

## Evidence of natural infections with *Trypanosoma*, *Anaplasma* and *Babesia* spp. in military livestock from Tunisia

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**Abstract.** Livestock constitute habitual hosts and carriers for several infectious pathogens which may represent a serious public health concern affecting the readiness of military forces and lead to wide economic losses. The present report aimed to investigate the prevalence of some haemopathogens infecting military livestock, particularly, dromedaries, sheep and horses using Giemsa-stained blood smears. A total of 300 animals (100 from each species) were selected, clinically examined and sampled. *Trypanosoma* spp. (22.0%), *Anaplasma* spp. (17.0%) and *Babesia* spp. (1.0%) were identified in camels' blood. Six dromedaries were found to be co-infected by *Trypanosoma* and *Anaplasma* organisms (6.0%). Camels of female gender, infested by ticks and showing clinical signs were statistically more infected by *Trypanosoma* spp., compared to those of male gender, free of ticks and apparently healthy ( $P=0.027$ , 0.000 and 0.004, respectively). *Babesia* spp. infection (1.0%) was identified, for the first time in Tunisia, in one adult female camel that presented abortion and anemia. *Anaplasma* spp. was the only haemopathogen identified in examined sheep (6.0%) and horses (17.0%). Horses infested by *Hippobosca equina* flies and sheep infested by *Rhipicephalus turanicus* ticks were more infected by *Anaplasma* spp. than other non-infested animals ( $P=0.046$  and 0.042, respectively). *Hyalomma dromedarii*, *H. impeltatum* and *H. excavatum* were the most prevalent diagnosed ticks removed from camels with an intensity of infestation of 1.2 ticks per animal. However, in sheep, only *R. turanicus* was identified. *H. equina* and *Tabanus* spp. were the potential hematophagous flies found in dromedaries and horses herds. This useful data must be taken into consideration during animal treatment and vectors' control programs in Tunisian military farms which help to limit the diffusion of vector-borne diseases, keep our livestock healthy and reduce economic losses.

### INTRODUCTION

Worldwide, infections by vector-borne haemopathogens are numerous and reach an alarming level, which represents a serious problem for both animal production and public health (Rosenberg *et al.*, 2018). The occurrence of these organisms was highly

dependent on vector activities, preferential hosts and pathogen virulence (Woolhouse, 2001). Interestingly, the epidemics of emerging and re-emerging diseases were mainly related to climatic changes represented by the rise in Earth's temperature and the substantial deficit in water resources. In addition, interactions between pathogens and

endosymbionts co-infecting arthropod vectors and/or hosts may cause the appearance of potential new vectors, virulent pathogens, and dominant animal hosts (Walther *et al.*, 2002; Moutailler *et al.*, 2016). Therefore, animals play the role of host and/or reservoir of several infectious pathogens responsible for significant economic losses (Narladkar, 2018). Among these animal species, we cite the one-humped camels, known by its hardiness and resistance to infections (Faye *et al.*, 2015), sheep characterized by a high receptivity and sensitivity (Bishop, 2015) and horses, for which vector-borne infections are poorly documented (Onmaz *et al.*, 2013).

The main bacterial vector-borne diseases observed in these domestic animal species are anaplasmosis (Ben Said *et al.*, 2018), rickettsiosis (Parola *et al.*, 2005), coxiellosis (Eldin *et al.*, 2017), bartonellosis (Erekat *et al.*, 2016a) and Lyme disease (Ben Said *et al.*, 2016). *Anaplasma* genus (Rickettsiales: Anaplasmataceae) is genetically diverse and contains six recognized species (Atif, 2016; Ben Said *et al.*, 2018). Generally, infections with these bacteria are responsible for anemia, loss of productivity and reduced production of milk and meat (Splitter *et al.*, 1956; Yasini *et al.*, 2012; Asseldonka *et al.*, 2013). To date, in Tunisia, two *Anaplasma* species have been identified in camels, i.e. *Anaplasma phagocytophilum* by immunofluorescence antibodies assay (Ben Said *et al.*, 2013) and strains genetically related to *A. platys* (*A. platys*-like) by sequencing after nested PCR based on 16S rRNA gene (Belkahia *et al.*, 2015). Regarding sheep, five *Anaplasma* species have been detected, namely *A. ovis* (Belkahia *et al.*, 2014; Belkahia *et al.*, 2017), *A. bovis* (Ben Said *et al.*, 2015; Belkahia *et al.*, 2017), *A. platys*-like (Ben Said *et al.*, 2017b), *A. phagocytophilum*-like 1 and *A. phagocytophilum*-like 2 (Ben Said *et al.*, 2015; Ben Said *et al.*, 2017a). However, only *A. phagocytophilum* was identified in horses by molecular and serological methods (M'ghirbi *et al.*, 2012; Ben Said *et al.*, 2014).

On the other hand, parasitic infections are numerous, adapted specifically to preferential hosts and lead to different clinical forms (Edman, 2004). Trypanosomiasis, or “surra”, is caused by *Trypanosoma evansi* belonging to the class of Kinetoplastida and the family of Trypanosomatidea. It was identified, for the first time, in Indian camels and horses (Röttcher *et al.*, 1987). This pathogen is mechanically transmitted by Tabanids after blood sucking and is responsible for abortions, anemia and weight loss (Olaho *et al.*, 1987). In Tunisia, no formerly published reports regarding camels' trypanosomiasis have been performed except the annual report on animal health in Tunisia, indicating that *Trypanosoma* infection rate was 10.0% in camels from the governorate of Tataouine (Lachtar *et al.*, 2017). Similarly, infection with *Trypanosoma* spp. has never been reported in sheep and horses from Tunisia. However, Sallemi *et al.* (2018) reported that 10.0% of examined cattle from the governorate of Ariana were infected with *T. evansi*. In dogs, the infection was identified only in one case from northern Tunisia (Rjeibi *et al.*, 2015).

Piroplasma organisms are intracellular apicomplexan parasites known to infect animals' erythrocytes (Aktas *et al.*, 2007; Khamesipour *et al.*, 2015). They belong to the phylum Apicomplexa, order Piroplasmida and include two main genera e.g. *Theileria* and *Babesia* (Preston, 2001). The infection with pathogenic organisms of piroplasma is fatal and causes serious economic losses. In Tunisia, these protozoa have been detected in three ticks feeding on horses (Ros-Garcia *et al.*, 2011) and sheep (Rjeibi *et al.*, 2016) but no report was performed on camels.

Following several series of abortions and anemia observed in dromedaries, diarrhea and weight loss recorded in sheep and horses from Tunisian military farms during summer season, we decided to investigate the occurrence of vector-borne haemopathogens and to identify ectoparasites' density in selected animals located in these farms.

## MATERIALS AND METHODS

### Areas of study

The present study was carried out during the summer (August 2017) in two governorates from Tunisia (Figure 1). Firstly, camel farms, located in the governorate of Kebili (latitude 33°27'2" N, longitude 9°01'2" E), were visited. These farms belonged to desertic climate from Southern Tunisia were characterized by dryness and low level of pluviometry. Secondly, sheep farms and horses stables belonged to the governorate of Manouba (latitude 36°48'2" N, longitude 9°52'2" E) from Northern Tunisia were examined. This region is situated in a sub-humid area with a mean annual rainfall varying between 400 and 800 mm. This study has interested in (i) camels from Tunisian military mehari unit of the second Saharan Territorial Grouping, (ii) horses belonging to the military cavalry center of Honor Regiment and (iii) sheep from the Directorate of Military Farms. Dromedaries were already used to control Tunisian desertic borders from Algerian and Libyan sides as well as camel racing activities. Horses serve for military protocols and athletic activities and, finally, sheep farming allows providing healthy and controlled meat for military staff. These animals were well maintained and managed.

### Blood samples

A total of 300 domestic animals (100 camels, 100 sheep and 100 horses) were randomly selected, examined and sampled referring to their sanitary status (animals apparently healthy and presenting clinical signs). Whole blood samples were collected from the jugular vein of each animal using sterile vacutainer® tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) as anti-coagulant. Samples were stored at +4°C until use.

### Ectoparasites collection

Ticks were removed manually from animals' bodies. Flies were caught from their close environment. Specimens were counted, morphologically identified on the genus and species level and stored in alcohol 75.0%. The identification of ectoparasites was



Figure 1. Map of Tunisia showing the two investigated governorates.

applied based on the taxonomical key of ticks' and flies' identification (Walker *et al.*, 2013; Tayloor, 2016). In order to characterize ticks' population infesting these animals, three epidemiological ratios were determined.

Infestation rates (%) =  $\frac{\text{number of infested animals}}{\text{total number of animals}} \times 100$

Infestation intensity =  $\frac{\text{Number of ticks}}{\text{number of infested animals}}$

Abundance =  $\frac{\text{Number of ticks}}{\text{total number of animals}}$

### Blood smears preparation

All samples were subjected to blood smears on a microscopic slide for direct search of eventual haemopathogens and identify which cell types were specifically colonized. A small drop of blood was used to perform a thin blood smear on a degreased slide and left to dry. Blood smear was fixed by dipping slide in absolute methanol for

three minutes and then stained using a 10.0% Giemsa solution during ten minutes. Haemopathogens were detected by microscopic observation at 100 x magnification with immersion oil. Photos were captured and saved directly using a high-resolution microscopic camera compound to computer (*Leica*® Microsystems Microscopy and Scientific Instruments, Wetzlar, Germany).

### Statistical analysis

Exact confidence intervals (CI) for prevalence rates at the 95% level were calculated. Comparison of the prevalence of each haemopathogen in camels, horses and sheep according to risk factors, and farms, and comparison of infestation prevalence rates of each tick or fly species found in tested animals were performed with Epi Info 6.01 (CDC, Atlanta), using the  $\chi^2$  test and Fisher's exact test with a threshold value of 0.05. In order to consider any confusion factor, a chi square Mantel-Haenszel test was performed.

## RESULTS

### Ectoparasites' identification and its prevalence (%)

Despite the regular use of acaricides, examined animals were found to be exposed and infested by both ticks and/or flies. In total, 35.0% of camels were infested by 42 tick specimens belonging to three species of *Hyalomma* genus (Figure 2). *Hyalomma dromedarii* (17.0%) and *H. impeltatum* (14.0%) were the most abundant tick species followed by *H. excavatum* that was rarely diagnosed (4.0%). The intensity of infestation was evaluated to 1.2 ticks per camel. Regarding sheep, 10.0% were infested by *Rhipicephalus turanicus* (n=10) with a mean intensity of infestation of one tick per animal (Table 1). In contrast, horses were not infested by ticks. Regarding flies, *Hippobosca equina* was found abundant in both camels and horses' herds, with 19 specimens removed from horses bodies (Figure 2). However, *Tabanus* sp. was captured only from dromedary herds and pastures (Figure 2).

### Morphological features of identified haemopathogens

Visualized by Giemsa stained blood films, trypanosomes are seen, generally, in the morphological stage of trypomastigote characterized by a slender form with approximately 20  $\mu\text{m}$  of length with a C or U shape. It is equipped with a thin and undulating membrane associated to the flagellum stems, a centrally positioned nucleus and a kinetoplast (Figure 3A). This morphological profile is reminiscent of *T. evansi*. *Anaplasma* organisms showed various features strictly dependant to *Anaplasma* strains, its evolutive stage and infected cell type. It appears as round purple inclusions of 0.3–1.0  $\mu\text{m}$  in diameter,

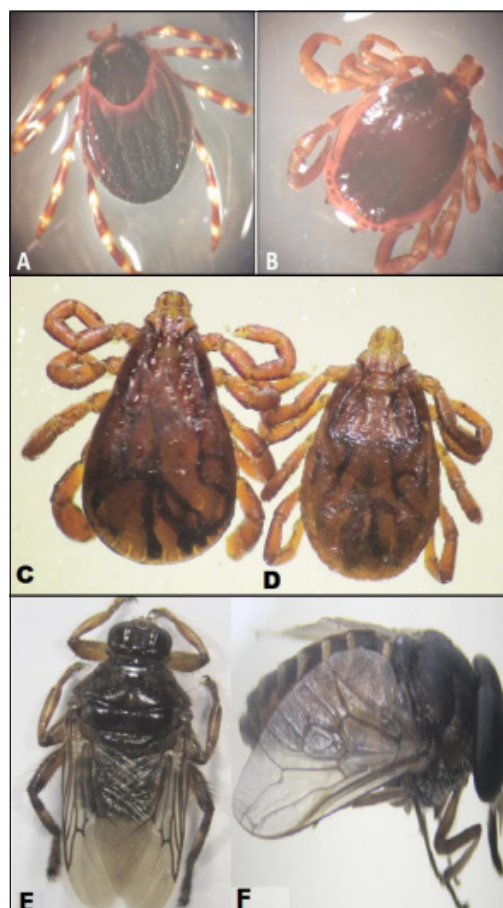


Figure 2. Ectoparasites collected and identified in this study.

A: *Hyalomma dromedarii* tick female; B: *Hyalomma dromedarii* tick male; C: *Rhipicephalus turanicus* tick male; D: *Rhipicephalus turanicus* tick female; E: *Hippobosca equina* flies and F: *Tabanus* spp. flies.

Table 1. Ectoparasites collected from camels, sheep, horses and their close environment in different Tunisian Military farms

Localization	Arthropod vectors	Number of Parasite	Infested animals	Prevalence (%)	Intensity of infestation	Mean abundance
Camels	<i>Hyalomma dromedarii</i>	19	17	17.0	1.11	0.19
	<i>Hyalomma impeltatum</i>	14	14	14.0	1	0.14
	<i>Hyalomma excavatum</i>	9	4	4.0	2.25	0.09
	Total	42	35	35.0	1.2	0.42
Environment close to camels	<i>Hippobosca equina</i>	3	NA	NA	NA	NA
	<i>Tabanus</i> spp.	5	NA	NA	NA	NA
Sheep	<i>Rhipicephalus turanicus</i>	10	10	10.0	1	0.1
Environment close to sheep	NF	-	-	-	-	-
Horses	<i>Hippobosca equina</i>	19	19	19.0	1	0.1
Environment close to horses	<i>Hippobosca equina</i>	6	NA	NA	NA	NA

NF: Not Found.  
NA: Not Applicable.

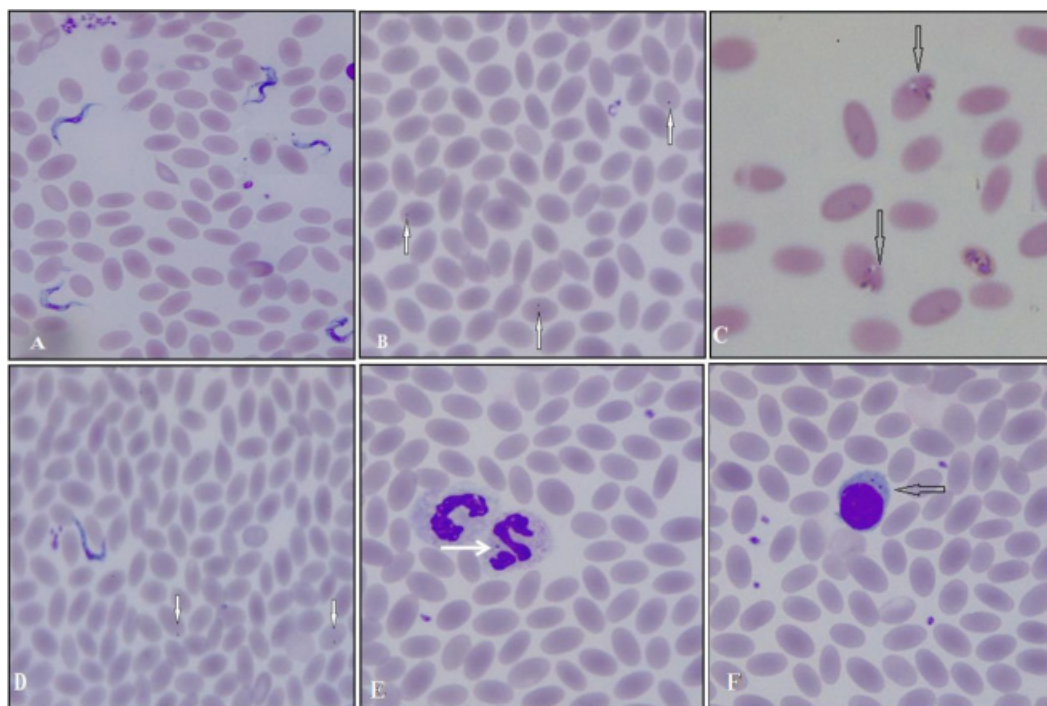


Figure 3. Haemopathogens identified in camels.

A: Blood film hyper-infested by *Trypanosoma* spp.; B: Inclusion of *Anaplasma* spp. in erythrocytes; C: *Babesia* spp. in erythrocyte; D: Co-infection by *Trypanosoma* spp. and *Anaplasma* spp.; E: Morulae of *Anaplasma* spp. in camel neutrophil granulocyte; F: *Anaplasma* spp. in camel monocytes.

located in erythrocytes of all examined blood smears but also in camels' neutrophils and monocytes. In sheep, these inclusions are dense and homogeneous with a polar or subpolar position within the outer margins of the erythrocytes (Figure 4A). However, in camels and horses, these erythrocytic inclusions are also in marginal position but appear smaller and thinner (Figure 3B and 4B). Besides, camels' neutrophils (Figure 3E) and monocytes (Figure 3F) were also found to be infected by *Anaplasma* spp. seen as purple, dense and homogeneous morulae positioned centrally. The intra-erythrocytic piroplasm, *Babesia* spp. occurs as purple inclusions that measure 1-3µm of length. It showed various distinguishing features varying in shape and size. In this study, *Babesia* spp. is seen in trophozoite stage associated in pairs with cyclical or ring forms (Figure 3C).

#### Haemopathogens identified in camels and risk factors survey

Globally, almost a third of camels (34.0%) were infected by at least one of these three following haemopathogens: *Trypanosoma* spp. (22.0%, 95% CI: 0.17-0.43), *Anaplasma* spp. (17.0%, 95% CI: 0.07-0.29) and *Babesia* spp. (1.0%, 95% CI: 0.02-0.06). Six camels were co-infected by *Trypanosoma* and *Anaplasma* organisms (6.0%, 95% CI: 0.02-0.18) and 11.4% from them were infested by ticks (Table 2

and Figure 3D). Camels infested by ticks were statistically more infected by *Trypanosoma* spp. (45.7%, 95% CI: 0.33-0.79) compared to those free of ticks (9.2%, 95% CI: 0.03-0.28,  $P \leq 0.001$ ). In addition, dromedaries showing clinical signs as abortion, emaciation and anemia were statistically more infected by *Trypanosoma* organism (32.7%; 95% CI: 0.25-0.63) compared to those apparently healthy (8.8%; 95% CI: 0.02-0.30,  $P=0.004$ ). Furthermore, males (12.5%; 95% CI: 0.01-0.25) were significantly less infected than females (30.7%; 95% CI: 0.29-0.71,  $P=0.027$ ) (Table 2). No significant difference was recorded regarding age class. Infection by *Anaplasma* spp. was not influenced by animal's age and gender or tick infestation. Besides, there was no significant difference between the prevalence rates observed in symptomatic and apparently healthy camels. *Babesia* spp. infection was identified, for the first time in Tunisian dromedary (*Camelus dromedarius*) (1.0%, 95% CI: 0.02-0.06). This only case was recorded in adult female camel that presented abortion and anemia.

#### *Anaplasma* spp. in sheep and horses and associated risk factors

*Anaplasma* spp. was the only haemopathogen identified in examined sheep (6.0%, 95% CI: 0.07-0.29) and horses (17.0%, 95% CI: 0.07-0.29). Horses infested by *Hippobosca equina* flies (15.7 %, 95% CI:

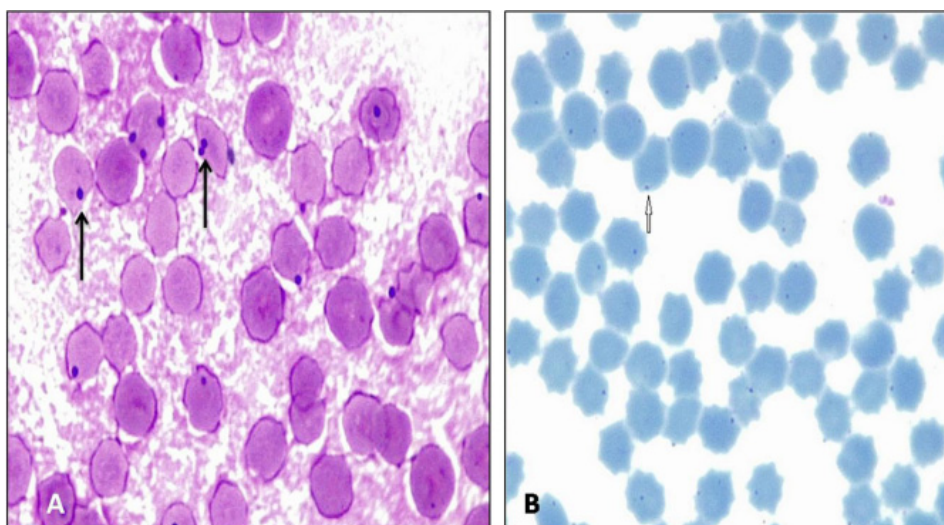


Figure 4. *Anaplasma* spp. organisms observed in sheep (A) and horses (B) blood smears.

Table 2. Prevalence of haemopathogens identified in camels according to gender, age, tick infestation and clinical signs

	<i>Trypanosoma</i> spp.			<i>Anaplasma</i> spp.			<i>Babesia</i> spp.		
	Positive/ Examined	% (±95% CI)	p-value	Positive/ Examined	% (±95% CI)	p-value	Positive/ Examined	% (±95% CI)	p-value
Gender									
Male	6/48	12.5 (0.01-0.27)	0.027*	8/48	16.6 (0.04-0.32)	0.932	0/48	0	0.336
Female	16/52	30.7 (0.29-0.71)		9/52	17.3 (0.02-0.34)		1/52	1.9 (0-0.13)	
Age									
< 5 years	5/24	20.8 (0.14-0.70)	0.976	3/24	12.5(0-0.21)	0.793	0/24	0	0.578
5–10 years	6/28	21.4 (0.10-0.70)		5/28	17.8 (0.02-0.58)		0/28	0	
> 10 years	11/48	22.9 (0.06-0.37)		9/48	18.7 (0.04-0.32)		1/48	2.0 (0-0.10)	
Tick infestation									
Yes	16/35	45.7 (0.33-0.79)	0.000*	6/35	17.1 (0-0.38)	0.977	1/35	2.8 (0-0.16)	0.172
No	6/65	9.2 (0.03-0.28)		11/65	16.9 (0.08-0.36)		0/65	0	
Clinical signs									
Symptomatic	18/55	32.7 (0.25-0.63)	0.004*	8/55	14.5(0.02-0.30)	0.472	1/55	1.8 (0-0.12)	0.365
Asymptomatic	4/45	8.8 (0.02-0.30)		9/45	20.0(0.04-0.36)		0/45	0	
Overall	22/100	22.0 (0.17-0.43)		17/100	17.0 (0.07-0.29)		1/100	1.0 (0-0.06)	

95% CI: 95% confidence interval; \*: Statistically significant test.

Table 3. Co-infection with identified haemopathogens in camels according to gender, age, tick infestation and clinical signs

		Co-infection ( <i>Trypanosoma</i> spp. / <i>Anaplasma</i> spp.)		
		Positive/Examined	% ( $\pm$ 95% CI)	P-value
Gender	Male	3/48	6.25 (0-0.17)	0.451
	Female	3/52	5.7 (0-0.28)	
Age	young (< 5 years)	2/24	8.3 (0-0.38)	0.654
	Adult (5-10 years)	2/28	7.14 (0-0.21)	
	Aged (> 10 years)	2/48	4.16 (0-0.17)	
Tick infestation	Yes	4/35	11.4 (0.03-0.41)	0.032*
	No	2/65	3.0 (0-0.09)	
Clinical signs	Symptomatic	3/55	5.4 (0-0.25)	0.640
	Asymptomatic	3/45	6.6 (0-0.19)	
Overall		6/100	6.0 (0.02-0.18)	

Abbreviations: 95% CI: 95% confidence interval. \*: Statistically significant test.

Table 4. Prevalence of *Anaplasma* spp. in horses and sheep according to gender, age, tick infestation and clinical signs

		Horses			Sheep		
		Positive/Examined	% ( $\pm$ 95% CI)	p-value	Positive/Examined	% ( $\pm$ 95% CI)	p-value
Gender	Male	1/37	2.7 (0.04-0.32)	0.289	8/48	16.6 (0.04-0.32)	0.932
	Female	5/63	7.9 (0.02-0.34)		9/52	17.3 (0.02-0.34)	
Age	< 5 years	2/32	6.25 (0-0.21)	0.942	6/66	9.0 (0.02-0.16)	0.003*
	> 5 years	4/68	5.8 (0.02-0.58)		11/34	32.0 (0.17-0.48)	
Tick infestation	Yes	3/19	15.7 (0-0.38)	0.046*	4/10	40.0 (0.04-0.32)	0.042*
	No	3/81	3.7 (0.08-0.36)		13/90	14.0 (0-0.38)	
Clinical signs	Symptomatic	2/26	7.6 (0.02-0.30)	0.674	8/55	14.5 (0.02-0.30)	0.472
	Asymptomatic	4/74	5.4 (0.04-0.36)		9/45	20.0 (0.04-0.36)	
Overall		6/100	6.0 (0.07-0.29)		17/100	17.0 (0.07-0.29)	

95% CI: 95% confidence interval; \*: Statistically significant test.

0.04-0.38) were statistically more infected by *Anaplasma* spp. than non-infested animals (3.7%, 95% CI: 0.08-0.36,  $P=0.046$ ) (Table 4). Similarly, sheep infested by *R. turanicus* ticks (40.0%, 95% CI: 0.04-0.32) were more infected by *Anaplasma* spp. than non-infested (14.0%, 95% CI: 0.04-0.38,  $P=0.042$ ). However, no significant difference was observed regarding other risk factors (Table 4).

## DISCUSSION

Livestock belonged to Tunisian military farms were found to be infected by various vector-borne pathogens. Dromedaries were statistically more infected (34.0%) with haemopathogens than sheep (17.0%) and horses (6.0%). *Anaplasma* spp. was the main bacterial microorganisms identified, microscopically, in all examined animal



species. Besides, *Trypanosoma* spp. and *Babesia* spp. were the major parasites identified in camel's blood.

The microscopic feature of *Trypanosoma* spp. observed in the current study was morphologically similar to *T. evansi* which is the causative agent of surra (Bruce, 1911). This prevalence (22.0%) was higher than that reported in camels from the governorate of Tataouine (10.6%) (Lachtar *et al.*, 2017) and in cattle from northern Tunisia (10.6%) (Sallemi *et al.*, 2018). Besides, infection by *T. evansi* was, occasionally, reported in dogs and horses (Desquesnes *et al.*, 2013; Rjeibi *et al.*, 2015). The pathogenic effect of this parasite is greatly important among camels manifested by abortions, anemia and an important decrease of animals' productivity (Röttcher *et al.*, 1987; Desquesnes *et al.*, 2013). In other African countries, the stained blood smears revealed that 14.0% and 15.8% of analyzed camels, respectively, from Algeria (Bennoune *et al.*, 2013) and Nigeria (Wakil *et al.*, 2016) were infected by *T. evansi*. However, in Mauritania, this prevalence was estimated at 1.3%, 16.2% and 25.2%, respectively, by microscopic examination, Card Agglutination Test (CAT) and Immuno-fluorescence Antibodies Assay (IFA) (Dia *et al.*, 1997). In Somalia, 1.7% of tested camels harbored *Trypanosoma* spp. by stained blood films, whereas seropositivity reached 56.0% using ELISA (Baumann & Zessin, 1992). Thus, the microscopic survey of haemopathogens is an interesting diagnostic test since it allows direct evidence of pathogens and identifies their cellular tropism. However, low sensitivity remains its major drawback compared to serological (IFA or ELISA) and molecular (PCR) methods (Baumann & Zessin, 1992; Dia *et al.*, 1997).

In this study, *Trypanosoma* spp. prevalence rate was significantly correlated to ticks infestation (45.7%; 95% CI: 0.33-0.79,  $P \leq 0.000$ ) and particularly associated to tabanid flies, despite its limited number, since it is known to be involved in the mechanic transmission of *T. evansi*. This is in line with previous results indicating a definite association between trypano-

somiasis outbreaks and abundance of ticks and tabanids (Losos, 1980; Luckins, 1998; Njiru *et al.*, 2002). However, *Hippobosca camelina* failed to transmit, experimentally, *T. evansi* despite the parasite surviving in the mouthparts and gut of the fly (Oyieke & Reid, 2003). Others biting flies like *Stomoxys* and *Haematobia minuta* as well as vampire bite were described in the mechanic transmission of *T. evansi*, but their role remains limited compared to ticks and tabanids (Sinha *et al.*, 1971; Soulsby, 1982; Luckins, 1998). *T. evansi* cannot grow and differentiate in insect tissues and its transmission can only be done mechanically (Gibson *et al.*, 1983; Songa *et al.*, 1990). Horses may also be infected by this parasite and usually die after an acute course of the disease (Jilo *et al.*, 2017). Dogs may be infected by *T. evansi* through eating infected meat (Röttcher *et al.*, 1987).

*Anaplasma* organisms were identified in camels' blood (17.0%). This result is in line with previous molecular reports indicating that 17.7% of Tunisian dromedaries (*Camelus dromedarius*) were infected by *Anaplasma* spp. identified probably as strains genetically related to *A. platys* (*A. platys*-like) by sequencing of some positive samples (Belkahia *et al.*, 2015). By the same way, *Anaplasma* spp. was identified in camels blood films from Iran (17.1%) (Ghazvinian & Khodaiean, 2016), Saudi Arabia (40.5%) (Ismael *et al.*, 2016), Nigeria (20.8%) (Wakil *et al.*, 2016) and Somalia (13.2%) (Abdalla *et al.*, 2017). These differences could be explained by the specific epidemiology of anaplasmosis in each country which is influenced by abiotic factors (such as air temperature, relative humidity and specific vegetation type), ticks' control programs, herd organization, breeding practices, and/or diversity of animal hosts that significantly affect the distribution of potential tick vectors (Hagras *et al.*, 1991).

In dromedaries, inclusions observed in neutrophils may correspond to *A. platys*-like (Bastos *et al.*, 2015; Belkahia *et al.*, 2015; Li *et al.*, 2015) as reported in ruminants and cats from Italia (Zobba *et al.*, 2014; Zobba *et al.*, 2015). However, the classified *A. platys*,

was preferentially found in canine platelets (Sainz *et al.*, 2015). Contrariwise, purple inclusions found in camels' erythrocytes, were considered as *Anaplasma* organisms in previous microscopic investigations (El-Naga & Barghash, 2016; Ghazvinian & Khodaiean, 2016; Ismael *et al.*, 2016; Abdalla *et al.*, 2017). Furthermore, camels can harbor others strains of *Anaplasma* spp. like *A. ovis* and *A. marginale* that may be observed in erythrocytes (El-Naga & Barghash, 2016; Noaman, 2018). Purple inclusions found in camel monocytes appear, morphologically, as *Anaplasma* organisms. This may consolidate the previous result indicating that *A. bovis* may infect ruminant monocytes (Sreekumar *et al.*, 1996; Rar & Golovljova, 2011; Zobba *et al.*, 2014). Therefore, detection, identification and interpretation of different blood smears features may provide useful data related to haemopathogens preferential cells, however, the great similarity of these various microscopic profiles makes it insufficient and needs to be completed by others serological and molecular assays.

In sheep, the observed intra-erythrocytic inclusions may correspond to *Anaplasma ovis* or *Anaplasma bovis* (Friedhoff, 1997; Liu *et al.*, 2012). These two *Anaplasma* species were earlier detected in Tunisian sheep using molecular methods (Belkahia *et al.*, 2014; Ben Said *et al.*, 2015). However, strains genetically related to *A. platys* were also identified in sheep from Tunisia (Ben Said *et al.*, 2017a) with a tropism in neutrophilic granulocytes as reported by Zobba *et al.* (2014). Among horses, thin and small round inclusions found in red cells, reminds the shape of intracellular bacteria of the Anaplasmataceae family. To date, among this group, only *A. phagocytophilum* (M'ghirbi *et al.*, 2012; Ben Said *et al.*, 2014) and *Neorickettsia risticii* (Coffman *et al.*, 2008) were detected in horses. Previous data indicated that horse neutrophils were specifically colonized by *A. phagocytophilum* (Alberti *et al.*, 2005). Thus, further studies, especially by using molecular methods, are needed to identify this classified or unclassified species belonging to Anaplasmataceae family found in infected horses.

To our knowledge, this is the first report describing *Babesia* spp. infection in camels (*Camelus dromedarius*) from Tunisia. *Babesia* spp. positive blood smear was identified in an adult female camel with previous history of abortion and presenting anemia. Our result shows a lower prevalence (1.0%) compared to previous microscopically findings reported in camels from Egypt (11.8%) (El-Naga & Barghash, 2016) and Nigeria (24.3%) (Wakil *et al.*, 2016). Similarly, the molecular prevalence of *Babesia* spp. among Iranian camels (6.6%) (Khamesipour *et al.*, 2015) was higher than that estimated in our study. Besides, the prevalence of this parasite in apparently healthy camels from Sudan reached 43.6% by microscopic examination and 74.5% using molecular tools (Ibrahim *et al.*, 2017). However, *Babesia caballii* is considered as the main species infecting camels (Qablan *et al.*, 2012; Al-Saad *et al.*, 2015). Otherwise, *Babesia* spp. was absent in examined sheep and horses' samples. In contrast, molecular survey of this parasite showed that prevalence rate was 2.9% in sheep (Rjeibi *et al.*, 2016). In addition, Tunisian cattle were found to be infected by *B. divergens* (Bouattour & Darghouth, 1996), *B. bovis* (6.8%) and *B. bigemina* (4.3%) (M'Ghirbi *et al.*, 2008). Regarding ticks, *Babesia* spp. was also identified in *Rhipicephalus annulatus*, *R. bursa* (M'Ghirbi *et al.*, 2010) and *Hyalomma marginatum* (Ros-Garcia *et al.*, 2011).

Our study revealed that *H. dromedarii*, *H. impeltatum* and *H. excavatum* were diagnosed as the most prevalent ticks infesting dromedaries and *R. turanicus* in sheep. In Tunisia, few studies described the potential tick vector of *Anaplasma* organisms (Ben Said *et al.*, 2018). In fact, *A. phagocytophilum* was isolated from *H. marginatum* (M'ghirbi *et al.*, 2012), *H. scupense* and *Ixodes ricinus* (Sarih *et al.*, 2005). However, *A. platys* was identified in *Rhipicephalus sanguineus sensu lato* (Sarih *et al.*, 2005). Under specific conditions and symbiotic interactions, some pathogens have been adapted to other animal hosts and vectors (Gharbi *et al.*, 2013). *Anaplasma ovis*, habitually associated with sheep, was identified in camel sharing the same

pasture (Moutailler *et al.*, 2016; Noaman, 2018).

Regarding flies, few number of tabanid (n=5) flies were collected in camel herds which may be explained by its reduced activity during summer (Jilo *et al.*, 2017). Whereas, *Hippobosca equina* was abundant during this season (Sokół & Michalski, 2015) which may explain the relatively important collected number (n=28). Moreover, horses found positive to *Anaplasma* spp. were infested by this latter fly species and not by ticks. This finding is consistent with previous reports suggesting that flies were increasingly included in the epidemiology of camel trypanosomosis and equine anaplasmosis by simple mechanical transmission which may consolidate the role of ticks (Hawkins *et al.*, 1982; Scoles *et al.*, 2008). The role of flies is proved in completely fed specimens and in highly bacteriemic host during acute infection. However, tick-borne biological transmission is more efficient than mechanical transmission by flies (Scoles *et al.*, 2008). This fly infested camel in a non-specific way, particularly in mixed herds, which makes the epidemiology of these vector-borne pathogens complex and difficult to manage (Woolhouse, 2001).

Our study provides new epidemiological data regarding haemopathogens infesting Tunisian military livestock. Unlike horses and sheep, camels were found to be intensively infested by several arthropod vectors and hosted both parasitic and bacterial pathogens. This data may be useful during vector-borne disease treatment and control programs. Further investigations, by using more sensitive tools, are required to define accurately the involved haemopathogens species and/or unclassified strains, and to establish the phylogenetic and epidemiological relations between pathogens, hosts and arthropod vectors.

#### **Conflicts of interest**

The authors declare that they have no conflicts of interest related to this work.

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