically, *T. camelensis* was detected in Giemsa-stained blood smears from 67 of 173 camels in the present study. However, species identification can be hampered due to developmental changes in the form and size of parasitic stages in the erythrocyte and because of some piroplasmid species may differ in form and size when infecting different vertebrate hosts (pleomorphism) (Homer *et al.*, 2000). Moreover, one vertebrate host may be infected by several different piroplasmid species, and similarly, “vertebrate host specificity” is not a reliable taxonomic criterion. This challenge is evident in the observation that several species are routinely found by molecular methods in a single wildlife animal and are often indistinguishable even by microscopic observation (Jinnai *et al.*, 2010).

In general, the pathogenesis of various forms of theileriosis is dependent on the production of schizonts in lymphocytes and piroplasms in erythrocytes. In our study, the *T. camelensis* produce numerous schizonts and piroplasms and all infected camels showed the clinical picture that might indicate the high pathogenicity and the 1st introduction of theileriosis in this herd. The description of *T. camelensis* lacks any information on developmental stages in the previous studies (Nassar, 1992). In other *Theileria* spp., *T. parva*, *T. annulata* and

T. hirci produce numerous schizonts and piroplasms and are highly pathogenic; *T. mutans*, *T. buffeli* and *T. ovis* rarely produce schizonts but may cause varying degrees of anaemia when there are large numbers of piroplasms in red blood cells; and with *T. velifera* and *T. separata*, no schizonts have been described, the parasitaemia is usually scanty and the infection is mild or subclinical (Radostits *et al.*, 2007).

Haematologically, the mean values of the haematological parameters in clinically healthy camels in the present study lie within the normal ranges previously reported by Mal *et al.* (2001). These parameters may vary from place to place and from time to time, which may affect the validity of the analysis. Affected camels in this study showed a highly significant reduction ($P < 0.001$) in the total RBC count, HGB concentration, HCT and MCV, reflecting a microcytic hypochromic type of anaemia (Table 1). Although many evidence has been presented to explain the mechanism of the anaemia, the exact underlying mechanism is currently unknown (Shiono *et al.*, 2004). Boulter and Hall (2000) reported that anaemia has been claimed to be a result of the removal of erythrocytes by phagocytosis rather than lysis induced by multiple invasion of parasite to the RBCs. Additionally, tumour necrosis factor- α (TNF- α) has also been implicated in the pathogenesis of anaemia by suppressing haematopoietic progenitors, resulting in decreased erythrocyte production and decreased erythrocyte survival (Boulter and Hall, 2000). A significant increase ($P < 0.01$) in platelets and PCT was observed in affected camels, which confirms the presence of anaemia. Mixed infections of *Theileria* with other pathogens showed no significant difference when compared with *Theileria* infection alone indicating that the changes in these parameters attributed mainly to *Theileria* infection.

The mean values of the biochemical parameters in clinically healthy camels lie within the normal ranges previously reported by Ayoub *et al.* (2003). A highly significant reduction ($P < 0.001$) in the mean values of iron was found in affected camels when compared with the mean values of control camels. Significant increase in the mean values of GGT, AST, ALT, total bilirubin, blood urea nitrogen and LDH was found in affected camels when compared with the mean values of control camels. The increase in the level of GGT, AST and ALT could be explained by damage to the skeletal or heart muscles, hepatic tissues and erythrocytes because bulk of those tissues throughout the body can be considered as an ample reservoir of enzymes liable to be released and detected during a pathological condition (Kataria and Bhatia 1991). The hyperbilirubinaemia could be attributed to excessive destruction of RBCs and the indirect hepatocellular damage. The increased level of blood urea nitrogen may indicate indirect damage of renal tissue and the presence of globin catabolites liberated from haemoglobin lysis by the reticulo-endothelial system through the process of erythrophagocytosis (Kataria and Bhatia, 1991; Qarawi, 1999).

We can conclude that theileriosis has a deleterious effect on the health of camels and affects their haematological and biochemical parameters. Our study represents the first description of *T. camelensis* developmental stages and can serve as the basis for

subsequent studies in dromedaries under natural and experimental conditions. The increasing identification and description of novel species in a great array of hosts allows us to foresee that *Theileria* research will become a highly dynamic field in the years to come. The piroplasmids reported so far from dromedaries are either specific for these hosts or may represent species known from other hosts that have been transmitted to camels via shared ticks. A synonymisation of *T. camelensis* with *Theileria equi* was proposed in 1926, and a more recent report indicates that both *Theileria equi* and *Babesia caballi* can indeed infect camels (Qablan *et al.*, 2012). Undoubtedly, the application of molecular diagnostic methods has promptly improved our understanding of the diversity of piroplasms in terms of increased diagnostic sensitivity, determination of new or cryptic species, and understanding the intraspecific genetic diversity (Criado-Fornelio *et al.*, 2003). DNA of *Theileria mutans* was also recently detected in camels (Tomassone *et al.*, 2012). Therefore, further study to distinguish between these scenarios using molecular diagnostic methods on the isolated *Theileria* of the present study is necessary. Moreover, studies focusing on the host specificity and genetic diversity of piroplasmids in camels are necessary.

Acknowledgement: The authors extended their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project RGP-VPP-282.

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