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Main Haemato-biochemical disorders in camel trypanosomiasis in Tunisia

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Abstract:
The aim of the present study was to evaluate the impact of *Trypanosoma evansi* infection on the blood profile of Tunisian dromedary camels. Serological, haematological and biochemical analysis were performed on blood samples collected from 220 dromedary camels (*Camelus dromedarius*). Giemsa-stained blood smears were used to establish a differential leucocyte count. Others haematological parameters were measured on whole blood collected in EDTA tubes. Sera were used both to perform CATT and to measure some biochemical parameters. More than half of samples (62.7%) were seropositive for trypanosomiasis infection. The comparison of haematological profiles between infected and non-infected camels revealed the presence of a microcytic hypochromic anaemia, neutrophilia, monocytosis and eosinophilia. The biochemical profile of the infected animals showed liver dysfunction (decreased urea and total proteins) and hypoferremia, the last abnormality may lead to microcytic anaemia. Results of the present study revealed that *T. evansi* was highly prevalent in Tunisian dromedary camels and may induce severe biological disorders. Haemato-biochemical parameters, markedly affected by *T. evansi*, may be used as biomarkers for the control of this infection in camels.

Key words: Biochemical parameters, Haematological parameters, *Trypanosoma evansi*, dromedary camel, blood profile, Tunisia.

1. Introduction

Camels are considered as an important source of protein for humans for their ability to convert the scant resources of the desert into milk and meat (Sazmand et al., 2022). According to the most recent data, the global population of dromedary camels (*Camelus dromedarius*, one-humped camel) is estimated to be around 35 million, of wich80% of them are bred in Africa. In Tunisia, The national cameline herd is approximated to 80,000 female units (OIE, 2021), mainly located in the southern part of the country (96%). the southern area of Tunisia is characterised by an arid climate, where the dromedary rearingis based on traditional pastoral production systems, and embodies an important activity for its ecological, economic and social positive impacts (Salmi et al., 2018). Even though the camels are the most adapted species to the harsh conditions of arid and semi-arid rangelands, and are relatively less prone to several of the devastating diseases affecting other animal species, they are littered with several specific illnesses. Trypanosomiasis is a serious disease in camels caused by *Trypanosoma evansi*, and is endemic in tropical and subtropical areas (Sazmand et al., 2022). It represents a major threat to cameline livestock production, and is the most pathogenic and economically important protozoan infection of camels, leading to an impaired milk and meat production, decreased performances or even the death of affected animal (Sazmand et al., 2022). The parasite is widely distributed in Asia, Central and South America and Africa, including Tunisia. It is mechanically transmitted by hematophagous biting flies and affects a large scale of domestic and wild mammals, but historically, the main host was the camel (Sazmand et al., 2022). The course of a *T. evansi* infection ranges from an acute disease with high mortality to a chronic infection characterized by non-specific signs such as subcutaneous oedema, fever, lethargy, weight loss and abortion (Sazmand et al., 2022). Clinical signs are linked to the several lesions observed in different tissues, and to the immune response induced by the parasite (Enwezor et Sackey, 2005), which leads to different alterations in blood profile (Hussain et al., 2016). Clinical and

parasitological diagnosis (Tehseen et al., 2015) are insufficient to monitor the disease, as the clinical signs are non-pathognomonic wen the parasites are absent in the blood, while the changes in blood parameters are biomarkers of various disease conditions including trypanosomiasis (Hussain et al., 2016). In Tunisia, despite the large cameline population and the reported outbreaks of trypanosomiasis, the disease is poorly documented and is underestimated. Only a few studies on the prevalence of trypanosomiasis in the camel population were conducted (Kalthoum et al., 2022; Selmi et al., 2019), while no studies on haemato-biochemical disorders were ever carried. The present study aims to evaluate the impact of *T. evansi* infection on some haematological and biochemical parameters, by comparing infected and non-infected animals. The results of this study will be useful for the diagnosis, the surveillance and the control of *T. evansi* infection.

2. Materials and methods

2.1. Ethical statement

the design and procedures of our study were approved by National Committee on Ethics in Animal Experimentation of the National School of Veterinary Medicine of Sidi-Thabet (CEEA-ENMV, Approval number CEEA-ENMV 13/20). The sampled animals were owned by private camel herders., who were aware of the objectives of the study, and their animals were sampled with their permission, in their presence under the supervision of a qualified veterinarian. The sampling procedure was conducted in accordance with the relevant national and international guidelines on animals handling, with a special care to respect the animal welfare. During or after the sampling process, no animal was injured or dead, and no female aborted.

2.2. Study area

The present study was carried from July 2019 to September 2020 in three Southern Tunisian governorates: Medenine, Tataouine and Kebili (Figure 1).

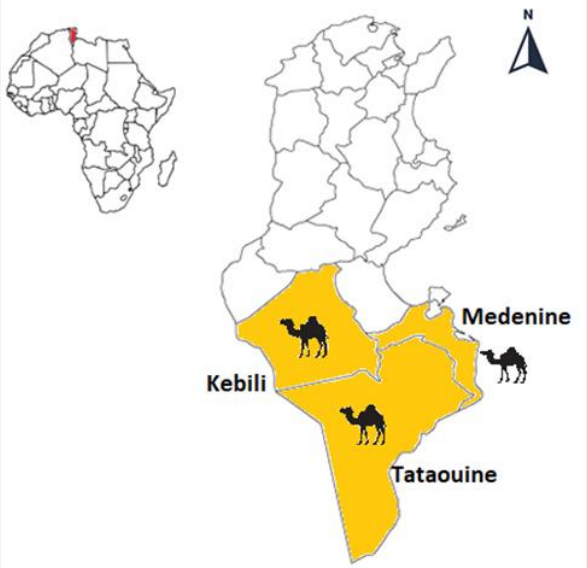

Figure 1. Sampling areas (Medenine, Tataouine and Kebili) showed on the map of Tunisia.

These regions are located in the southern part of Tunisia, a transitional region towards the Sahara, and includes lowlands spreading south of high steppes, from the Algerian border to the Gulf of Gabes. According to Köppen climate classification, the south of Tunisia has a hot semi-arid climate (BSh) and a hot desert climate (BWh), with an extremely hot summer, a warm winter and a very low annual rainfall (84 – 219 mm). All the selected regions are located between 33°52’ and 33°16’N and 10°46’ and 8°58’E, with an altitude varying between 6 and 238 m above sea level, and annual temperatures ranging from 19°.8 to 21.4°C. These regions were selected for the high number of dromedaries, and the sampled animals were chosen by the willing dromedary herdsmen.

2.3. Study animals

A total of 220 dromedary camels of both sex (157 females and 63 males, with a sex ratio F:M of 2.5), randomly selected by their handlers were sampled. Their age varied between 10 months and 25 years (mean age: 8.8 ± 5.6 years). Animals were indexed according to their age into 3 groups: 57 young animals (less than

4 years), 78 adults (between 5 and 11 years) and 85 old animals (more than12 years). According to their origin, 92 camels were from Medenine, 78 from Kebili and 50 from Tataouine.

2.4. Blood collection and diagnostic tests

All the camels were clinically examined, and blood samples were withdrawn from the jugular vein into three 5 ml vacutainer tubes, with and without anticoagulant. Giemsa-stained thin blood smears (GST) were performed for each collected EDTA blood sample (OIE, 2021). Sera and heparinized plasma were obtained by centrifugation of the blood collected on dry and heparinized tubes respectively, at 3,000 rpm for 10 minutes and stored at -20°C until used. Card Agglutination Test for Trypanosomiasis (CATT) was performed on sera as per the manufacturer's instructions (Institute of Tropical Medicine, Antwerp, Belgium). A specimen was considered positive when blue agglutinates were visible after 5 minutes of agglutination (Bajyana and Hamers, 1988).

2.5. Haematological studies

Blood samples collected in EDTA tubes were used to analyse different haematological parameters using an Auto Hematology analyzer BC-2800Vet® (Shenzen Mindray BioMedical Electronics Co., Ltd., Shenzhen, China). The haematological study included red blood cell count (RBC, $\times 10^9$/ml), haemoglobin concentration (Hb, g/dl), packed cell volume (PCV, %), mean corpuscular volumes (MCV, fl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, %Hb), red cell distribution (RDW, %) and white blood cell count (WBC, 10^6/ml). Animals are considered anaemic when haemoglobin was below 10.2 g/dl threshold value (Islam et al., 2019).

Differential leukocyte counts such as lymphocytes, neutrophils, monocyte, eosinophils and basophils were estimated as cross sectional method according to Blumenreich (1990). Obtained haematological findings were compared to the usual values reported by Islam et al. (2019). Abnormal RBC were detected in Giemsa-stained thin blood smears examined under a microscope at 1000× magnification with immersion oil.

2.6. Biochemical studies

Plasma and sera were used to measure biochemical parameters with an automatic biochemical analyser (Abott Architect Ci8200, Abbott Park, Illinois, U.S.A.). Urea (mmol/l), creatinine (Crea, µmol/l), total protein (TP, g/l) and Iron (Fe, µmol/l) levels were evaluated for each blood sample. Obtained levels were compared to the usual values reported by Ben Romdhane et al. (2003) and Abdalmula et al. (2018).

2.7. Statistical analysis

The descriptive study was performed using Microsoft Excel 2016. All data were analysed by Statistica 6.0.0 software (Tibco, California, USA). First, the distribution of each variable was tested by the Kolmogorov–Smirnov test. Then, quantitative values were expressed as median and range, for each parameter. Statistical differences between infected and non-infected camels were evaluated using Student t test for Haematological parameters (RBC, Hb, PCV, MCV, MCH, MCHC, RDW) and biochemical parameters (Urea, Crea, TP and Iron). Chi square test was used to compare proportions of neutrophils, lymphocytes, monocytes, eosinophils and basophils between two animal groups. All statistical tests were considered significant at a threshold of 0.05.

3. Results

3.1. Serological findings

More than the half (62.7%; 138/220) of blood samples were tested positives to *T. evansi* by CATT. The highest seroprevalence was observed in camels from Kebili governorate (75.6%; 59/78), followed by Medenine governorate (40.2%;37/92) and Tataouine governorate (54 %;27/50) (p=0.091).

According to the grade of CATT, 39.1% (54/138) of the seropositive samples were classed as grade 1, 29.7% (41/138) as grade 2 and 31.2% (43/138) as grade 3,and the difference was statistically significant (p=0.007). Male camels showed a significantly higher seroprevalence (79.3%, 50/63) than females (48.4%, 76/157) (p=0.006), while age had no significant effect (p=0.558) on *T. evansi* infection. The seroprevalence was significantly higher during dry season (73.5%, 125/170) compared to wet season (54%, 27/50) (p=0.006) (Table 1).

Table 1. Number and percentage of positive samples using serological test.

Variable	Category	Total of examined animals	Positive samples	Prevalence (%)	Chi-square	p value
Sex	Male	63	50	79.3	**7,547**	**0.006**
	Female	157	76	48.4		
Age group	Young ≤ 5 years	57	25	43.8	1,163	0,558
	Adult of 5 to 11 years	78	53	67.9		
	Old ≥ 12 years	85	60	70.6		
Season	Dry season	170	125	73.5	**7,330**	**0,006**
	Wetseason	50	27	54		
Governorates	Medenine	92	37	40.2	**8,032**	**0,091**
	Tataouine	50	27	54		
	Kebili	78	59	75.6		
CATT grade	Grade 1	138	54	39.1	**7.880**	**0.007**
	Grade 2	138	41	29.7		
	Grade 3	138	43	31.2		
Overall		220	138	62.7		

3.2. Blood profile

The haematological parameters showed a significant decrease in Hb, PCV, MCV and MCH (p=0.026; 0.005; <0.001 and <0.001, respectively) and a significant increase of RDW (p=0.039) in infected camels compared to non-infected ones. However, RBC, MCHC and WBC did not vary between infected and non-infected camels (p=0.356, 0.995 and 0.078, respectively). Significant decrease in Hb confirmed the occurrence of anaemia in more than the third of seropositive camels (32.6± 4.4 %, 45/138). Their haemoglobin concentration ranged between 4.9 and 9.4 g/dl (mean 7.3 ± 1.2 g/dl). In 18.1% (25/138) of the seropositive samples, decreased Hb was associated to a decreased RBC. Moreover, decreased Hb was associated both with a decreased RBC and a decreased PCV in 5.1% (7/138) of animals. A significant decrease in MCV and MCH confirmed that anaemia was microcytic and hypochromic in the infected animals. Significant increased RDW confirmed the anisocytosis in 63.8± 4.5 % (88/138) of the infected camels. Leukogram showed a leucocytosis in both infected and non-infected camels (p=0.078). the leucocytosis was mainly related to neutrophilia, observed in 72.4 ± 3.8 % (100/138) of infected camels. Although, monocytes and eosinophils proportions were within reference values of the species, they were significantly higher in the infected animals compared to the non-infected ones (p<0.001). Whilst basophils proportion did not vary between the two animal groups (p=0.779). Nitrogen and protein parameters (Urea, Creatinine, TP) were significantly lower in the infected compared to the non-infected animals (p<0.01; 0.006 and 0.036, respectively). Decreased Urea, Creatinine and TP was recorded in 13.9± 3 % (18/129), 11.6 ± 2.8 % (16/138) and 6.2 ± 2 % (8/138) of the infected camels, respectively. Serum iron concentration was lower in the infected than in the non-infected camels (p=0.048). Severe iron deficiency was confirmed in 18.8± 3.5 % (26/138) of the infected animals and reached 3.8 µmol/l. The lowest iron concentration recorded during this study in an infected animalwas of 6.8 µmol/l (Table 2).

Table 2. Results of blood parameters in infected (Positive CATT) and non-infected camels (Negative CATT)

		Non-infected camels (n=102)		**Infected camels (n=138)**		
	Standards	**Median**	**Range**	**Median**	**Range**	**P value**
RBC (×10^9/ml)	6.7 – 17.3[(a)]	10.3	6.6-12.9	10.2	6.1 – 12.9	0.356
Hb (g/dl)	10.2 – 15.3[(a)]	12.2	5.5-14.2	12	4.9 – 14.2	**0.026**
PCV (%)	27 – 45 [(a)]	40.3	29.3-50	39	15 – 50.3	**0.005**
MCV (fl)	28 – 45 [(a)]	38.8	31.7-46.9	37.5	24.8 – 46.3	**<0.001**
MCH (pg)	8.6 – 13[(a)]	11.6	7.9-13.9	10.8	7.8 – 13.6	**<0.001**
MCHC (%Hb)	33 – 41 [(a)]	29.9	31-32.1	29.9	21.4 – 33.3	0.995
RDW (%)	13 – 18[(a)]	18.2	15.7-22.5	18.7	14.8 – 24.7	**0.039**
WBC (10^6/ml)	8.2 – 16.5 [(a)]	20.3	5.2-42.2	18.3	8 – 44	0.078
Lymphocytes (%)	39.5 – 57.8 [(a)]	61.7	14.5 – 66.4	53.5	12.1 – 77.6	**<0.001**
Neutrophils (%)	23.7- 43.2 [(a)]	33.7	26.2 – 77	38.9	14.4 – 77.3	**<0.001**
Monocytes (%)	1.5 – 6.6[(a)]	1.9	0.7 – 19.1	1.9	0.7 – 25.7	**<0.001**
Eosinophils (%)	2 – 8.8 [(a)]	0.9	0.4 – 16	0.9	0.4 – 27.4	**<0.001**
Basophils (%)	0 – 1 [(a)]	1.7	0-1.9	0.9	0 – 2.9	0.779
Urea (mmol/l)	3 – 9.9 [(b)]	5	1.7 – 10.8	4	1.7 – 8.6	**<0,001**
Creatinine(µmol/l)	70.7 – 167.9 [(b)]	78.7	47.7 – 268.7	67	55.7 – 202.4	**0,006**
TP (g/l)	56 – 78 [(b)]	65.5	29 – 80	64	37 – 75	**0,036**
Iron (µmol/l)	12.5 – 21.5 [(c)]	14.9	3.4 – 33.5	16.6	6.8 – 34.4	0,048

RBC: Red Blood Cell; **HB**: Haemoglobin; **PCV**: Packed Cell Volume; **MCV**: Mean Corpuscular Volume; **MCH**: Mean Corpuscular Haemoglobin; **MCHC**: Mean Corpuscular Haemoglobin Concentration; **RDW**: Red cell Distribution Width; **WBC**: White Blood Cell. **TP:** Total Protein**.**Standards: [(a)] Islam et al. (2019), [(b)] Ben Romdhane et al. (2003), [(c)]Abdalmula et al. (2018). Statistically significant differences are indicated in bolded characters.

4. Discussion

In Tunisia, despite the large population of camels, especially in the south of the country, and the reported outbreaks of Surra, limited data on the disease is available. To our knowledge, this study is the first report on haematological and biochemical modifications occurring during *T. evansi* infection in the Tunisian dromedary camels.

The present study was conducted on camels from the south of Tunisia and especially in the three governorates, Kebili, Medenine and Tataouine, where most of the Tunisian dromedary herds (91%) are located (Salmi et al., 2018). Serology was used to evaluate the prevalence of this disease as it is one of the most routinely used techniques, simple and with a higher specificity than the other serological tests (Verloo et al., 2001).

Seroprevalence obtained by CATT in the present study was (57.3%, 138/241) are similar to the onereported by Benaissa et al. (2020) in southern Algeria (45,9%), it is higher than that recorded by Kalthoum et al. (2022) in Tunisia (30.8%) and Boushaki et al. (2019) in Algeria (32.4%); and lower than the seroprevalence found by Zayed et al. (2010) in Egypt (82%). The infection rate by *T. evansi* decreases with camel age (Lemecha et al., 2008) which may explain the difference between our results and those reported by Kalthoum et al. (2022), where only adult animals older than 4 years were sampled, while in the present study, all ages were included and animals younger than 4 years represented 29.9% (72/241). The high seroprevalence found in Tunisian camels may be due to the persistence of antibodies during several months (OIE, 2021; Verloo et al., 2001). The seroprevalence of *T. evansi* is highly associated with the breeding mode: animals under extensive breeding mode seem to have the highest rate of infection with *T. evansi*, in comparison to the others production systems. Important transhumance movements of dromedaries between different areas and countries in extensive management systems explain most likely the wide distribution of *T. evansi* infection (Benaissa et al., 2020). In Tunisia, dromedary camels are essentially bred in an extensive mode (Salmi et al., 2018): the animals transhumed between several areas to graze and use the same watering points. This particularity and promiscuity of camel herds lead to potentialize the risk of *T. evansi* transmission between camels reared indifferent regions and herds (Benaissa et al., 2020). Finally, the disparity of seroprevalence levels could be due to the difference in sample sizes, climatic conditions, management practice, endemicity of the disease, vector ecology and co-infection by other pathogens.

The changes in haematological parameters are biomarkers of various disease conditions such as trypanosomiasis (Ohaeri et Eluwa, 2011). The main haematological modifications observed in the present study were anaemia, neutrophilia, monocytosis and eosinophilia. Comparatively, anaemia is a major symptom of animal trypanosomiasis (Enwezor et Sackey, 2005). In the present study, anaemia was confirmed by significant decreased Hb and PCV, considered to be reliable indicators for anaemia (Padmajia, 2012). Reduced PCV seem to be associated with a hemodilution as it was confirmed by the decreased Hte found in the present study, and not a result of reduced circulating RBC, as have been suggested by Hussain et al. (2016). Indeed, RBC revealed normal values in infected camels of the present study. Significant reduced MCV and MCH indicated microcytic and hypochromic anaemia in infected camels of the present study, which is similar to the results of Anode et al. (2019), while other studies indicated a macrocytic and hypochromic anaemia in infected camels (Hussain et al., 2016; Enwezor et Sackey, 2005). Reduced MCV is considered as an indicator of erythrophagocytosis occurring in camel trypanosomiasis (Ohaeri et Eluwa, 2011) caused by the mechanical damage of erythrocyte with the severe action of the flagella and microtubules of millions of *T. evansi* (Vickerman et Tetley, 1978). Reduced MCH values typically indicate an iron-deficiency anaemia in infected camels (Lalonde et Holbein, 1984). In the present study, anisocytosis, commonly observed in anaemia, was confirmed by a significantly increased RDW.

Added to the anaemia, leukocytosis is one of the major features of *T. evansi* infection (Sivajothi et al., 2015). In the present study, the leukogram evaluation showed a significant neutrophilia, monocytosis and eosinophilia in the infected camels compared to non-infected. Leukocytosis, observed in 85.7% (81/138) of infected camels, is mainly related to neutrophilia (Hussain et al., 2016). Neutrophils act together with lymphocytes and monocytes to repair tissues damages occurring during the chronic phase of the infection (Luna-Gomes et al., 2014), while the leukocytosis related to lymphocytosis is frequent during the acute phase (Sivajothi et al., 2015), and is followed by leucopoenia during the chronic phase as a result of the immunosuppressive action of trypanosomes (Sazmand et al., 2022). Although monocytes and eosinophils proportions were within the reference values of the species, they were significantly higher in infected camels compared to non-infected ones. Monocytosis occurring in camel trypanosomiasis (Hussain et al., 2016; Luna-Gomes et al., 2014, is a result of an increase in the activity of the mononuclear phagocyte system aiming to eliminate trypanosomes, the damaged red blood cells and the dead cells (Enwezor et Sackey, 2005). Moreover, eosinophilia, reported also by Hussain et al. (2016) and Padmajia (2012), is a characteristic feature

of different parasitic infections, including *T. evansi*, since it is a part of the hypersensitivity reaction (Njiru et al., 2000).

To investigate the functional status of various organs and tissues, some serum biochemical parameters were measured in the present study. a significant decrease in TP and urea were the most important findings and indicate the liver failure. a decreased TP was also reported by Azeem et al. (2019) and is due to an insufficient liver synthesis function thereby inducing a decreased urea concentration, as well as an erythrogenesis depression and a severe anaemia as reported by Kramer (2000). In blood, creatinine is directly proportional to the individual's muscle mass (Deen, 2013). the decrease in creatinine level, observed in 33.3% (46/138) of infected animals in the present study may be explained by the reduced muscle bulk, occurring in trypanosomiasis. In humans, a reduced creatinine level is also related to liver diseases and significant fluid overloads (Ostermann et al., 2016)

Our findings suggest that blood profile disorders observed in dromedary camels affected by *Surra* could contribute to the disease pathogenesis as a consequence of some haematological changes such as anaemia, where haemoglobin reduction may lead to tissue hypoxia (Baldissera et al., 2015). They may lead also to severe clinical disturbances such as oedema as observed in camel trypanosomiasis. These disorders depend on the parasite virulence, the susceptibility of the host and the sampling period (Akinseye et al., 2020).

5. Conclusion

In Tunisia, a few data on prevalence of camel trypanosomiasis was presented but none regarding its impact on blood profile and animal health was ever published. To our knowledge, this study is the first report focused on the measurement of several blood parameters in infected camels with *T. evansi*. The present study shows a high *T. evansi* infection rate in Tunisian dromedary camels and severe disorders in their blood profile. Parameters, markedly affected in *T. evansi* infection, can be commonly used as biomarkers for the control and the management of this disease in camels, and especially for control programs planification. Further studies are necessary to establish reference values for Tunisian dromedary camels for several blood parameters.

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