Original Article

Seroprevalence of *Theileria ovis* in Goats from M'Sila Region, Central Algeria

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ABSTRACT

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

Objectives: The current study investigates the seroprevalence of *Theileria* spp. in goats from the M'Sila region, Central 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 to test the goats' sera 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% on farms. The seropositivity rates did not differ significantly with age, sex, or breeding system of goats. Tick-infested goats were significantly more seropositive than no-infested goats.

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

Keywords: The indirect fluorescence antibody test (IFAT), Sera, Risk factors, Goats, *Theileria* spp

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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 one of the major constraints of small ruminant production, causing considerable economic losses mainly in Asia, Africa and the southern part of Europe (Schnittger et al., 2004). 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 virulent or non-pathogenic such as *Theileria ovis*, *Theileria separata*, *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, the Middle East, Asia and eastern and southern Europe (Zhang et al., 2015). Various tick species belonging 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 essential and reliable means for carrying out epidemiological surveys. Indirect fluorescent antibody test (IFAT) is the most widely used method for serodiagnosis of the common important species (WOAH, 2018). However, these serological tests have certain drawbacks, such as cross-reactivity of genetically closely related species. Thus, molecular methods remain an essential line of inquiry in determining species and genetic variations (Mans et al., 2015; Nagore et al., 2004; Tahaa 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 and some tick species. Based on the microscopic examination of Giemsa-stained blood smears, the prevalence of bovine tropical theileriosis has been estimated at 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). *T. annulata* has also been characterized in cattle with a prevalence 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 an infection rate of 6.70% (Ziam et al., 2015). Both *T. annulata* and *T. buffeli* were 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). *T. ovis* was detected in sheep, goats and *Rhipicephalus bursa* and *Rhipicephalus turanicus* from northeastern Algeria (Sadeddine et al., 2020; Aouadi et al., 2017). Additionally, *T. annulata* was detected in sheep from the 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 requires exhaustive studies and data to understand the epidemiology of this economically important parasitic disease. To support previously reported data, the present study aimed to investigate the seroprevalence of *Theileria* spp. in goats from the M'Sila region (central 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 a Steppic region located in the central part of Algeria occupying an area of 18175 km^2 (GPS: 35°42'7"N and 4°32'49"E) (Figure 1). This region is characterized by a semi-arid climate with very hot and dry summers and relatively cold winters. The average annual temperature ranges from 16.90 -18.40 °C. Breeding of small ruminants represents the main agricultural activity in this region.

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

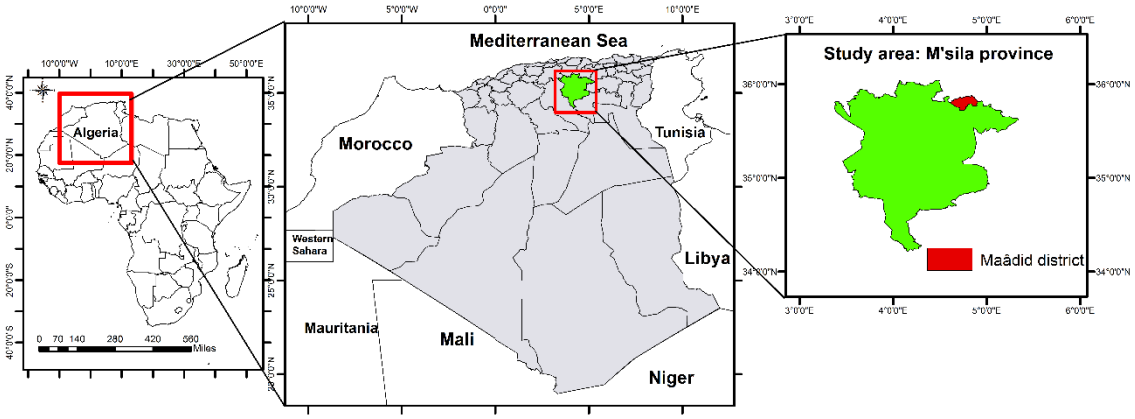

Figure 1. The location of the study area in Algeria

Note: Map was constructed using ArcGIS software, version 10.5.

Based on the institutional review board statement, the animal study, and the blood sample collection were carried out in accordance with the current Algerian Regulations No. 88-08 of January 26, 1988, related to Veterinary Medicine Activities and the protection of animal health (N°004 JORA of 27-01-1988).

Serological analysis

An indirect fluorescence antibody test (IFAT) was performed to screen the goats as previously described (Papadopoulos et al., 1995). Antibodies against *T. lestoquardi* were detected using Teflon-coated slides fixed with *T. lestoquardi* schizont stage antigens. Slides with blood smears infected by *T. ovis* were used as antigens to detect antibodies against this species. The blood smears were fixed with 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 (20 µL/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 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. The slides were examined under objective 40× (total magnification of 400×) using a fluorescent microscope. Sera were considered positive at titer ≥1/20 and ≥1/40 for *T. ovis* and *T. lestoquardi*, respectively. Moreover, positive sera with titer ≥1/20 were tested for other titers (1/100, 1/200, 1/400).

Statistical analyses

Statistical analyses were performed using online MedCalc's free statistical calculators website. The Fisher exact and chi-square tests were exploited to assess the seroprevalence variation according to previously recorded animal attributes (independent variables). The $P<0.05$ was regarded as 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*. 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).

Furthermore, females revealed slightly higher seroprevalence than males (18.18% vs 13.72%). The highest seroprevalence was recorded in goats over 24 months (20.73%) compared to those aged 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 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 $P>0.05$). Tick-infested goats were significantly more seropositive (40%) than those not infested (12.03%) (Table 3).

Table 1. Seroprevalence of *T. ovis* in goats from the M'Sila region, Algeria

Farm	Samples No.		Seroprevalence (%), (95% CI)
	Screened	Seropositive	
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)

Discussion

The present study is based on IFA test reports for the first time on the seroprevalence of *T. ovis* in goats from the M'Sila region, central Algeria. The IFA test is commonly used as a serological method for detecting specific antibodies of *Theileria* spp. in small ruminates. This recognition of a specific humoral response benefits the epidemiological surveys compared to the microscopic examination of blood smears, which remains challenging, particularly for chronic infections. However, cross-reactions or weak specific-immune responses represent the main disadvantages of serological methods (WOAH, 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 that in goats from Greece (Papadopoulos et al., 1996). A comparable result has been reported in Turkey (Sayin et al., 2009). These previous studies have documented higher seroprevalences in sheep (Papadopoulos et al., 1996; Sayin et al., 2009). Different serological investigations have also reported variable prevalence rates of *Theileria* spp. in sheep and goats (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 were detected with a titer of 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 the seroprevalence variations. The seroprevalence did not vary significantly with age, sex, or breeding sys-

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

IgG Antibody	Serum Titration	Screened Samples (n)	Seropositive Samples (n)	Seroprevalence (%), (95%CI)
Anti-*T. 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-*T. Lestoquardi*	1/40	128	0	0, (0.00-0.00)

tem (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., 2014). 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 belonging to *Hyalomma*, *Haemaphysalis* and *Rhipicephalus*, which are distributed in Africa's tropical and subtropical regions, could transmit *Theileria* spp. in small ruminants (Stuen, 2020). The presence of *T. ovis* in sheep and goats has been confirmed in North African countries such as Algeria and Tunisia using molecular approaches (Sadeddine et al., 2020; Aouadi et al., 2017; Rjeibi et al., 2014; M'ghirbi et al., 2013). *T. ovis* was a non-pathogenic species for infected animals (Aouadi et al., 2017; M'ghirbi et al., 2013).

Similarly to our findings on the 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., 2016; Salih et al., 2003; Ahmed et al., 2018; Hassan et al., 2019). The absence of seropositive

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

Risk Factor	No.		Seroprevalence (%), (95% CI)	P
	Screened Samples	Seropositive Samples		
Sex	128	21	16.40, (13.79-28.20)	
Male	51	7	13,72, (3.58-22.41)	0.5
Female	77	14	18,18, (9.24-26.75)	
Age (m)	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 association 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)	

cases should not be excluded, as it may be related to the detection threshold of the test for a weak specific-immune response. *T. 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., 2016; El Imam & Taha, 2015; Saeed et al., 2015; Awad et al., 2018; Habibi et al., 2020; Rassim Mohammed & Al-Saadi, 2023). The occurrence of this species has been confirmed molecularly in sheep from Tunisia (Rjeibi et al., 2016). Its geographical extent is likely broader than previously reported since this species has occurred, for example, in the Maghreb region.

Conclusion

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

Ethical Considerations

Compliance with ethical guidelines

The animal study protocol was approved by the Institutional Review Board of Research Center in Agropastoralism of Ziane Achour University, Djelfa, Algeria, in accordance Ministry of Higher Education and Scientific Research, for studies involving animals.

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The paper was extracted from the master's thesis of Laadjal Soumia, approved by the Department of Veterinary Sciences, Higher National Veterinary School, Algiers.

Authors' contributions

Conceptualization: Ghalmi Farida, Hafsi Fella, and Cantekin Zafer; Samples collection: Laadjal Soumia, and Reghaissia Nassiba; Experiments: Cantekin Zafer; Statistical analyses: Dahmane Abdeldjalil, and Samari Houssem; Initial draft preparation: Reghaissia Nassiba, Laadjal Soumia, and Laatamna AbdElkarim; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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