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Seroprevalence of *Theileria Ovis* in Goats from M'sila Region, Central of Algeria

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Abstract

BACKGROUND: In Algeria, data on the epidemiology of theileriosis in small ruminants are limited.

OBJECTIVES: The current study aims is investigating on the seroprevalence of *Theileria* spp. in goats from M'sila region, Central of Algeria.

METHODS: Blood samples of 128 goats from 19 farms were collected from the locality of Maâdid. The indirect fluorescence antibody test (IFAT) was performed in order to test the sera of goats for antibodies against *Theileria ovis* and *Theileria lestoquardi*.

RESULTS: Out of 128 tested samples, 21 sera (16.40%) were positive for *T. ovis* antibodies. All samples were seronegative for *T. lestoquardi*. The seroprevalence of *T. ovis* varied from 10% to 30% in the farms. The seropositivity rates did not vary significantly with age, sex, or breeding system of goats. Tick infested goats were significantly more seropositive than no infested goats.

CONCLUSIONS: The present study reports important data on the epidemiology of caprine theileriosis from Central of Algeria.

Keywords: IFA test, sera, risk factors, goats, *Theileria* spp.

Introduction

Theileriosis, caused by the haemoprotozoan *Theileria*, is one of the most common tick-borne diseases in livestock worldwide (Gharbi et *al*., 2020). This parasitic disease is considered as one of the major constraints of small ruminant production, causing considerable economic losses mainly in Asia, Africa and southern part of Europe (Schnittger et *al*., 2004). Currently, more than 185 species are known within the genus *Theileria* in domestic and wild animals (Al-Fahdi et *al*., 2017). *Theileria annulata* and *Theileria parva* are the most important pathogens for cattle (Gharbi et *al*., 2020;Al-Fahdi et al., 2017), while *Theileria lestoquardi* (formerly known as Theileria hirci), *Theileria luwenshuni*, and *Theileria uilenbergi* are pathogenic for small ruminants (Mans et *al*., 2015;Aydin et *al*., 2013; Altay et *al*., 2012). Other species less or non-pathogenic such as *Theileria ovis*, *Theileria seperata*, *Theileria recondita*, *T. annulata*, and uncharacterized isolates of *Theileria* (*Theileria* sp. OT1, *Theileria* sp. OT3, and *Theileria* sp. MK) have been reported in sheep and goats (Aydin et *al*. 2013; Altay et *al*. 2012; Zaeemi et *al*. 2011; Defaye et *al*. 2022; Stuen, 2020). *Theileria* species of small ruminants are distributed particularly in the tropical and subtropical regions of Africa, Middle East, Asia, and eastern and southern parts of Europe (Zhang et *al*., 2015). Various tick species belong to *Hyalomma*, *Haemaphysalis*, and *Rhipicephalus* genera are known as vectors of this protozoan (Mans et *al*., 2015; Stuen, 2020). *Theileria* species discrimination is of epidemiological and clinical significance. The species identification on microscopic examination of blood smears is limited. Serological tests are important and reliable means for carrying out epidemiological surveys. Indirect fluorescent antibody test (IFAT) is the most widely used method for sero-diagnosis of the common important species (OIE, 2018). However, these serological tests have certain drawbacks such as cross-reactivity of genetically closely related species. Thus, the molecular

methods remain an essential line of enquiry in the determination of species and genetic variations (Mans et *al*., 2015; Nagore et *al*., 2004, Tahir et *al*., 2023).

In Algeria, the epidemiology of theileriosis in livestock is poorly known. Few studies, limited to some geographical regions, have been conducted on the prevalence and molecular characterization of *Theileria* spp. in ruminants as well as in some tick species. Based on the microscopic examination of Giemsa-stained blood smears, the prevalence of bovine tropical theileriosis has been estimated of 10.4% to 45.5% in central-east of Algeria (Ayadi et *al*., 2016; Benchikh Elfegoun et *al*., 2017; Ziam et *al*., 2020; Foughali et *al*., 2021a).*Theileria annulata* has been also characterized in cattle with prevalence varying of 25.4% to 50%(Ayadi et *al*., 2016; Ziam et *al*., 2015). Moreover, Bovine Theileria isolates closely related to *Theileria buffeli* have been detected with infection rate of 6.70% (Ziam et *al*., 2015). The occurrence of both *T. annulata* and *T. buffeli* was confirmed in cattle and *Rhipicephalus annulatus* (Sadeddine et al., 2020). In sheep, an infection rate of 2.32% of *Theileria* spp. has been recorded using microscopic examination (Foughali et al., 2021b). *Theileria ovis* were detected in both sheep and goats as well as in *Rhipicephalus bursa* and *Rhipicephalus turanicus* from north-eastern Algeria (Sadeddine et *al*., 2020; Aouadi et *al*., 2017). Additionally, *T. annulata* was detected in sheep from east of Algeria (Sadeddine et *al*., 2020). In horses, microscopic examination and competitive ELISA test revealed an overall prevalence of *Theileria equi* of 15.9% and 29.12%, respectively (Benfenatki et *al*., 2016). Recently, *T. equi* was genetically identified in equines and cattle (Sadeddine et *al*., 2020). Algeria has a large area with climate diversity, which makes necessary exhaustive studies and many data for understanding the epidemiology of this economically important parasitic disease. As such, to support previously reported data, the present study aimed to investigate on the seroprevalence of *Theileria* spp. in goats from the M'sila region (central of Algeria) using the IFA test.

Materials and methods

Study area and sampling

The present study was carried out in the Maâdid locality from M'sila province, which is steppic region located in the central part of Algeria occupying an area of 18,175 km^2 (GPS: 35°42'7''N and 4°32'49''E) (Fig.1). This region is characterized by a semi-arid climate with very hot and dry summers and relatively cold winters. The average annual temperature ranging from 16.90 ºC to18.40 ºC. Breeding of small ruminants represents the main agricultural activity in this region.

Blood samples were collected directly from the jugular vein of 128 goats during Jun to September 2016. Goats belong to 19 semi-extensive farms associated or not with sheep breeding. Approximately, 10% of reared goats in each farm were selected randomly for blood sampling. Goats were in good health and no infection signs were detected during sampling. Data related to animal characteristics, including gender, age, breeding system, and tick infestation were recorded during sampling. All samples were collected in EDTA tubes, subsequently were centrifuged and the obtained sera were preserved in -20°C until serological analysis.

Institutional Review Board Statement: "The animal study and the blood sample collection were carried out in accordance to the current Algerian Regulations No. 88-08 of 26 January 1988 related to Veterinary Medicine Activities and the protection of animal health (N° 004 JORA of 27-01-1988).

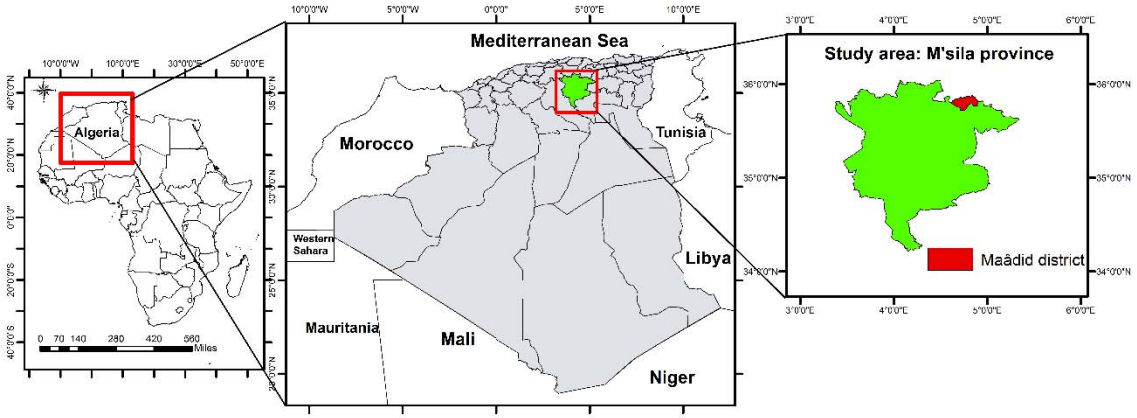

Figure 1: Geographic map showing the location of study area in Algeria (map was constructed using ArcGIS software).

Serological analysis

Indirect fluorescence Antibody test (IFAT) was performed for screening the goats as previously described (Papadopoulos et *al*., 1995). The detection of antibodies against *T. lestoquardi* was performed using Teflon-coated slides fixed with antigens of *T. lestoquardi* schizont stage. Slides with blood smears infected by *T. ovis* were used as antigens to detect antibodies against this species. The blood smears were fixed with the acetone as previously described (Ludford, 1969). Positive and negative control sera were included for each reaction.

Sera were diluted in PBS at 1/20 and 1/40 for antibodies IgG detection of *T. ovis* and *T. lestoquardi*, respectively. Subsequently, they were deposited in wells of teflon coated slides (20µl/well) and incubated at room temperature for 20 min. After three washes in PBS, specific antibodies for targeted *Theileria* species could be detected using anti-goat IgG conjugated to fluorescein isothiocyanate (FITC) diluted at 1/50 in PBS. Finally, sera and conjugates were incubated for 20 min at room temperature. Three washes in PBS were then carried out. The slides were covered with coverslips using glycerol 50% in PBS. Examination of the slides was performed under objective 40x (total magnification of 400) using a fluorescent microscope. Sera were considered positive at titer $\geq$ 1/20 and $\geq$ 1/40 for *T. ovis* and *T. lestoquardi*, respectively. Moreover, positive sera with titer $\geq$ 1/20 were tested for other titers (1/100, 1/200, 1/400).

Statistical analyses

Statistical analyses were performed using an online site SciStat® (https://www.scistat.com/index.php). Fisher's exact and Chi-Square tests were exploited to assess the seroprevalence variation according to animal attributes (independent variables), previously recorded. The *P*-value <0.05 was regarded statistically significant.

Results

Seroprevalence and associated risk factors

Out of 128 tested samples, 21 goats (16.40%) were seropositive for *T. ovis* (Table 1). All tested goats were seronegative for *T. lestoquardi*. Out of the 19 screened farms, 12 (63.15%) were seropositive. The seroprevalence of *T. ovis* in these farms varied from 10% to 30% (Table 1). Among the positive sera with the titer 1/20 (21 samples; 16.40%), 14 (10.93%), 5 (3.90%), and 2 (1.56%) were positive with titers of 1/100, 1/200, and 1/400, respectively (Table 2).

Table 1. Seroprevalence of *Theileria ovis* in goats from M'sila region, Algeria

Farms	No. of screened samples	No. of seropositive samples	Seroprevalence (%) (CI 95%)
1	4	0	0 (0.00 - 0.00)
2	4	1	25 (0.00 - 67.44)
3	4	1	25 (0.00 - 67.44)
4	2	0	0 (0.00 - 0.00)
5	7	2	28 ,57 (0.00 - 62.04)
6	10	0	0 (0.00 - 0.00)
7	10	3	30 (1.60 - 58.40)
8	6	1	16.16 (0.00 - 46.49)
9	9	1	11.11 (0.00 - 31.64)
10	12	2	16.16 (0.00 - 37.75)
11	9	2	22,22 (0.00 - 49.38)
12	10	3	30 (1.60 - 58.40)
13	3	0	0 (0.00 - 0.00)
14	5	0	0 (0.00 - 0.00)
15	10	1	10 (0.00 – 28.59)
16	13	3	23,07 (0.17 - 45.98)
17	3	0	0 (0.00 - 0.00)
18	4	1	25 (0.00 - 67.44)
19	3	0	0 (0.00 - 0.00)
Total	128	21	16.40 (13,79 - 28,20)

CI: confidence interval

Table 2. Seroprevalence rates according to the different dilutions of sera

IgG antibodies	Serum Titration	No. of screened samples	No. of seropositive samples	Seroprevalence (%) (CI 95%)
anti-*Theiliria ovis*	1/20	128	21	16.40 (13,79 - 28,20)
	1/100	128	14	10.93 (5.53 - 16.34)
	1/200	128	5	3.90 (0.55 - 7.26)
	1/400	128	2	1.56 (0.00 - 3.71)
anti-*Theiliria Lestoquardi*	1/40	128	0	0 (0.00 - 0.00)

Furthermore, females revealed slightly high seroprevalence as compared to males (18.18% vs 13.72%). The highest seroprevalence was recorded in goats more than 24 months (20.73%) as compared to those aged of 12-24 months and less than 12 months, which showed seroprevalence of 8.57% and 9.09%, respectively. The seroprevalence was higher (17.52%) in farms where sheep breeding was absent as compared to farms where sheep breeding was present (12.90%). The seroprevalence did not vary significantly with age, sex, or breeding system (presence or absence of sheep) (all results showed *P*-value > 0.05). Tick infested goats were significantly more seropositive (40%) than those no infested (12.03%) (Table 3).

Table 3. Distribution of *T. ovis* seroprevalence by sex, age, tick infestation, and breeding system

Risk factors	No. of screened samples	No. of seropositive samples	Seroprevalence (%) (CI 95%)	*P*-value
Sex	128	21	16.40 (13,79 - 28,20)	
Males	51	7	13,72 (3,58 - 22,41)	0,5
Females	77	14	18,18 (9,24 - 26,75)	
Age (months)	128	21	16.40 (13,79 - 28,20)	
< 12	11	1	9,09 (0 - 26,25)	0.2
12-24	35	3	8,57 (0 - 17,17)	
> 24	82	17	20,73 (11,16 - 28,83)	
Tick infestation at sampling	128	21	16.40 (13,79 - 28,20)	
Presence	20	8	40.00 (18,09 - 61,90)	0.01
Absence	108	13	12,03 (5,74 - 18,25)	
Breeding system	128	21	16.40 (13,79 - 28,20)	
Extensive associated with sheep	31	4	12,90 (1.10 - 24.70)	0.5
Extensive not associated with sheep presence	97	17	17.52 (9.96 - 25.09)	

Discussion

The present study based on IFA test reports for the first time the seroprevalence of *T. ovis* in goats from M'sila region, central of Algeria. The IFA test is commonly used as a serological method for the detection of specific antibodies of *Theileria* spp. in small ruminates. This recognition of a specific humoral response remains useful in the epidemiological surveys as compared to the microscopic examination of blood smears that remains difficult, particularly for chronic infections. However, cross-reactions or weak specific-immune responses represent the main disadvantages of serological methods (OIE, 2022). Data on the seroprevalence of *Theileria*

spp. in small ruminants are relatively limited worldwide. The seroprevalence of *T. ovis* in the present study was higher than the reported one in goats from Greece (Papadopoulos et *al*., 1996). Relatively, a comparable result has been reported in Turkey (Sayin et *al*., 2009). In sheep, these previous studies have documented higher seroprevalences (Papadopoulos et *al*., 1996; Sayin et *al*., 2009). Variable prevalence rates of *Theileria* spp. in sheep and goats have also been reported from different serological investigations (Alyasino & Greiner, 1999; Guo et *al*., 2007; Luo et *al*., 2017; Li et *al*., 2017). All seropositive samples were detected with a titer ≥ 1/20, of which only two samples were detected with a titer 1/400.Various factors such as the study design, sample size, sampling period and tick infestation of animals, as well as the infection status and intensity of the specific humoral response, could influence on the seroprevalence variations. The seroprevalence did not vary significantly with age, sex, or breeding system (presence or absence of sheep breeding associated with the examined farms). In goats, no difference in the seroprevalence of *Theileria* infection was reported between females and males (Luo et *al*., 2017). In sheep, the molecular prevalence of *T. ovis* did not vary significantly for age categories, while females were significantly more infected than males (Rjeibi et *al*., 2014a). The effect of associated host factors on the susceptibility to infection is not fully well understood. Tick infested goats were more seropositive than no infested goats. Different species belong to *Hyalomma*, *Haemaphysalis*, and *Rhipicephalus*, which are distributed in the tropical and subtropical regions of Africa as an example, could transmit *Theileria* spp. in small ruminants (Stuen, 2020). The presence of *T. ovis* in both sheep and goats has been confirmed in North African countries such Algeria and Tunisia using molecular approaches(Sadeddine et *al*., 2020; Aouadi et *al*., 2017; Rjeibi et *al*., 2014a; M'ghirbi et *al*., 2013). *Theileria ovis* was known as non-pathogenic species for infected animals (Aouadi et *al*., 2017; M'ghirbi et *al*., 2013).

Similarly to our findings on seroprevalence of *T. lestoquardi* (0.0%), all goats and even sheep were tested to be seronegative in Turkey (Sayin et *al*., 2009). Seroprevalence rates of this species ranging from 1.2% to 33.8% were revealed in sheep from Tunisia and Sudan (Rjeibi et *al*., 2014b; Salih et *al*., 2003; Ahmed et *al*., 2018; Hassan et *al*., 2019). It should not be excluded that the absence of seropositive cases may be related to the detection threshold of the test for a weak specific-immune response. *Theileria lestoquardi*, the causative agent of malignant ovine theileriosis (MOT), is distributed particularly in some countries from sub-Saharan Africa (Sudan and Tanzania) and Asia (Iraq, Iran, India, Pakistan, Saudi Arabia, and Oman) (Rjeibi et *al*., 2014b; El Imam & Taha, 2015; Saeed et *al*., 2015; Awad et *al*., 2018; Habibi et *al*., 2020; Rassim Mohammed and Al-Saadi, 2023). Occurrence of this species has been confirmed molecularly in sheep from Tunisia (Rjeibi et *al*., 2014b). It is likely that its geographical extent is broader than previously reported since this species has occurred for example in the Maghreb region.

In conclusion, the current study reports valuable data on the seroprevalence of *T. ovis* in goats from central of Algeria. Further exhaustive studies in different regions through the national territory are required to better understand the epidemiology of theileriosis in small ruminants, particularly regarding the prevalence, clinical impact, and molecular characterization of infecting species.

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