Epidemiological survey of vector-borne infections in equids from Northern Tunisia

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Summary

Leishmaniosis (*Leishmania infantum* infection) and piroplasmoses (*Theileria equi* and *Babesia caballi* infections) are vector-borne diseases with significant economic and public health impacts. Despite their importance, there is a lack of data concerning these infections in equids from Tunisia. The present study was carried out to estimate the prevalence of *L. infantum*, *T. equi* and *B. caballi* in 104 equids from Northern Tunisia. The authors reported for the first time on the seroprevalence of anti-*Leishmania* antibodies in equids in Tunisia (6.7%). The study reported a high infection prevalence of piroplasms (23.1%), revealed for the first time *T. equi* and *B. caballi* infections in Tunisian donkeys, and showed that these animals act as reservoirs for the maintenance and dissemination of piroplasms.

Keywords

Babesia caballi – Equid – *Leishmania infantum* – Prevalence – *Theileria equi* – Tunisia.

Introduction

Equids still play an important role in transportation and labour in Tunisian rural areas. Moreover, parasitic infections represent a major

health problem in equids, since the parasites involved decrease the productivity of equids leading to considerable economic losses and a dramatic alteration of the animals' well-being (1, 2). Leishmaniosis (*Leishmania infantum* infection) and piroplasmoses (*Theileria equi* and *Babesia caballi* infections) are vector-borne diseases affecting equids. Despite their significant effect on equids and humans, there is a lack of data from Tunisia on these infections. *Leishmania infantum* is the causative agent of visceral leishmaniosis, a life-threatening endemic disease in Tunisia, with a high infection rate in children under five years of age (3). This type of infection is strongly correlated with environmental changes as well as with dogs and sand flies, which serve as reservoirs and vectors, respectively (4, 5, 6). So far, no species in Tunisia, other than dogs and humans, has shown signs of infection by *L. infantum*. Since leishmaniosis commonly occurs in equids from Southern and Central America, pointing to *L. braziliensis* as the likely causative agent (7), equids are gaining increased importance in *L. infantum* epidemiology in endemic areas. Furthermore, cases of equine leishmaniosis due to *L. infantum* have been reported in Europe suggesting a potential role for equids in the epidemiology of this parasite (8, 9, 10, 11). Equine piroplasmosis is a widely distributed tick-borne disease affecting wild and domestic equids with an economic worldwide impact on the equine industry (12). *Theileria equi* and *B. caballi* are intra-lymphocytic and intra-erythrocytic protozoa, respectively (13, 14). Infected animals can exhibit no clinical signs but can become carriers of parasites (1). In Tunisia, donkeys represent 65.5% of the total equine population (www.onagri.tn). These animals cohabit closely with horses in Tunisian rural areas but their role as a potential reservoir of *T. equi* and *B. caballi* has not yet been elucidated.

In the present study, an epidemiological survey was carried out to estimate the molecular prevalence of *L. infantum*, *T. equi* and *B. caballi* in Tunisian equids. In addition, a serology was performed to estimate the seroprevalence of anti-*Leishmania* immunoglobulin G (IgG).

Materials and methods

Study area and animals sampling

This study was carried out in four districts of Northern Tunisia: Bizerte (37°16′N 9°52′E; sub-humid region); Jendouba (36°30′N 8°47′E; humid region), Ben Arous (36°44′50″N 10°20′0″E; semi-arid region) and Ariana (36°51′45″N 10°11′44″E; sub-humid region) (Fig. 1). These districts are characterised by a Mediterranean climate with hot, dry summers and cold, wet winters. During wintertime, blood samples were collected from the jugular vein of 104 randomly selected equids (59 Arab purebred horses and 45 donkeys) into sterile vacuum tubes, with and without ethylenediamine tetra-acetic acid (EDTA). The animals had access to free grazing, which was supplemented by hay, alfalfa and crushed barley. Clinical examinations were performed for all equids and only those animals that appeared to be healthy were included in the survey. Equids were ranked into groups based on different parameters (species, age and district).

DNA extraction and polymerase chain reaction

For the detection of *Leishmania* and the other parasites, DNA was extracted from the whole blood samples (15) using the Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, United States of America), according to the manufacturer's instructions. The DNA was stored at –20°C until use. Polymerase chain reactions (PCRs) were carried out to assess equids for the presence of *Leishmania* spp., *T. equi* and *B. caballi.* Primers as described by Degrave *et al.* and Alhassan *et al.*, respectively (16, 17) (Table I), were used as part of the PCR process. In each PCR run, a negative control (distilled sterile water) and positive controls (DNA samples for the parasites in question) were included. All PCR products were electrophoresed in 1.5% agarose gels stained with ethidium bromide and checked with an ultraviolet (UV) trans-illuminator.

Indirect immunofluorescence antibody test

To investigate anti-*Leishmania* IgG antibodies in equids sera, an indirect immunofluorescence antibody test (IFAT) was carried out using a kit (IDvet, Grabels, France) according to the manufacturer's instructions. Sera were tested at a 1:20 dilution cut-off point (18).

Statistical analysis

Infection prevalence among different parameters (i.e. species, age and district) was compared using Epi Info™ Version 6 software and the Chi-square Mentel-Haenszel test (19). Results were considered significant at a *p* value threshold of 0.05.

Results

Polymerase molecular reaction

The overall infection prevalence of piroplasms was 23.1 ± 4% (24/104). Positive equids were found in the three-studied bioclimatic zones (sub-humid, humid and semi-arid regions). The infection prevalence of *T. equi* was estimated at 22.1 ± 4%, and that of *B. caballi* was low at 2.9 ± 1%. Nonetheless, only two equids were co-infected by both parasites (1.9 ± 1%). Equids from the Ariana district showed the highest molecular prevalence (36.3 ± 9%), while the Bizerte district adjacent to it, and belonging to the same bioclimatic zone, displayed an infection prevalence of zero ($p < 0.05$) (Fig. 1). All the screened equids were PCR negative to *Leishmania* spp.

Indirect immunofluorescence antibody test

Anti-*Leishmania* IgG antibodies were detected in 6.7% ± 3 (7/104) of equids sera. Only horses from the Jendouba district showed a positive result with IFAT.

Discussion

To our knowledge, only one study concerning equine piroplasmosis in Tunisia has previously been published (20); however, no study in Tunisia targeted piroplasm infection in donkeys. The piroplasm

infection prevalence found in the current study (23.1%) was higher than that reported by Ros-García *et al.* (20) (12.5%), but lower than that reported in equids from Jordan (27.1%) (21), Italy (33%) (22), Sudan (35.9%) (23), Iran (45%) (24) and Venezuela (66.2%) (15). Moreover, the *T. equi* infection prevalence (22.1%) was significantly higher than that of *B. caballi* (2.9%) ($p < 0.05$). The authors' results are consistent with Qablan *et al.* who reported the same trend (18.8% and 7.3% of *T. equi* and *B. caballi* prevalence, respectively) (21) and Sgorbini *et al.* (2015) who reported a high infection prevalence of *T. equi* (41%) in horses from central Italy but no positive results for *B. caballi* infection among the same group (25). The higher infection prevalence of *T. equi* compared to *B. caballi* can be explained by the difference in parasitaemia levels. Other studies reported that this difference is the result of the persistence of *T. equi* in equids for its entire lifetime in comparison to *B. caballi* that remains in equids from only one to four years (26).

The present study revealed, for the first time in Tunisia, the occurrence of *T. equi* and *B. caballi* infections in donkeys (22.2%). The prevalences of these infections were similar to those reported in donkeys from Italy (21%) (27). The high infection rates of piroplasms in donkeys demonstrate the involvement of these animals in the epidemiology of piroplasmoses. Thus, donkeys should be considered in the control programmes of equine piroplasmosis in Tunisia as they are reservoirs. There was a statistically significant difference between infection prevalence and districts ($p < 0.001$). The highest prevalence was found in the Ariana district (36.3%), while the lowest was found in the Bizerte district (0%) (Fig. 1, Table II). The present study showed no significant association between equids species and piroplasm infection rate ($p > 0.05$). Other studies showed that donkeys are less receptive to piroplasms than horses (21, 28). There was a statistically significant difference between age and piroplasm infection prevalence ($p < 0.001$) (Table II). This result is consistent with one study from Mongolia (29) but inconsistent with that reported in Italy (22).

This paper provides the first report of *Leishmania* infection in equids from Tunisia. The IFAT showed a seroprevalence of 6.7% of anti-

Leishmania IgG antibodies in equids sera. The seroprevalence found in the present study (6.7%) was higher than that observed in Greece (0.3%) (30), and Portugal (4%) (11), but closer to that reported from Italy (6.5%) (18). One horse that showed skin lesions was *L. infantum* positive using IFAT but was PCR negative. This could be explained by the low parasitaemia in horses owing to the fact that they are not very receptive to *L. infantum* infection and by the effective immune response of horses in the elimination of the *Leishmania* parasite (31). In the present study, no animal was positive to *Leishmania* DNA in Northern Tunisia where the 24 pure-bred horses closely cohabite with 17 dogs suffering of leishmaniosis. However, all but one IFAT-positive equids did not exhibit any clinical signs of leishmaniosis, which may mean that they can serve as carriers. Our study confirmed that equids could be frequently infected with *L. infantum* in Tunisian endemic areas for leishmaniosis where a population density of infected dogs is high.

The aim of this study was to estimate the prevalence of three protozoa (*L. infantum*, *T. equi* and *B. caballi*) in equids from Northern Tunisia. The authors' study reported high infection prevalence by piroplasms. In addition, it reported for the first time in Tunisia, *T. equi* and *B. caballi* infections in donkeys and revealed that this species serves as a reservoir.

In addition, the authors recorded anti-*Leishmania* antibodies in equids sera for the first time in Tunisia. Thus, the involvement of equids in the epidemiology of this parasite cannot be excluded, and further molecular and serological studies are necessary in a larger sample in the most endemic areas of leishmaniosis in Tunisia to corroborate the current study's findings.

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Table I

Primers used in the present survey for detection by polymerase chain reaction of *Leishmania* spp., *Theileria equi* and *Babesia caballi* DNA in equids

Pathogen	Primer	Type	Sequence 5'-3'	Target gene	Product size (bp)	Reference
Leishmania spp.	Leish F	Forward primer	(C/G) (C/G) (G/C) CC (C/A) CTA T (T/A) T TAC ACC AAC CCC	kDNA mini-circles	120	(16)
	Leish R	Reverse primer	GGG GAG GGG CGT TCT GCG AA	kDNA mini-circles		
Babesia caballi and *Theileria equi*	Bec-UF2	Universal forward primer	TCGAAGACGATCAGATACCGTCG	18S RNA	867–913	(17)
	Cab-R	*B. caballi* specific reverse primer	CTCGTTCATGATTTAGAATTGCT	18S RNA	540	
	Equi-R	*T. equi* specific reverse primer	TGCCTTAAACTTCCTTGCGAT	18S RNA	392	

A: adenine
bp: base pair
C: cytosine
G: guanine
kDNA: Kinetoplast deoxyribonucleic acid
T: thymine
18S RNA: 18S ribosomal ribonucleic acid

Table II

Risk factors of *Theileria equi* and *Babesia caballi* infections in equids from Northern Tunisia

Species	Parameter		*Theileria equi*			*Babesia caballi*			*T. equi* + *B. caballi*		
			Positive/examined (%)	*p* value	OR (95% CI)	Positive/examined (%)	*p* value	OR (95% CI)	Positive/examined (%)	*p* value	OR (95% CI)
Horses	Age group (years)	≤2	0/10 (0)	0.002*	NA	0/10 (0)	0.95	NA	0/10 (0)	0.57	NA
		3–5	3/10 (30)			0/10 (0)			0/10 (0)		
		6–10	4/14 (28.5)			1/14 (7.1)			0/14 (0)		
		11–17	5/19 (26.3)			1/19 (5.2)			1/19 (5.2)		
		≥18	1/6 (16.6)			0/6 (0)			0/6 (0)		
	Districts	Jendouba	8/24 (33.3)	< 0.001*	NA	0/24 (0)	0.16	NA	0/24 (0)	0.12	NA
		Ariana	4/8 (50)			1/8 (12.5)			1/8 (12.5)		
		Ben Arous	1/8 (12.5)			1/8 (12.5)			0/8 (0)		
		Bizerte	0/19 (0)			0/19 (0)			0/19 (0)		
Donkeys	Age group (years)	≤2	3/11 (27.2)	< 0.001*	NA	0/11 (0)	0.36	NA	0/11 (0)	0.36	NA
		3–5	2/13 (15.3)			1/13 (7.7)			1/13 (7.7)		
		6–10	0/10 (0)			0/10 (0)			0/10 (0)		

		11–17	1/4 (25)			0/4 (0)			0/4 (0)		
		≥18	4/7 (57.1)			0/7 (0)			0/7 (0)		
	Districts	Ariana	3/14 (21.4)	0.47	NA	0/14 (0)	0.42	NA	0/14 (0)	0.42	NA
		Ben Arous	6/22 (27.2)			1/22 (4.5)			1/22 (4.5)		
		Bizerte	0/9 (0)			0/9 (0)			0/9 (0)		
Overall	Age group (years)	≤2	3/21 (14.2)	0.13	1.98 [0.84; 4.84]	0/21 (0)	0.29	NA	0/21 (0)	0.52	NA
		3–5	5/23 (21.7)			1/23 (4.3)			1/23 (4.3)		
		6–10	4/24 (16.6)			1/24 (4.1)			0/24 (0)		
		11–17	6/23 (26)			1/23 (4.3)			1/23 (4.3)		
		≥18	5/13 (38.4)			0/13 (0)			0/13 (0)		
	Districts	Jendouba	8/24 (33.3)	< 0.001*	NA	0/24 (0)	0.29	NA	0/24 (0)	0.50	NA
		Ariana	8/22 (36.3)			1/22 (4.5)			1/22 (4.5)		
		Ben Arous	7/30 (23.3)			2/30 (6.6)			1/30 (3.3)		
		Bizerte	0/28 (0)			0/28 (0)			0/28 (0)		

CI: confidence interval
NA: not applicable
OR: odds ratio
*statistically significant ($p \leq 0.05$)

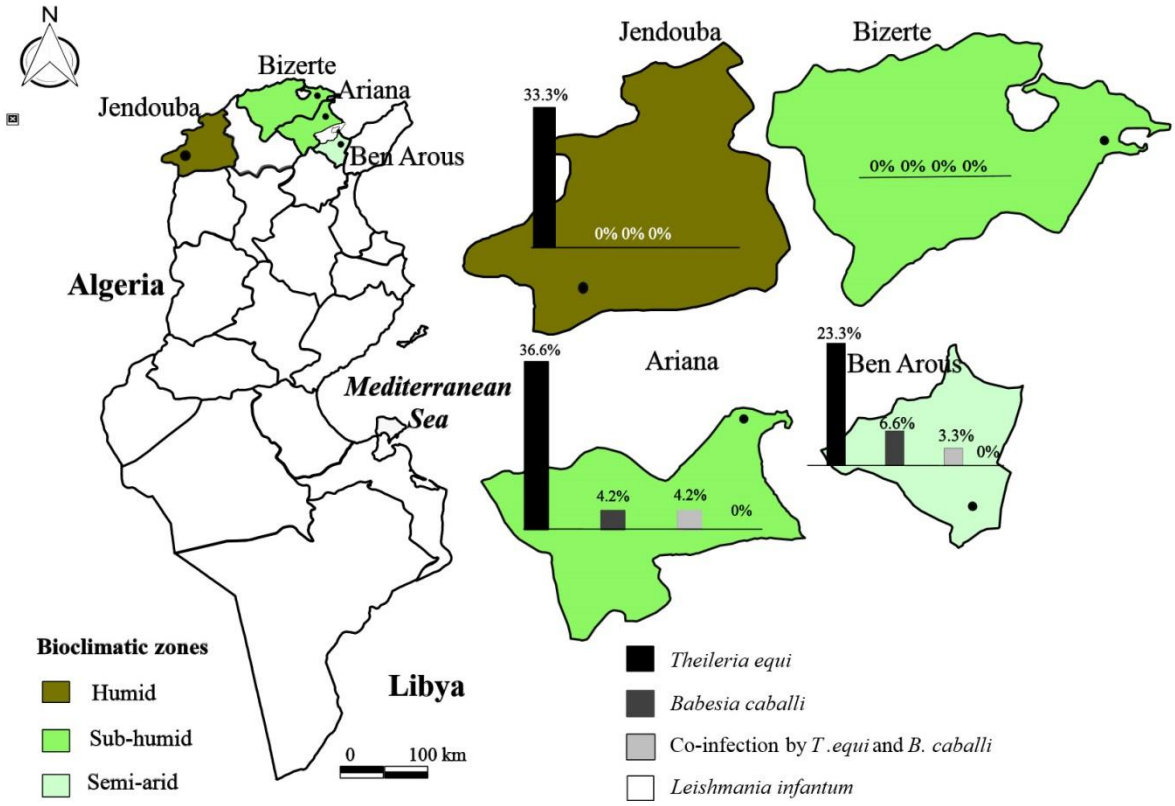

Fig. 1

Molecular prevalence of *Theileria equi*, *Babesia caballi* and *Leishmania infantum* in equids from four districts in Northern Tunisia