Haematological profile of dromedary camels naturally infected with *Trypanosoma evansi*

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**INTRODUCTION**

In arid and desert areas, camels are essential for the economy and food security of local populations as they ensure meat, milk, and hair fibre production as well as for transportation and agriculture labour’s (Faye & Bonnet, 2012). The environmental aridity linked to climatic changes and the globalization of the world economy (Faye, 2016) makes camels a major player in the international animal production context as it is a considerable protein source for humans (Zarrin et al., 2020). Their adaptation traits would lead to more sustainable animal production systems with fewer inputs compared with conventional livestock species (Faye, 2016). The major constraints to enhance camel production is parasitic diseases (Sazmand et al., 2019). *Trypanosoma evansi*, agent of Surra, is the most pathogenic and economically important protozoan parasite of camels that is the causes of impaired milk and meat production, decreases in performance or even death. The parasite is mechanically transmitted by bloodsucking flies of Tabanidae (Enwezor & Sackey, 2005) to a large number of domestic and wild mammals, but historically, the main host was the camel (Desquesnes et al., 2013). Unfortunately, it is prevalent in many African and Asian countries where most of camel population is present. The course of 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.

**ABSTRACT**

Background: Trypanosomiasis is widely distributed in African and Asian camel livestock. It is among the major constraint to camel production and causes serious economic losses. The disease control and management required knowledge about its impact on animal health. **Objectives:** The present study aims to confirm *T. evansi* infection in studied animals and to determine the modifications of haematological parameters in infected camels compared to non-infected ones. **Methods:** A total of 241 dromedary camels were randomly selected. Parasitological and serological tests were performed to confirm the infection. Haematological parameters (Red Blood cell Count, White Blood Cells, haemoglobin concentration, Packed Cell Volume, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration, Red cell Distribution Width and White Blood cell Count) were automatically analysed, differential leukocyte counts was manually carried out and morphological abnormalities of erythrocytes were recorded. **Results:** *Trypanosoma evansi* infection was confirmed in 3.6% of animals by microscopy and in 57.3% by serology. In seropositives camels, the most important haematological alterations were microcytic hypochromic anaemia, neutrophilia, monocytosis, eosinophilia and anisocytosis. **Conclusion:** On the basis of these findings, haematological parameters, markedly affected in *T. evansi* infection, are good biomarkers for the diagnosis and monitoring of the disease. They may be used for planning control programs to reduce its impact on camel livestock production.

**Key words:** Camel; Haematological profile; Natural infection; *Trypanosoma evansi*
and abortion (Desquesnes et al. 2013, Lukins & Dwinger, 2004). Clinical signs are related to several damages caused to different tissues and to the immune response induced by the parasite (Desquesnes et al., Enwezor & Sackey, 2005) leading to different alterations in haematological profile (Hussain et al., 2016). These alterations are biomarkers of various disease conditions including trypanosomiasis (Ohaeri & Eluwa, 2011). However, in Asia and Africa, few data on haematological profile of camels naturally infected by *T. evansi* are available. Especially for Tunisian dromedary camels, there’s no published data on haematological profile. Hence, the present study was conducted to determine the impact of natural *T. evansi* infection on haematological profile in order to highlight the severity of the disease. The results of this study will be useful for the diagnosis, the surveillance and for planning control programs of *T. evansi* infection in camel livestock.

**MATERIALS AND METHODS**

**Study area**

This study was carried out from July 2020 to September 2021. Five regions belonging to three South Tunisian governorates: Medenine (Djerba and Ben Guerdane), Tataouine, and Kebili (Kebili and Douz) were included in this study (Fig. 1). These regions are characterized by a traditional pastoral livestock production and included most of the Tunisian dromedary herds (91%) (Jemli et al., 2017).

According to Köppen climate classification, the south of Tunisia is a Hot desert climate (BWh), with extremely hot summer, warm winter and very low annual rainfall (Table 1).

**Study animals**

A total of 212 dromedary camels of both sex (112 males and 100 females with a sex ratio, M: F of 1.12) were randomly selected from volunteer dromedary herdsmen. Their age varied from 10 months to 25 years (mean age: 8.14 ± 6.08 years). Animals were ranked according to their age to 3 groups: 48 young animals of less than 4 years, 73 adults aged between 5 and 11 years and 91 old animals of more than12 years age. According to their origin, 88 camels were from Medenine, 74 camels were from Kebili and 50 camels were from Tataouine.

**Blood collection and diagnostic tests**

Blood samples were collected from jugular vein by a competent veterinarian into two tubes with and without anticoagulant. Whole blood collected on tube with anticoagulant (Ethylene diamine tetra acetic acid, EDTA) was used for haematological analysis and to perform Giemsa-stained thin blood smears (GST) (Soulsby, 1982). Thin smear was considered positive when at least one trypanosome was observed (OIE, 2018). Sera were obtained by centrifugation of blood collected on dry tubes at 3,000 rpm for 10 minutes. They are used for Card Agglutination Test for Trypanosomiasis (CATT/*T. evansi*), performed as per the manufacturer’s instructions (Institute of Tropical Medicine, Antwerp, Belgium). For CATT, a specimen was considered positive when blue agglutinates were visible after 5 minutes of agglutination (Bajyana and Hamers, 1988; Verloo et al., 2001).

**Haematological studies**

Blood samples collected in EDTA tubes were used to determine 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) (×10<sup>6</sup>/ml), Haemoglobin concentration (Hb) (g/dl), Packed Cell Volume (PCV) (%), Mean Corpuscular Volume (MCV) (fl), Mean Corpuscular Haemoglobin (MCH) (pg), Mean Corpuscular Haemoglobin Concentration (MCHC) (%Hb), Red cell Distribution Width (RDW) (%) and White Blood cell Count (WBC) (10<sup>6</sup>/ml). Differential leukocyte counts such as monocyte, lymphocyte, neutrophils, eosinophils and basophils were estimated as cross sectional method according to Blumenreich (1990). Obtained haematological values were compared to usual values established by Islam et al. (2019). The assessment of erythrocyte morphology was done through microscopic examination of GST under magnification of x1000 of an Olympus light microscope. The quantification of the morphological abnormalities was as described by Adekola et al. (2015). The values obtained were then converted to percentage abnormalities using the formula: Echinocytes (%) = number of echinocytes counted in 200 erythrocytes ÷ 200 x 100. This was repeated for all the observed morphological abnormalities to obtain the percentage count of each abnormality.
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for each parameter. Statistical differences between infected and non-infected camels were evaluated using: Mann-Whitney test for RBC, HB, PCV, MVC, MCH, MCHC, RDW and WBC and Chi square to compare proportions of neutrophils, lymphocytes, monocytes, eosinophils and basophils. All statistical tests were considered significant at a threshold of 0.05.

RESULTS

Parasitological and serological findings

Giemsa-stained thin blood smears (GST) revealed slender and flagellated trypomastigote morphologically compatible with T. evansi (Fig. 2).

Trypanosoma evansi was present in 3.8 ± 1.2 % (8/212) of examined samples by GST. All these positive samples were positive by CATT. Infection prevalence by GST was of 4.5 ± 1.1 % (4/97) in Medenine and of 5.4 ± 2.1 % (4/74) in Kebili. While, all blood samples from Tataouine were negative.

Table 1: Main geographical and climatic characteristics of the study area

<table>
<thead>
<tr>
<th>Locality</th>
<th>South-East</th>
<th>South – West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Governorate</td>
<td>Medenine</td>
<td>Tataouine</td>
</tr>
<tr>
<td>Region</td>
<td>Djerba</td>
<td>Tataouine</td>
</tr>
<tr>
<td>Latitude</td>
<td>33°52’N</td>
<td>33°55’N</td>
</tr>
<tr>
<td>Longitude</td>
<td>10°46’E</td>
<td>10°27’E</td>
</tr>
<tr>
<td>Altitude in m (asl)</td>
<td>6</td>
<td>238</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>219</td>
<td>116</td>
</tr>
<tr>
<td>Annual temperature (°C)</td>
<td>20.3</td>
<td>19.8</td>
</tr>
<tr>
<td>Köppen climate type</td>
<td>BSh</td>
<td>BWh</td>
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</tbody>
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BSh: Hot semi-arid (steppe) climate, BWh: Hot deserts climate, asl: above sea level

Table 2: Results of haematological parameters in positive CATT and negative CATT camels

<table>
<thead>
<tr>
<th>Standards</th>
<th>Non-infected camels</th>
<th>Infected camels</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>Erythrogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (x10^9/ml)</td>
<td>6.7 – 17.3</td>
<td>10.3</td>
<td>10.2</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.2 – 15.3</td>
<td>12.2</td>
<td>12</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>27 – 45</td>
<td>40.3</td>
<td>39</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>28 – 45</td>
<td>38.8</td>
<td>37.5</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>8.6 – 13</td>
<td>11.6</td>
<td>10.8</td>
</tr>
<tr>
<td>MCHC (%Hb)</td>
<td>33 – 41</td>
<td>29.9</td>
<td>29.9</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>13 – 18</td>
<td>18.2</td>
<td>18.7</td>
</tr>
<tr>
<td>Leukogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (10^6/ml)</td>
<td>8.2 – 16.5</td>
<td>20.3</td>
<td>18.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>39.5 – 57.8</td>
<td>61.7</td>
<td>53.5</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>23.7-43.2</td>
<td>33.7</td>
<td>38.9</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1.5 – 6.6</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2 – 8.8</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0 – 1</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>NLR</td>
<td>0.45</td>
<td>0.1 – 3.3</td>
<td>0.75</td>
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</table>


Fig2. Giemsa-stained blood smear from a camel showing Trypanosoma evansi (x1000)

Statistical analysis

Descriptive study was performed using Microsoft Excel 2016. 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.
More than half (57.1 ± 3.2 %; 121/212) of blood samples were positives by CATT. The highest seroprevalence was obtained in Kebili (77 ± 4.2%; 57/74) followed by Medenine (42 ± 5 %; 37/88) and Tataouine (54 ± 7 %; 27/50) (p=0.019).

**Haematological profile**

Although they were within the references values of species, Hb, PCV, MCV and MCH exhibited significant decrease in infected camels compared to non-infected ones (p= 0.026; 0.005; <0.001 and <0.001, respectively), while RDW was higher than references value and it was significantly increased (p=0.039) in infected camels compared to non-infected ones. The concentration of Hb was particularly decreased in camels with positive GST. However, RBC, MCHC and WBC did not vary between infected and non-infected camels (p=0.356, 0.995 and 0.079, respectively) (Table 2).

Significant decrease in Hb concentration confirmed the occurrence of anaemia in more than third of seropositive camels (32.2 ± 4.4 %, 39/121), having a haemoglobin concentration between 4.9 and 9.4 g/dl (mean: 7.3 ± 1.2 g/dl). In 18.2 ± 3.6 % (22/121) of seropositive samples, decreased Hb was associated between infected and non-infected camels (p=0.356, 0.995 and 0.079, respectively) (Fig. 3).

Significant decrease MCV and MCH in infected animals confirmed that anaemia was microcytic and hypochromic. Significant increased RDW confirmed anisocytosis in 63.6 ± 4.5 % (77/121) of infected camels. It was confirmed also by microscopic examination of thin blood smears showing poikilocytosis with different abnormalities in red blood cells (microcytes, macrocytes, pale cells, tear droplet cells, elliptical cells, helmet cells and lemon-shaped cells) (Fig. 3).

The most frequent abnormal red blood cells in infected camels were microcytes (26.6 ± 4.2%) followed by macrocytes (13.5 ± 3.1 %), pale cells (17 ± 2.6 %), tear droplet cells (3.3 ± 2.8 %) and lemon shaped cells (0.8 ± 0.4 %) (Fig. 4).

Leukogram evaluation showed leucocytosis in both infected camels and non-infected ones (p=0.079). Neutrophilia, monocytes and eosinophilia were recorded in infected camels compared to non-infected ones. In infected camels, the neutrophils to lymphocytes ratio (NLR) was significantly higher in infected animals compared to non-infected ones (p<0.001) which indicated that leucocytosis was mainly related to neutrophilia. Monocytes and eosinophil proportions were significantly higher in infected animals compared to non-infected ones (p<0.001). Whereas, basophil proportion did not vary between the two animal groups (p=0.778).

**DISCUSSION**

Surra is a severe disease that may lead to health deterioration and causes economic losses in camels. Despite, it was prevalent in Tunisian dromedary camels, the disease was underestimated. As far we know, the present study is the first report on haematological modifications occurring during *T. evansi* infection in Tunisian dromedary camels.
For prevalence estimation, both microscopy and serology are used to detect the infection by *T. evansi* in the present study. Microscopy is one of the most routinely used technique as it is simple, inexpensive and specific test but lack sensitivity, especially in chronic and subclinical phases when parasitemia is low (Tehseen et al., 2015). Card Agglutination *Trypanosoma* Test (CATT) was used as it is simple with higher sensitivity than GST and it has higher specificity compared to other serological tests (Gutierrez et al., 2000; Verloo et al., 2001). In the present study, *T. evansi* was present in 3.8% of sampled animals. This prevalence was higher than that found by Boushaki et al. (2019) in Algeria (2.4%) and by Dia et al. (1997) in Mauritania (1.3%). In Tunisia, the only work conducted on prevalence of *T. evansi* infection in camels using GST was that of Selmi et al. (2019) but it was limited to the military livestock of Kebili. They reported a prevalence of 22% in only 100 camels from Kebili which is higher than those obtained in our study for the same region (5.4%, 4/74).

More than half of studied animals (57.1%) were seropositive for *T. evansi* by CATT which is similar to those recorded by Benaissa et al. (2020) in south Algeria (45.9%), Kyari et al. (2021) in Kenya (45.9%) and Njiru et al., (2004) in Nigeria (44%). While, it was higher than that found by Kalthoum et al. (2022) in Tunisia (30.8%), Boushaki et al. (2019) in Algeria (32.4%), Ararhouch et al. (2003) in Morocco (16%) and Dia et al. (1997) in Mauritania (14.2%). On the other hand, it was lower than the seroprevalence observed in Egypt (82%) (Zayed et al., 2010). The infection rate by *T. evansi* decreases with camel age (Delafosse and Doutoum, 2004; Lemecha et al., 2008) which may explain the higher seroprevalence found in this study compared to that of Kalthoum et al. (2022). Indeed, only adult animals older than 4 years were sampled in the study of Kalthoum et al. (2022), while in the present study, animals of different ages were included and animals younger than 4 years represented 22.6% (48/212). The high seroprevalence found in Tunisian camels may be due to the persistence of antibodies during several months despite the treatment of efficient trypanocide molecules (OIE, 2018; Verloo et al., 2001). Indeed, 18.4% (39/212) of the sampled camels of the present study were treated with melasormine hypochloride (Cymelarsan®, Merial, Maroc).

The seroprevalence of *T. evansi* is highly associated with the breeding mode. Animals under extensive mode seem to have the most important number of animals infected with *T. evansi*, in comparison to the others production systems (Elamin et al., 1998). In Tunisia, dromedary camels are essentially bred in an extensive mode (Salmi et al., 2018); the animals travel several areas in a phenomenon called transhumance to graze and valorise different vegetation and they used the same animal watering points. 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). This particularity leads to highlight the risk of the transmission of *T. evansi* between camels of different regions, added to the promiscuity of camel herds (Benaissa et al., 2020). The high prevalence may be due also to the variations in the ecology of the study areas and seasons of the year. Indeed, season has a direct effect on the distribution of biting flies, mechanical vectors of *T. evansi* (Luckins, 1988).

The changes in haematological parameters are biomarkers of various disease conditions such as trypanosomiasis (Pandey et al., 2015; Ohaeri & Eluwa, 2011). The main haematological modifications observed in the present study was anaemia, neutrophilia, monocytosis and eosinophilia. Anaemia is a major symptom of animal trypanosomiasis (Enwezor & Sackey, 2005). Reliable indicators for anaemia are decreased Hb and PCV (Eyob & Matios, 2013; Padmaja, 2012) which were confirmed in the present study. Reduced PCV may be associated with increased plasma volume. Hemodilution could be responsible for anaemia (Jenkins & Facer, 1985), especially in trypano-tolerant animals (Stijlemans et al., 2018; Radwanska et al., 2018) and not a result of reduced circulating RBC (Hussein et al., 2016). Reduced RBC count revealed normal values in infected camels of the present study. Based on significant reduced MCV and MCH, anaemia was microcytic and hypochromic in infected camels. Similar observations were confirmed by Anode et al. (2019) who indicated microcytic and hypochromic anaemia in camels due to iron deficiency. While, others studies indicated macrocytic and hypochromic anaemia in infected camels (Hussain et al., 2016; Enwezor & Sackey, 2005). Reduced MCV is considered as an indicator of erythropagocytosis occurring in camel trypanosomiasis (Ohaeri & Eluwa, 2011; Derakhshanfar, 2010). Reduced MCH value typically indicates an iron deficiency anaemia in infected camels (Lalonde & Holbein 1984) due to its involvement in the hematopoietic process (Wolkmer et al., 2007) and in many enzymatic systems (Da Silva et al., 2009). All pathogenic microorganisms, including *T. evansi*, require iron for optimal growth and virulence (Al-Rubaie et al., 2020). It may be attributed to the decrease of iron-transporting proteins since they are used by parasites for their own metabolism (Taylor et al., 2013). During infection, iron sequestration by the reticuloendothelial system was also responsible for iron deficiency and anaemia (Roesser 1980; Letendre and Holbein 1983; Gutierrez et al., 2006). Microcytic and hypochromic anaemia may result to iron-restricted erythropoiesis (Ganz & Nemeth, 2009) indicating that animals were in either in chronic or recovery phases of anaemia (Mbaya et al., 2012).
In the present study, anisocytosis, poikilocytosis and hypochromia were recorded in infected animals. Similar changes were reported by Abd El-Baky and Salem (2001). Anisocytosis, which is commonly observed in anaemia, was confirmed both by significant increased RDW and by morphological changes of RBC observed in GST as reported by Hussain et al. (2016). The most frequent RBC abnormal red blood cells in infected camels of the present study were microcytes (46.6 ± 4.2%) followed by macrocytes (23.5 ± 3.1 %). Emeribe and Anosa (1991) indicated that in experimental T. evansi infected rabbits, the macrocytic cells shifted terminally to microcytic hypochromic cells, with evidence of moderate anisocytosis and poikilocytosis erythrocytes.

In the present study, leucocytosis was observed both in infected and non-infected camels. Hence, leucocytosis was not a reliable indicator for T. evansi infection as has long been considered (Hussain et al., 2016; Sivajothi et al., 2015; Chaudhary & Iqbal, 2000). Similar observation has been reported in experimentally infected goats (Dargantes et al., 2005). Leucogram evaluation showed significant neutrophilia, monocytes and eosinophilia in infected camels compared to non-infected ones (p<0.001). The same trend was reported by Hussain et al. (2021). In infected camels of the present study, leucocytosis was mainly related to neutrophilia as have been reported previously (Hussain et al., 2016; Abd El-Baky & Salem, 2001; Chaudhary & Iqbal, 2000). Neutrophils act together with lymphocytes and monocytes to repair tissue damage during the chronic phase of infection (Luna-Gomes et al., 2014). While, leucocytosis related to lymphocytosis is frequent during the acute phase (Sivajothi et al., 2015) and is followed by leucopenia during chronic phase due to immunosuppressive action of trypanosomes (Desquesnes et al., 2013). 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 (p<0.001). 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 phagocytic system tending to eliminate trypanosomes, damaged red blood cells and dead cells (Enwezor & Sackey, 2005). Otherwise, eosinophilia, which was also reported by Hussain et al. (2016) and Padmaja (2012), is a characteristic feature of different parasitic infections, including T. evansi, since it is a part of the hypersensitivity reaction (Abd El-Baky and Salem, 2011, Njiru et al., 2000).

Therefore, infected camels included in the present study seems to be already in chronic phase of disease as their haematological profile was dominated by microcytic hypochromic anaemia, neutrophilia and monocytosis.

**CONCLUSION**

On the basis of the present study, we concluded that T. evansi infection is highly prevalent in Tunisian dromedary camels and induces severe disorders in haematological profile. The mains changes are anaemia, neutrophilia, monocytosis, anisocytosis and poikilocytosis. Haematological parameters, markedly affected during the disease, are good biomarkers for control and management of T. evansi infection and for planning control programs aiming to reduce its prevalence and its impact on camel production.

**Authors contribution**

Sihem Ismail-Hamdi designed the experiment with Samir Ben Romdhan, performed most of the experiments, analysed the data and wrote the original paper; Mohamed Gharbi review and edit final version; Nabil Hamdi perform the statistical analysis and interpret the results; Sirine Ben Yahia, Houcine Ben Yahia, Boubaker Ben Smida and Walid Chandoul perform sampling and field work; Samir BEN ROMDHANE designed the experiment and supervise the study.

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