Seroprevalence of Antibodies against *Anaplasma* spp. in Algerian

Sheep and Associated Risk Factors

3

2

Mira Rima Haddoum^{1,2}, Omar Salhi^{3,4}, Mohamed Rahal⁴, Abdelaziz Lounas³, Safia
 Zenia², Nassim Ouchene³, Nadjet Amina Khelifi Touhami*³, Farida Ghalmi^{1,2}, Naouelle
 Azzag^{1,2}

7

8

- 9 1. Research Laboratory for Management of Local Animal Resources (GRAL), Higher
- National Veterinary School of Algiers, BP 161, El Harrach, Algiers, Algeria.
- 2. Higher National Veterinary School of Algiers, BP 161, El Harrach, Algiers, Algeria.
- 3. Institute of Veterinary Sciences, University of Blida 1, BP 270, Blida, Algeria.
- 4. Laboratory of Biotechnology and Valorization of Biological Resources, Faculty of
 Sciences, University of Medea, BP 166, Medea, Algeria.

15

- *Corresponding author: khelifi nadjet@univ-blida.dz
- 17 ORCID: Mira Rima Haddoum <u>0009-0003-7637-2986</u>
- 18 ORCID: Nadjet Amina Khelifi Touhami: <u>0000-0002-1383-5002</u>

19

20 21

22

23 24

25

26 27

28

29

30

31 32

33

34

35 36

37

38

39

40

41

Abstract

This study aimed to determine the seroprevalence and identify the main risk factors associated with exposure to Anaplasma spp. in sheep from two Algerian regions, Médéa and Bordj Bou Arréridj. Between March and November 2021, a total of 361 blood samples were collected from sheep during both spring and autumn seasons. The sera were analyzed using a competitive enzyme-linked immunosorbent assay (MSP5-cELISA), which detects antibodies against Anaplasma spp. but does not distinguish between species. The overall seroprevalence reached 73.13%, with a significantly higher rate observed in Médéa (81.0%) compared to Bordj Bou Arréridj (70.12%) (odds ratio [OR] = 1.82). Multivariate logistic regression analysis revealed that age (12-24 months), sampling season (spring), sub-humid climatic conditions, and tick infestation were significantly associated with Anaplasma spp. seropositivity. Among these, tick infestation emerged as the strongest predictor (adjusted OR = 11.98, p < 0.0001). No significant associations were detected with sex or breeding system. These results demonstrate a high level of exposure to *Anaplasma* spp. among Algerian sheep and provide the first cELISA-based serological evidence for these regions. The findings underscore the role of environmental factors—particularly climatic conditions and vector presence—in shaping the epidemiological dynamics of anaplasmosis in small ruminants. As MSP5-based serology detects antibodies rather than active infection, these data reflect historical exposure rather than current infection status. Future research should integrate molecular confirmation (PCR and sequencing) and longitudinal follow-up to identify 42 circulating Anaplasma species, assess infection seasonality, and design effective, region-

43 specific vector control strategies to mitigate the economic impact of ovine anaplasmosis in

44 Algeria.

Keywords: Algeria, *Anaplasma* spp., cELISA, Risk factors, Sheep

47 48

49

45 46

1. Introduction

50 Sheep farming plays a crucial role in North African agriculture, contributing significantly to the

- local economy and the livelihoodsof rural communities [1]. In Algeria, it also supports the
- 52 preservation of the steppe ecosystem by providing essential animal products that sustain
- 53 human well-being [2].
- However, sheep are exposed to numerous health challenges, and their productivity is often
- 55 compromised by infectious and parasitic diseases, many of which are vector-borne and
- 56 transmitted by hematophagous arthropods such as ticks. These pathogens cause major
- economic losses by reducing flock productivity and animal health [3].
- 58 Among tick-borne pathogens, bacteria of the genus Anaplasma have gained increasing
- 59 attention because of theire global distribution, economic impact and zoonotic potential [4].
- 60 Anaplasma spp. are Gram-negative, obligate intracellular bacteria belonging to the family
- 61 Anaplasmataceae, order Rickettsiales, and class Alphaproteobacteria. They infect various
- host cells, particularly erythrocytes, leukocytes, and endothelial cells and cause anaplasmosis,
- a disease characterized by fever, anemia, weight loss, and, in severe cases, death.
- 64 Small ruminants can serve as hosts for several *Anaplasma* species, most notably *Anaplasma*
- ovis, and occasionally Anaplasma marginale or Anaplasma phagocytophilum under specific
- 66 epidemiological conditions [5]. However, A. ovis remains he primary etiological agent of
- ovine Anaplasmosis. It is a strict intraerythrocytic bacterium that infects sheep, goats, and
- 68 certain wild ungulates [6].
- 69 Ovine Anaplasmosis is mainly transmitted by ticks of the order Ixodida, particularly
- 70 Rhipicephalus bursa, Hyalomma lusitanicum, and Dermacentor silvarum [7]. Clinical signs
- are often nonspecific and may include fever, lethargy, anorexia, anemia, jaundice, nasal
- discharge, weight loss, and abortion [8]. These symptoms, however, can overlap with those of
- other tick-borne hemoparasitic infections such as babesiosis and theileriosis, which makes clinical
- 74 diagnosis alone unreliable.
- 75 The disease is widespread among small ruminants and has a broad geographical distribution,
- having been reported in Mediterranean countries, the Middle East, the USA, Asia, and Africa
- 77 [9]. Animals that survive the acute phase of the disease often remain chronically infected,
- with low bacteremia levels undetectable by conventional stained blood smears [10]. In such
- 79 cases, diagnosis relies mainly on indirect methods such as serological testing. Therefore, the
- 80 detection of anti-A. ovis antibodies through serology is an essential tool for effective
- 81 monitoring and management of flock health [11].

82

- 83 Serological approaches, particularly competitive ELISA (cELISA) are valuable for epidemiological
- surveillance.. The test used in this study (Anaplasma Antibody Test Kit, version 2, VMRD, Pullman,
- WA) detects antibodies against the major surface protein MSP5, which is conserved across several

Anaplasma species [12]. Although initially developed for detecting antibodies to A. marginale, this assay also identifies A. ovis due to the shared MSP5 epitope. However, it does not allow species differentiation and may cross-react among A. marginale, A. ovis, and A. phagocytophilum. This limitation should therefore be considered when interpreting serological results.

This study aims to address existing knowledge gaps by assessing the seroprevalence of antibodies against *Anaplasma ovis* in sheep using the MSP5-based cELISA, an approach still underused in Algeria. In contrast to previous research mainly focused on the northeastern part of the country, this work investigates geographically underrepresented regions to provide a more comprehensive epidemiological overview. It also explores potential risk factors associated with seropositivity, such as management practices, season, and sanitary conditions. By combining sensitive diagnostic tool with a multifactorial risk analysis, this study contributes novel insights into the epidemiology of ovine anaplasmosis and supports the development of targeted control strategies addapted to local contexts.

2. Materials and methods

2-1- Study area

This study was conducted from March to November 2021 in two regions of Algeria: Medea and Bordj Bou Arreridj (Figure 1). Located respectively in the north and central-north of the country, both regions present distinct agro-ecological characteristics influencing tick dynamics and, consequently, the epidemiology of *Anaplasma* spp. Infection. Bordj Bou Arreridj, situated in the eastern High Plateaus southeast of Algiers, has a semi-arid climate with cold winters and hot, dry summers. Temperatures vary significantly by season, with moderately warm springs (15–25 °C) and a gradually cooling autumn (25–10 °C). Such dry conditions are known to favor *Rhipicephalus* and *Hyalomma* tick species, which are important *Anaplasma* vectors. Medea, a predominantly mountainous area in the Tellian Atlas, has a subhumid climate characterized by cooler temperatures (10–22 °C in spring; 10–20 °C in autumn) and higher humidity, which provides suitable conditions for tick survival and activity.



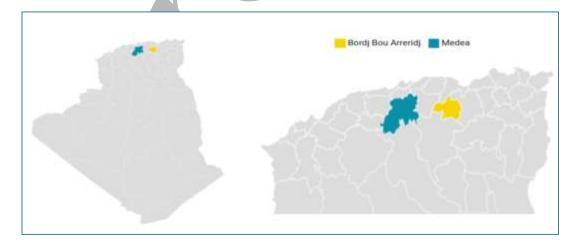


Figure 1: Study area showing the sampled regions (Medea and Bordj Bou Arreridj).

2-2- Samples collection

- 118 A total of 361 blood samples were collected from sheep: 261 from Bordj Bou Arreridj region
- and 100 from Medea during two distinct seasons (spring and autumn). The difference in
- sample size reflects flock population density and accessibility to farms, as the Bordj Bou
- Arreridj region has a larger sheep population and greater farm availability. This distribution
- was nevertheless considered in subsequent statistical analyses to minimize potential bias in
- 123 prevalence comparisons.
- For each animal, a data collection form was completed to record individualand environmental
- factors, including age, sex, farming system, general health status, tick infection at the time of
- sampling, season, and local climatic conditions.
- Among the 361 animals sampled, 258 were females (71.46%) and 103 were males (28.53%).
- 128 Age was categorized as: <12 months (34.90%), 12–24 months (43.49%), and >2 years
- 129 (21.60%). In terms of season, 241 samples (66.75%) were collected in spring and 120 samples
- 130 (33.24%) in autumn.
- 131 Blood was drawn from the jugular vein into plain tubes without anticoagulant. After
- coagulation, samples were centrifuged at 2,000 rpm for 10 minutes (adjusted from 1,000 rpm to
- ensure complete serum separation). Serum was then aliquoted and stored at -20 °C until
- serological analysis.

136 **2-3- Competitive ELISA (cELISA)**

- 137 All sera were tested for the antibodies against *Anaplasma* spp. using a commercial
- competitive ELISA kit (Anaplasma Antibody Test Kit, cELISA v2, VMRD, Pullman, WA,
- USA). The assay targets antibodies directed against the major surface protein MSP5, which is
- highly conserved among A. marginale, A. ovis, and A. phagocytophilum [12]. The test was
- performed following the manufacturer's instructions.
- Optical density (OD) was measured at 630 nm using an ELX800 ELISA microplate reader
- 143 (BioTek Instruments, Inc., USA). The percentage of inhibition was calculated formulas
- 144 follows:

135

- 145 % inhibition = $100 \times (1 OD \text{ of sample } / OD \text{ of negative control})$.
- Samples were classified as Negative if % inhibition < 30% and Positive if % inhibition ≥
- 147 30%.

156

157

- Positive and negative controls supplied with the kit were used to validate each assay plate. No
- additional field controls were required, as the commercial controls provided by the
- manufacturer are species-independent and recommended for small ruminants.
- 151 Although initially developed for bovine anaplasmosis, the MSP5-based cELISA has been
- validated for A. ovis detection in sheep, showing high sensitivity and specificity (>90%) in
- previous studies [11, 13]. Nevertheless, it does not allow differentiation between A.
- marginale, A. ovis, or A. phagocytophilum, nor does it distinguish current infection from past
- exposure. This limitation was considered in data interpretation.

2-4- Data analyses

- 158 Seroprevalence was calculated as the proportion of positive samples among all tested animals.
- Associations between categorical variables were evaluated using the Chi-square (χ^2) test.
- Univariate and multivariate logistic regression analyses were performed to assess associations
- between Anaplasma spp. seropositivity (binary outcome) and potential risk factors (age, sex,
- season, region, climate, farming system, tick infestation, and health status). Odds ratios (ORs)

- with 95% confidence intervals (CIs) were reported. The ORs presented in the abstract
- 164 correspond to adjusted values derived from the final multivariate model.
- A Directed Acyclic Graph (DAG) was constructed to identify confounders and guide variable
- selection for multivariate analysis. The DAG included variables such as region, season,
- 167 climate, age, tick infestation, and management system. It was developed using the "dagitty"
- package in R (version 4.3.2, 64-bit) to clarify causal assumptions and strengthen model
- interpretation.
- All analyses were conducted in R software (version 4.3.2; R Foundation for Statistical
- 171 Computing, Vienna, Austria). A p-value < 0.05 was considered statistically significant.
- The geographical map was generated using GADM (http://gadm.org/)
- te http://gadm.org/.

174175

176

3. Results

3-1- Geographical Distribution of *Anaplasma* Seropositivity in Sheep

- Out of a total of 361 sheep tested for antibodies against *Anaplasma* spp., 264 were positive,
- yielding an overall seroprevalence of 73.13% (95% CI: 69.6%–75.7%) (Table 1). In Bordi
- Bou Arreridj, 183 of 261 samples were positive (70.12%; 95% CI: 64.5%-75.7%), while in
- 180 Medea, 81 of 100 samples tested positive (81.0%; 95% CI: 73.3%–88.7%). These results
- indicate widespread circulation of Anaplasma spp. in both regions, with a higher
- seroprevalence observed in Medea (Table 1).

183 184

Table 1. Serological prevalence of *Anaplasma spp.* infection in sheep by region

185	

Region	Number of sheep tested	Prevalence (%)	95% CI
Bordj Bou Arreridj	261	70.12 (183/261)	64.50 – 75.70
Medea	100	81.00 (81/100)	73.30 - 88.70
Total	361	73.13 (264/361)	69.60 – 75.70

186 187

188

3-2- Univariate Analysis of Risk Factors Associated with Seropositivity

- A univariate analysis was conducted to explore the association between seropositivity and potential risk factors (Table 2). Variables significant at p < 0.20 were further assessed using
- multivariate logistic regression to control for confounding effects identified in the DAG (region, age,
- season, climate, and tick infestation)..
- 193 Seroprevalence of *Anaplasma* spp. antibodies was significantly higher in Medea (81.0%)
- compared to Bordj Bou Arreridj (70.1%), with an adjusted odds ratio (aOR)= 1.82 (95% CI:
- 195 1.03-3.20, p = 0.037), indicating a higher risk of exposure in Médéa.
- 196 Age significantly influenced antibody prevalence. The 12–24 months age group showed the
- highest seropositivity (80.9% aOR=2.07 , 95% CI: 1.26–3.39, p=0.005),, while animals under 12
- months showed lower prevalence (63.5%).
- 199 Gender and breeding type were not significantly associated with seropositivity (p > 0.05).
- Males and females had comparable rates (71.8% vs 73.6%), as did animals raised under extensive and
- semi-extensive systems (73.4% vs 71.4%). Season had a notable effect, with animals sampled in

spring showing higher positivity rate (76.8%) than those sampled in autumn (65.8%), with an aOR of 1.72, 95% CI: 1.06–2.77, p = 0.027, suggesting a seasonal influence likely related to tick activity.

Climatic conditions were also significant. In sub-humid areas, seroprevalence reached 76.4%, compared to 59.4% in semi-arid zones (aOR = 2.21, 95% CI: 1.27–3.83, p = 0.004), indicating higher

risk in wetter environments favoring tick survival..

202

203204

211

212

213214

215216

Health status showed a positive but non-significant association with seropositivity (p = 0.076).

Animals in moderate or poor condition had higher odds of exposure (aOR = 1.75, 95% CI: 0.99-

210 3.09), suggesting a potential trend worth further investigation.

Tick infestation emerged as the most influential risk factor. Among 241 infested animals,

89.2% were seropositive, compared to 40.8% of non-infested animals (aOR = 11.98, 95% CI:

6.94–20.68, p < 0.0001), confirming the major role of ticks in *Anaplasma spp.* transmission.

Table 2: Risk factors analysis for *Anaplasma spp.* seropositivity by cELISA

	Number	Positive number	95 % CI on	aOR	95 % CI on	<i>p</i> -value	
	sampled	(%)	prevalence	aUK	aOR	p-value	
Overall Total	361	264 (73.13)	68.6 -77.6	-	-	-	
Region							
Bordj Bou Arreridj (BBA)	261	183 (70.12)	64.5 - 75.7	0.55	0.31 - 0.97	0.037	
Medea	100	81 (81.0)	73.3 - 88.7	1.82	1.03 - 3.20	-	
		Age					
< 12 Months	126	80 (63.50)	55.1 - 71.90	0.482	0.29 -0.77	0.005	
12-24 Months	157	127 (80.90)	74.7 - 87.00	2.070	1.26-3.39	-	
> 2 years	78	57 (73.10)	63.2 -82.9	0.997	0.56-1.75	0.995	
		Gender	*				
Male	103	74 (71.8)	63.2 - 80.5	0.813	0.55 -1.52	0.728	
Femele	258	190 (73.6)	68.3 - 79	1.095	0.656 -1.825	-	
		Season					
Spring	241	185 (76.8)	71.4 - 82.1	1.715	1.059-2.77	0.027*	
Autumn	120	79 (65.8)	57.3 - 74.3	0.583	0.36-0.94	-	
		Type of bree	eding				
Extensive	312	229 (73.4)	68.5 - 78.3	1.104	0.56-2.15	0.773	
Semi-Extensive	49	35 (71.4)	58.8 - 84.1	0.906	0.46- 1.77	-	
		Climate	2				
Semi-arid	69	41 (59.4)	47.8 - 71.0	0.453	0.26-0.77	0.004**	
Sub-humid	292	223 (76.4)	71.5 - 81.2	2.207	1.27- 3.83	-	
•		Health sta	tus				
Good	234	162(69.2)	63.3 - 75.1	0.551	0.33-0.93	0.076	
Moderate	98	79(80.6)	72.8 - 88.4	1.753	0.99-3.09	-	
Poor	29	23(79.3)	61.6 -90.15	1.447	0.57-3.67	0.447	
		Ticks infests					
Yes	241	215(89.2)	85.3 - 93.1	11.98	6.94- 20.68	<0.0001**	
No	120	49 (40.8)	32.0 - 49.6	0.083	0.04- 0.14		

OR: Odds Ratio; CI: Confidence Interval (95%); p-value: Probability value indicating statistical significance (p < 0.05 significant)

3-3- Causal Framework: Directed Acyclic Graph (DAG)

The Directed Acyclic Graph (Figure 2) illustrates hypothesized relationships between variables influencing seropositivity. The model identifies **tick infestation**, **age**, **season**, **and climate** as direct determinants, while **region** and **breeding type** act indirectly through their effects on environmental exposure

and

animal

health.

The DAG guided the selection of confounders included in the multivariate logistic regression model,

ensuring that associations were adjusted for key interacting variables.

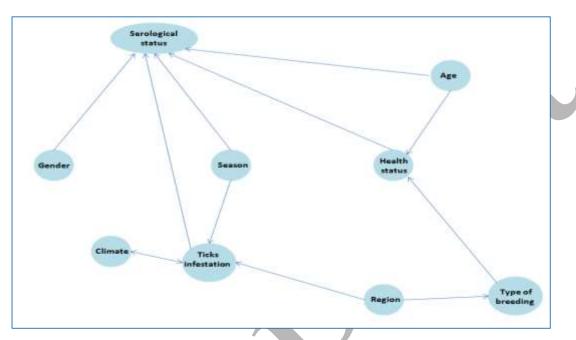


Figure 2: Directed Acyclic Graph (DAG) representing hypothesized causal pathways influencing seropositivity.

3-4- Multiple Correspondence Analysis (MCA): Risk Group visualization

The biplot showed in Figure 3 presents the projection of individuals onto the first two dimensions obtained from a Multiple Correspondence Analysis (MCA), using simulated data categorized into three risk groups. These groups are defined as follows: the High Risk group (in red) includes animals infested with ticks, living in a sub-humid climate, and aged between 12 and 24 months; the Moderate Risk group (in orange) includes animals with various other combinations of factors; and the Low Risk group (in green) consists of animals not infested with ticks, living in a semi-arid climate, and younger than 12 months.

The individuals in the high-risk group are tightly clustered in a specific area of the factorial space. This strong spatial concentration suggests a robust association between this profile and risk indicators such as seropositivity. The consistency of this group's position implies that its defining characteristics contribute significantly to explaining the variability in the dataset.

In contrast, individuals in the low-risk group are located in a clearly distinct region, well separated from the high-risk group. This spatial separation reflects a protective profile, likely due to the absence of ticks, the favorable environmental conditions, and the younger age of the animals. Their positioning on the plot supports the assumption that these factors are associated with lower risk levels.

The moderate-risk group appears more dispersed across the factorial space. This wide distribution indicates a high degree of heterogeneity within the group, encompassing diverse combinations of intermediate or less clearly defined risk factors. The overlap of this group with both the low and high-risk regions reflects its transitional nature and the uncertainty surrounding its classification.

The ellipses surrounding each group represent the concentration and variability of individuals within each category, likely corresponding to 95% confidence intervals around group centroids. While the high and low-risk groups form relatively well-defined clusters, the moderate group shows substantial overlap with the others, reinforcing the idea of less distinctive profiles.

In summary, this MCA biplot provides a useful visual tool for exploring the structure of categorical data and understanding the relationships between different risk profiles. It highlights clear separations between high and low-risk groups, while also emphasizing the complexity and diversity within the moderate-risk category.

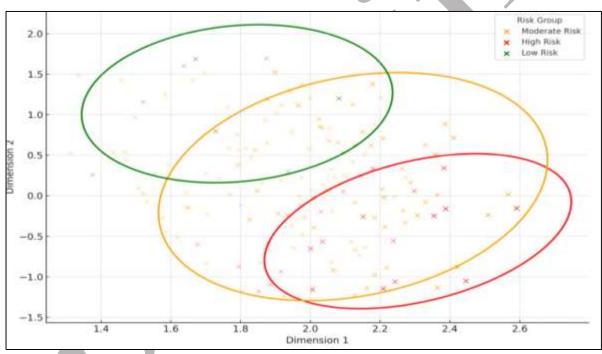


Figure 3: Multiple Correspondence Analysis (MCA) Biplot Showing Risk Groups: the High Risk group (in red); the Moderate Risk group (in orange) and the Low Risk group (in green).

4. Discussion

To our knowledge, this study provides the first sero-epidemiological data on antibodies against *Anaplasma spp*. in sheep from Medea and Bordj Bou Arreridj, two major sheep-farming regions in northern Algeria. It follows on from previous work carried out in other regions of Algeria and contributes to a better understanding of the geographical distribution of anaplasmosis in the Algerian sheep herd. However, as similar surveys have already been performed in other parts of the country [16–18], our findings should be interpreted as a regional extension of previous work rather than a nationwide first report.

The competitive ELISA (cELISA) employed in this study targets the major surface protein 5 (MSP5), which is conserved among A.marginale, A. centrale, and A. ovis [13]. Although the test has shown excellent sensitivity (100%) and specificity (99.7%) in detection A. marginale

infections in cattle [14], such performance values cannot be directly extrapolated to A. ovis in 278 sheep, as the test was not originally validated for this host-pathogen pair. Furthermore, MSP5-based 279 assays may cross-react with other Anaplasma species, notably A. phagocytophilum. Compared to 280 molecular diagnostic techniques such as PCR, which detect only active infections, cELISA 281 also detects exposure to Anaplasma spp. rather than active infection and cannot differentiate between 282 283 species or infection stages [15].

The overall seroprevalence observed (73.12%), 81% in Medea and 70.12% in Bordi Bou 284 Arreridi, indicates widespread exposure of sheep populations to *Anaplasma spp.* in both areas. These 285 findings are consistent with previous Algerian studies reporting 78.02% in goats from El Tarf 286 and Guelma [16], and 61.7% in sheep and 54.2% in goats from Souk Ahras [17]. This alignment 287 suggests a broadly similar circulation of the pathogen among small ruminants across different 288 regions, despite variations in host species and environmental conditions. Conversely, a recent 289 meta-analysis by Nahal et al. [18] estimated an average prevalence of 30% in Algerian sheep, 290 291 , with strong regional disparities (26% in the northeast vs. 9% in the north-central area).

Such high seroprevalence in our study, particularly in Medea, may be explained by local 292 ecological and farming factors favoring tick vectors. 293

Recent investigations have provided important insights into the epidemiology of Anaplasma 294 295 ovis across North Africa. In Tunisia, M'ghirbi et al. [30] reported that A. ovis is endemic in small ruminants across three bioclimatic zones. In their survey of 263 apparently healthy 296 sheep and goats, the prevalence reached 80.4% in sheep, as determined by duplex PCR 297 298 targeting the msp4 gene, with an overall Anaplasma spp. prevalence of 78.3%. Complementary findings were obtained by ElHamdi et al. [31], who conducted a five-month 299 longitudinal study in Tunisian flocks. They documented fluctuating molecular prevalence and 300 incidence of new infections ranging from approximately 2% to 11%, along with a steady 301 increase in seroprevalence reaching 52.6% in lambs by November, indicating ongoing 302 transmission within herds. In Algeria, a recent meta-analysis covering studies from 2004 to 303 304 2023 estimated an overall Anaplasma spp. infection rate of 28% (95% CI = 17-41), with infection levels of approximately 30% in sheep; A. ovis was among the species identified [32]. 305 Furthermore, a molecular survey conducted in the Oum El Bouaghi region of Algeria revealed 306 A. ovis infection in 10.8% of sheep, while A. marginale and A. platvs were detected at lower 307 rates (1.7% and 0.2%, respectively), and A. phagocytophilum was not observed in the sampled 308 population [33]. 309

The differences among studies are largely due to variation in diagnostic techniques 310 (microscopy, PCR, or cELISA), sampling season, and management conditions [15,26]. For 311 clarity, prevalence values from selected North African and Mediterranean studies using 312 different diagnostic methods are summarized in Table S1 (Supplementary). 313

314 At the international level, wide disparities have been reported, ranging from 71.8%-88.9% in Morocco [19], to 43.9%–58.8% in Turkey [20,21], and 66.6%–41.7% in Iraq and Sudan [9] 315 to 37%-82.5% in European countries [9]. Such variability reflects differences in ecological 316 317 settings, animal management, vector abundance, and diagnostic methods and host populations.

Age was identified as a significant factor in our study: animals aged 12-24 months showed 318 the highest seropositivity (80.9%), followed by those over 2 years (73.1%), and the lowest in 319 320 animals under 12 months (63.5%) (p < 0.01).

This gradient is consistent with results from Algeria [16,24] and Nigeria [23], and likely 321 reflects cumulative exposure to infected ticks and gradual immune maturation in older 322 323 animals.

No significant difference was found between males (71.8%) and females (73.6%). This agrees 324 325 with previous Algerian findings [16,18,24] and supports the notion that sex plays a minor role compared to environmental or management factors [22]. 326

- Season was shown to be an important determinant, with higher prevalence in spring (76.8%)
- 328 than in autumn (65.8%). This likely corresponds to increased tick activity during spring under
- favorable climatic conditions [25]. However, in the absence of entomological or climatic data (e.g.,
- tick density, rainfall, or temperature records), this explanation remains hypothetical and should be
- verified in future investigations incorporating environmental monitoring [29].
- Climate was another risk factor: prevalence was significantly higher in the sub-humid zone
- 333 (76.4%) than in the semi-arid zone (59.4%) (OR = 2.21; p = 0.004). This observation is
- consistent with the fact that humid environments favor tick survival and reproduction [19,30].
- By contrast, the breeding system (extensive vs. semi-extensive) had no significant effect in our
- study, with similar prevalence in extensive and semi-extensive systems. Nonetheless, studies
- 337 conducted in Morocco and Tunisia have reported higher infection rates in extensively managed herds,
- probably due to increased contact with tick habitats [19].
- Although not statistically significant (p = 0.076), animals in moderate or poor body condition
- exhibited slightly higher seroprevalence (80.6% and 79.3%, respectively) than those in good
- 341 condition (69.2%). This pattern may suggest that compromised health could increase
- susceptibility, or conversely, that chronic infection contributes to poorer body condition [10].
- 343 However, this interpretation remains hypothetical and warrants further research combining
- 344 clinical and molecular data. Tick infestation emerged as the strongest predictor of
- seropositivity, with an odds ratio of 11.98 (p < 0.0001), confirming the central role of tick
- vectors in *Anaplasma spp.* transmission.
- 347 The use of Multiple Correspondence Analysis (MCA) for visualizing and interpreting
- 348 categorical risk profiles remains a powerful exploratory tool, especially when combined with
- biplots for cluster visualization [26] In Figure 3, the distinct spatial grouping of individuals
- supports the hypothesis that risk-related variables co-vary systematically, producing clearly
- delineated profiles across the factorial space.
- 352 The tight clustering of the high-risk group indicates a strong co-association between
- infestation status, climatic conditions, and age, consistent with findings by Abdi and Valentin
- 354 [27], who demonstrated that MCA effectively captures multidimensional dependence
- 355 structures in epidemiological datasets.
- 356 The dispersion of the moderate-risk group aligns with patterns observed in other ecological or
- veterinary risk models, where mixed or transitional categories often reflect heterogeneous
- exposure levels [28]. Such intermediate clustering patterns suggest that the moderate-risk
- 359 category might encompass animals under partial protective conditions or variable exposure
- 360 environments, warranting further multilevel modeling.
- Finally, the use of confidence ellipses to visualize intra-group variability is a methodological
- enhancement that aids in quantifying uncertainty around category centroids [29]. This
- approach has gained traction in recent applied research for its clarity in representing risk
- gradients and category overlap zones in complex ecological datasets.
- In conclusion, this study provides new insights into the exposure of sheep to *Anaplasma* spp.
- in northern Algeria, revealing a high seroprevalence and clear associations with age, season,
- 367 climate, and tick infestation. These findings highlight the need for strengthened tick
- 368 surveillance and targeted control programs, including regular acaricide treatment, improved
- pasture management, and farmer awareness campaigns on tick prevention. Veterinarians
- should consider anaplasmosis in differential diagnoses of anemia or wasting syndromes in
- 371 small ruminants, especially during high-risk seasons.
- 372 Because the MSP5-based cELISA detects antibodies at the genus level, the results mainly
- 373 reflect previous exposure rather than ongoing infection. Therefore, molecular confirmation
- 374 (PCR and sequencing) is needed to identify circulating *Anaplasma* species and genotypes. In
- 375 addition, longitudinal monitoring of herds would help to assess infection dynamics,
- seasonality, and reinfection patterns, providing essential data for sustainable control strategies
- in Algerian sheep populations.

378 Acknowledgements

- 379 The authors would like to express their sincere gratitude to the farmers and veterinarians who
- facilitated sample collection and provided valuable field assistance. We are also thankful to
- 381 the laboratory staff for their technical support and to our colleagues for their constructive
- 382 comments throughout this work.

383 384

- **Authors' Contribution**
- 385 MRH: Investigation, Data curation, Conceptualization, Writing review & editing, OS, MR,
- 386 AL: Writing review & editing, SZ: Statistical analysis, NO, KTNA: Data analysis, Statistical
- analysis, Writing Final manuscript, FG, NA: Validation, Supervision.

388

- 389 Conflict of interests
- 390 The authors declare that they have no known competing financial interests or personal
- 391 relationships that could have appeared to influence
- 392 the work reported in this paper.

393

- 394 Ethics approval
- 395 Blood samples were collected with the consent of sheep owners. No animal was harmed, and
- all procedures followed national veterinary regulations and good animal welfare practices.

397

- 398 Grant support or Funding/Support
- 399 Not applicable

400 401

- Data Availability
- The datasets generated and analyzed during this study are available from the corresponding
- 403 author upon reasonable request.

404 405

406

407

References

- 1. Nefzaoui H, Ketata M, El Mourid M. Changes in North Africa production systems to meet climate uncertainty and new socioeconomic scenarios with a focus on dryland areas. In:
- 410 Car A, López-Francos A, Porqueddu C, editors. New Approaches for Grassland Research in a
- 411 Context of Climate and Socio-economic Changes. CIHEAM, Options Méditerranéennes: Série
- 412 A. Séminaires Méditerranéens No. 102; 2012. p. 403–21.
- 2. Boussaada D, Yerou H. Sustainability indicators of sheep farming systems in the eastern
- 414 steppe ecosystem of Algeria. Genet Biodivers J. 2022;6(1):16–24.
- 415 https://doi.org/10.46325/gabj.v6i1.193
- 416 3. Bentounsi R, Trad N, Gaous K, Kohil K, Cabaret J. Gastrointestinal nematode resistance
- 417 to benzimidazoles on a sheep farm in Algeria. Vet Rec. 2006;158(18):634-5.
- 418 https://doi.org/10.1136/vr.158.18.634
- 419 4. Kocan KM, de la Fuente J, Blouin EF, Garcia-Garcia JC. Anaplasma marginale
- 420 (Rickettsiales: Anaplasmataceae): recent advances in defining host–pathogen adaptations of a
- 421 tick-borne rickettsia. *Parasitology*. 2004;129(S1):S285–300.
- 422 <u>https://doi.org/10.1017/S0031182003004735</u>

- 423 5. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, et al.
- 424 Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order
- 425 Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with
- 426 Ehrlichia and Ehrlichia with Neorickettsia. Int J Syst Evol Microbiol. 2001;51(6):2145–65.
- 427 <u>https://doi.org/10.1099/00207713-51-6-2145</u>
- 428 6. de la Fuente J, Atkinson MW, Naranjo V, Fernández de Mera IG, Mangold AJ, Keating
- 429 KA, et al. Genetic characterization of *Anaplasma ovis* strains from bighorn sheep in Montana.
- 430 J Wildl Dis. 2006;42(2):381–5. https://doi.org/10.7589/0090-3558-42.2.381
- 7. Friedhoff KT. Tick-borne diseases of sheep and goats caused by Babesia, Theileria or
- 432 *Anaplasma* spp. *Parassitologia*. 1997;39(2):99–109.
- 8. Gharbi M, Rekik M, Darghouth MA. Epidemiological study of sheep anaplasmosis
- 434 (Anaplasma ovis infection) in Kairouan, Central Tunisia. J Adv Parasitol. 2015;2(2):30–4.
- 9. Renneker S, Abdo J, Salih DA, Karagenç T, Ahmed JS. Can Anaplasma ovis in small
- ruminants be neglected any longer? Transbound Emerg Dis. 2013;60(Suppl 2):105-12.
- 437 <u>https://doi.org/10.1111/tbed.12093</u>
- 10. Shompole S, Rurangirwa FR, McGuire TC. Cloned DNA probes identify *Anaplasma ovis*
- in goats and reveal a high prevalence of infection. J Clin Microbiol. 1989;27(12):2730–5.
- 440 https://doi.org/10.1128/jcm.27.12.2730-2735.1989
- 11. Ndung'u LW, Aguirre DH, Knowles DP. Detection of Anaplasma ovis infection in goats
- by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. J Clin
- 443 Microbiol. 1995;33(3):675-9. doi:10.1128/jcm.33.3.675-679.1995
- 12. Mason KL, Washburn KE, Drake C, Kocan KM, Whitworth LC. Validation of an
- 445 improved Anaplasma antibody competitive ELISA for detection of Anaplasma ovis antibody
- in domestic sheep. J Vet Diagn Invest. 2017;29(5):763-6. doi:10.1177/1040638717723704
- 13. Torioni de Echaide S, Knowles DP, McGuire TC, Palmer GH, Suarez CE, McElwain TF.
- Detection of cattle naturally infected with Anaplasma marginale in a region of endemicity by
- nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major
- 450 surface protein 5. J Clin Microbiol. 1998;36(3):777-82. doi:10.1128/JCM.36.3.777-82.1998
- 451 14. Carelli G, Decaro N, Lorusso A, Elia G, Lorusso E, Mari V, Ceci L, Buonavoglia C.
- Detection and quantification of Anaplasma marginale DNA in blood samples of cattle by real-
- 453 time PCR. Vet Microbiol. 2007;124(1-2):107-14. doi:10.1016/j.vetmic.2007.03.022
- 15. Belkahia H, Ben Said M, Sayahi L, Alberti A, Zhioua E, Hattab D, Darghouth MA.
- 455 Seasonal dynamics, spatial distribution and genetic analysis of Anaplasma species infecting
- 456 small ruminants from northern Tunisia. Infect Genet Evol. 2017;54:66-73.
- 457 doi:10.1016/j.meegid.2017.06.008
- 458 16. Reghaissia N, Dahmane A, Boularias G, Ghalmi F, Azzag N. Epidemiological and
- 459 comparative diagnostic study of Anaplasma spp. infection in goats from north-eastern
- 460 Algeria. Folia Vet. 2020;64(3):61-74.
- 461 17. Aouadi N, Hakem A, Harrat Z, Dahmani M, Hadjadj D, Abdi R, Righi S. Molecular
- evidence of tick-borne hemoprotozoan parasites (Theileria ovis and Babesia ovis) and bacteria
- in ticks and blood from small ruminants in northern Algeria. Comp Immunol Microbiol Infect
- 464 Dis. 2017;50:34-9. doi:10.1016/j.cimid.2016.11.005
- 18. Nahal N, Benrabah A, Chouikha R, Khelef D. Current status of ruminant anaplasmosis in
- Algeria: A systematic review and meta-analysis. Trop Anim Health Prod. 2024;56(4):164.
- 467 doi:10.1007/s11250-024-04347-9
- 468 19. Ait Lbacha H, Alali S, Zouagui Z, El Mamoun L, Rhalem A, Petit E, Dugat T, Maillard
- 469 R. High prevalence of Anaplasma spp. in small ruminants in Morocco. Transbound Emerg
- 470 Dis. 2017;64(1):250-63. doi:10.1111/tbed.12365

- 20. Dülek Ö, Kandemir Ç, Koçkaya ES, et al. Molecular investigation of Anaplasma spp. and
- 472 genotype profile of A. ovis in sheep from different farms in Türkiye. Acta Parasitol.
- 473 2025;70:88. doi:10.1007/s11686-025-01021-2
- 474 21. Öter K, Inci A, Yildirim A, Duzlu O, Ciloglu A. Molecular detection and typing of
- 475 Anaplasma species in small ruminants in Thrace Region of Turkey. Kafkas Univ Vet Fak
- 476 Derg. 2016;22(1):55-60. doi:10.9775/kvfd.2015.13723.
- 477 22. Ali A, Ijaz M, Durrani AZ, Ali MM, Mehmood K, Sabir AJ. Prevalence and
- 478 chemotherapy of anaplasmosis in clinically affected small ruminants in the River Ravi
- 479 Region, Lahore. Pak J Zool. 2014;46(3):775-80.
- 480 23. Nyifi ID, Jatau IA, Lawal UB, Abubakar O, Olugbenga OO. Prevalence of anaplasmosis
- in small ruminants in some selected livestock farms in Wukari Metropolis, Taraba State,
- 482 Nigeria. FUDMA J Agric Agric Technol. 2023;9(3):52-7.
- 483 24. Foughali AA, Bendahmane M, Benaissa MH, Boulkaboul A. Infection by
- 484 haemopathogens and tick infestation of sheep during summer season in Constantine region,
- 485 Northeast Algeria. Vet Med Sci. 2021;7(5):1769-77. doi:10.1002/vms3.556.
- 486 25. Elfegoun MCB, Benakhla A, Zouache B. Cinétique d'infestation par les tiques des bovins
- de la région subhumide de Constantine en Algérie. Rev Elev Med Vet Pays Trop.
- 488 2019;72(1):41-5. doi:10.19182/remvt.31612.
- 489 26. Greenacre M. Correspondence Analysis in Practice. 3rd ed. Chapman & Hall/CRC; 2021.
- 490 DOI: 10.1201/9781315369983
- 491 27. Abdi H, Valentin D. Multiple Correspondence Analysis. Wiley Interdiscip Rev Comput
- 492 Stat. 2022;14(4):e1553.
- 493 28. Smetana M, Salles de Salles L, Sukharev I, Khazanovich L. Highway construction safety
- 494 analysis using large language models. Appl Sci. 2024;14(4):1352. DOI
- 495 10.3390/app14041352

513

514

515

516

- 496 29. Husson F, Lê S, Pagès J. Exploratory Multivariate Analysis by Example Using R.
- 497 Chapman & Hall/CRC; 2017. DOI: 10.1201/b10345
- 498 30. Nahal A, Ben Said M, Ouchene N. Current status of ruminant anaplasmosis in Algeria: a
- 499 systematic review and meta-analysis. *Trop Anim Health Prod.* 2024;56(4):164.
- 500 <u>https://doi.org/10.1007/s11250-024-04010-1</u>
- 31. M'ghirbi Y, Oporto B, Hurtado A, Bouattour A. First molecular evidence for the presence of
- 502 Anaplasma phagocytophilum in naturally infected small ruminants in Tunisia, and confirmation of
- Anaplasma ovis endemicity, Pathogens. 2022;11(3):315. https://doi.org/10.3390/pathogens11030315
- 32. ElHamdi S, Mhadhbi M, Ben Said M, Mosbah A, Gharbi M, Klabi I, Daaloul-Jedidi M, Belkahia
- 505 H, Selmi R, Darghouth MA, et al. Anaplasma ovis prevalence assessment and cross validation using
- multiparametric screening approach in sheep from central Tunisia. *Pathogens*. 2022;11(11):1358.
- 507 https://doi.org/10.3390/pathogens11111358
- 33. Chadi H, Moraga-Fernández A, Sánchez-Sánchez M, Chenchouni H, Fernández de Mera IG,
- 509 Garigliany MM, de la Fuente J, Tennah S, Sedrati T, Ghalmi F. Molecular detection and associated
- 510 risk factors of Anaplasma marginale, A. ovis and A. platys in sheep from Algeria with evidence of the
- 511 absence of A. phagocytophilum. Acta Trop. 2024;249:107040.
- 512 https://doi.org/10.1016/j.actatropica.2023.107040

Table S1. Comparative prevalence of *Anaplasma ovis* infection in small ruminants from different regions and diagnostic methods

Country / Region	Host species	Diagnostic method	Reported prevalence (%)	Reference
Algeria (El Tarf, Guelma)	Goats	cELISA	78.02	[16]
Algeria (Souk Ahras)	Sheep / Goats	cELISA	61.7 / 54.2	[17]
Algeria (National meta- analysis)	Sheep	Mixed methods	30	[18]
Morocco	Sheep	PCR	71.8	[19]
Morocco	Sheep	Microscopy	88.9	[19]
Tunisia	Sheep	PCR	52.3	[19]
Italy (Sicily)	Sheep	PCR	37	[10]
Turkey	Sheep	PCR	43.9–58.8	20;21
Iraq	Sheep	PCR	66.6	[9]
Sudan	Sheep	Microscopy	41.7	[9]