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EPIDEMIOLOGICAL AND COMPARATIVE DIAGNOSTIC STUDY OF *ANAPLASMA* SPP. INFECTION IN GOATS FROM NORTH-EASTERN ALGERIA

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ABSTRACT

Anaplasmosis is an infectious disease affecting goats and other ruminants. Our goal was to estimate the prevalence of *Anaplasma* spp. infections, and to identify different tick species in goa ts along with their associated risk factors. The epidemiology of *Anaplasma* spp. (*A. ovis*/*A. marginale*) was investigated from April to September 2016 in dairy goats from three different agro-ecological areas in the northeast region of Algeria (Guelma and El-Taref). We tested 182 goat sera using a MSP5 competitive ELISA (cELISA) test for IgG antibody screening, and by microscopic examination using the Giemsa (May-Greunwald-Giemsa) stain of 128 blood smears to detect intraerythrocytic inclusions bodies. The results demonstrated a total infection rate of 78.02 % and 42.18 % using cELISA and MGG, respectively. Only two species of ticks collected were identified, i. e. *Rhipicephalus sanguineus* (85.39 %) and *Rhipicephalus bursa* (14.60 %). Our study revealed that factors, such as the season, the type of farming, the hygienic status and the climatic conditions of the studied areas were found to be significantly associated with the tick infestation of goats ($P < 0.05$). The *Anaplasma* spp. infection prevalence was found to be highly dependent on the climatic conditions, the season ($P < 0.05$) and most closely related to the type of breeding and herd management ($P = 0.000$).

The evaluation of the Giemsa technique showed specificity and sensitivity of 60.71 % and 45.16 % respectively. Calculating the concordance between the two techniques revealed Cohen's Kappa value of 0.038 in the range 0.21—0.40, which corresponded to a very

low agreement. The McNemar test results showed that both tests gave significantly different results ($P < 0.05$). This work provides evidence for *Anaplasma* spp. in goats from north-eastern Algeria.

Key words: caprine anaplasmosis; cELISA; epidemiology; MGG staining; ticks

INTRODUCTION

Anaplasmoses are common tick-borne zoonotic bacterial diseases of domestic animals. Goats and sheep on pastures are often infested with ticks which carry the anaplasmoses that constitute important diseases of livestock that is endemic in tropical and subtropical regions of the world [11, 33]. Anaplasmoses have been reported as diseases of major economic importance, as they cause heavy losses due to mortality, decreased production and lowered working efficiency of the affected animals [16, 62]. The causative agents are intracellular gram-negative bacteria that belong to the family *Anaplasmataceae* which is divided into six recognized *Anaplasma* species: *A. ovis, A. marginale, A. centrale, A. platys, A. bovis,* and *A. phagocytophilum* [62]. Small ruminants host several *Anaplasma* species, including *A. ovis, A. marginale* and the zoonotic agent *A. phagocytophilum* causing human granulocytic anaplasmosis [58]. Clinical manifestation depends on the immune status, as well as the welfare of the animals. *A. ovis* infects primarily sheep and goats causing a subclinical infection, while severe infections may involve anaemia, abortion, and mortality when combined with other stress factors, such as co-infection, hot weather, vaccination, and heavy tick infestation [57]. The acute disease caused by the intraerythrocytic *Anaplasma marginale* consists of: anaemia, icterus, fever, anorexia, and lethargy which they are most notable in cattle [52]. The transmission to the hosts (numerous species of mammals including humans) occurs via vectors, such as the hard ticks of the Ixodidae family. *A. marginale* and *A. ovis* are mainly transmitted to mammalian hosts by *Rhipicephalus* spp. and *Dermacentor* spp. [30].

Anaplasmosis diagnosis is usually based on the microscopic examination of Giemsa-stained blood smears, or serological and molecular diagnostic procedures. Microscopic diagnosis may be difficult in carrier animals, thus various serological techniques have been used for the detection of *Anaplasma* specific antibodies such as indirect immunofluorescence antibody (IFA), enzyme-linked immunosorbent assay (ELISA), and complement fixation tests (CF) [20]. The competitive ELISA (cELISA) is dependent on the use of a monoclonal antibody ANAF16C1 that recognizes the conserved antigen MSP-5 of different *Anaplasma* species, and it has high sensitivity and specificity values [41].

The study of *Anaplasma* spp. infection in goats was the main topic of this survey. Currently, limited data is available concerning this bacterial infection in Algeria. The aims of our work were as follows: (i) the estimation of *Anaplasma* spp. prevalence in goats based on microscopy and serological screening; (ii) the analysis of some risk factors for the *Anaplasma* infection; (iii) the identification of ticks infesting goats; and (iv) the performance evaluation, including the sensitivity and specificity determination of the different diagnostic tools such as microscopy and serologic methods used for detecting caprine anaplasmosis.

MATERIALS AND METHODS

Presentation of the study area

We carried out our study in two provinces in north-eastern Algeria (Guelma and El-Taref). The territory of Guelma is characterized by a sub-humid climate in the centre and the North, and semi-arid towards the South. This climate is mild and rainy in the winter and warm in the summer. The temperature varies from 4 °C in the winter to more than 35 °C in the summer. The geography of the district is characterized by a diversified relief (mountains, plains and plateaus, hills, and foothills) which mainly retains significant forest cover and the passage of wadi Seybouse which constitutes the main watercourse.

The El Tarf province is located in the far northeast of Algeria along the Tunisian border. The climate is generally humid. The humidity decreases from North to South in the following way: a coastal zone that presents a hot and humid climate; a mountainous area that occupies most of the region and has a mild humid climate in the north and cool in the south. The annual precipitation rate is 900 ml to 1200 ml. The study area has a total of 112,740 goats (69,200 in Guelma and 43,540 in El-Taref). The farming system is generally extensive. Animals are fed hay, bran, and grass during the grazing season; this goes from March to December with variations depending on weather conditions.

Study design and the target population

The choice of regions consisted of an appropriate sample that was governed by the objective to include the different agro-ecological strata of Algeria and regions representative of the variety of different indigenous goat breeds.

Blood samples were collected from goats from the different local farms in Guelma and El-Taref provinces of Algeria. Eligibility criteria involved a suspicion of anaplasmosis among goats in areas with sub-humid, humid and sub-arid climates. The animals were surveyed for anaplasmosis from April to September 2016, during the dry and wet seasons.

A questionnaire was developed and administered at the household level to collect individual animal and flock-level data for risk factor evaluation. A standardized questionnaire was developed for each sample in order to document the: age, sex, goat breeding, health status (tick infestation, agalactia, abortion, and sterility) of the animal, characteristic of the farm and climatic conditions. The goats were kept on pastures from spring to autumn and were stabled during the winter months. Animals from the farms had a history of frequent abortions.

The farms were mainly made up of goats of local breeds (100 %). The samples included 128 females (64.64 %) and 70 males (34.34 %). The animals were divided into three age classes: < 6 months (4 %), 6 to 12 months (30.8 %) and more than 12 months (65.6 %). Our samples concerned 15 municipalities distributed in the 3 agroecological zones of the northeast region of Algeria. The choice of goats sampled from each farm was made at random. This process allowed us to carry out a random sampling of 198 blood samples from goats aged between 4 months and 7 years old coming from 15 municipalities located in the provinces of Guelma and El-Taref and from 23 goat farms (14 from Guelma and 9 from El-Taref).

The cELISA test is the most commonly used serological method to detect antibodies against *Anaplasma* spp. and used as a reference diagnosis among microscopic examination of blood smears.

The required sample size was calculated according to the following formula with an expected prevalence of 10 % and a 95 % confidence interval (CI) [56]:

$$N = [Z^2 \times P(1 - P)]/d^2$$

Where:

N—is the number of samples to be collected in the study,

Z—is the value of the normal distribution for the confidence interval of 95 % [$Z^2 = (1.96)^2$],

P—is the expected prevalence,

d—is the absolute error of 10 %

A minimum of 100 samples was required. To increase the power of the statistical analysis, the sample size was multiplied by 2. Twenty-three herds were randomly selected and the herd sizes ranged from 5 to 120 heads.

At the individual level, the sample size was determined for each flock to detect the existence of the disease. The calculations were performed according to the formula commonly used in veterinary epidemiological surveys by Thrusfield [56]:

$$n = [1 - (1 - p)^{1/d}] \times [N - (d/2)] + 1$$

Where;

n—is the size of the sample in each flock,

p—is the detection probability of at least one seropositive goat,

N—is the size of the flock,

d—is the number of seropositive goats in the herd.

The probability of detecting at least one seropositive goat in a flock was 95 % (p = 0.95), while the number of seropositive goats in each flock (d) was calculated assuming that the herd was equal to 10 %. Finally, 10 to 30 blood samples were collected from each flock, making a total of 210 goats.

Sample collection

Blood sampling

A total of 198 blood samples were collected from goats of different gender and age groups (4 months to 7 years). Jugular venous blood samples were collected from each individual animal into two 5 ml vacutainer tubes (BD vacutainer, BD-Plymouth, UK), one containing EDTA and one additive-free. The blood smears were prepared and examined immediately. To separate the sera, the additive-free blood was allowed to clot for about 15—30 min at room

temperature. The tubes were then centrifuged at 1000—2000 rpm for 10 min and the serum was collected. The sera specimens were stored at –20 °C for further use.

Tick sampling

Once the blood was collected, the entire body of each of the 198 animals was inspected for ticks. In order to reduce the time of restraint and handling of the animals, we focused particularly on the ears, neck, udders and external genitalia (predilection sites of the tick's attachment). A minimal of three ticks were collected from the infested animals selected for blood sampling using entomological blunted clockmakers forceps through the examination of the perineum surface according to Baker and Ducasse [11]. All collected specimens were immediately placed in 70 % ethanol inside tubes labelled with the identification number and the date of collection and stored at 4 °C. In the Laboratory of parasitology of ENSV, Algeria, each tick was observed under a binocular magnifier for morphological identification and classified up to the species, sex, and life stage according to the taxonomic keys developed by Walker et al. [60], Meddour-Bouderda and Meddour [42].

Laboratory analysis

Microscopic examination of blood smears

Among the 198 goats sampled, only 128 smears were subjected to microscopic examination.

May-Greunwald-Giemsa (MGG) was chosen for staining and microscopic examination of blood smears accordingly the standard protocol [16]. The slides were allowed to air-dry before being fixed with absolute methanol. Fixed smears were stained with 10 % Giemsa and examined using a compound microscope under oil immersion lens. About 2 fields were examined from each slide for the presence of *Anaplasma* spp. by the same operator. Inclusion bodies consistent with anaplasmosis (due to *A. marginale* or *A. ovis*) were identified based on morphology and intra-erythrocytic localization [19].

Competitive ELISA (cELISA) assay

Sera (from 182 goats) were screened for the presence of *Anaplasma* spp. antibodies by using the *Anaplasma* Antibody Test Kit, cELISA from VMRD Inc. (Pullman, WA, USA) following the manufacturer's instructions.

The optical density (OD) was measured at 620 nm with an ELISA microplate reader (Immunoskan ELISA, BDSL). The results were calculated according to the formula:

100 [1 — (Sample OD ÷ Negative Control OD)]. The positive sample OD must be > 30 %. The optical densities (OD) obtained were converted into percentages of inhibition, calculated according to a formula indicated by the manufacturer. The cut off threshold was determined at 30 %. Samples with a percentage below 30 % were negative and those with a percentage above 30 % were positive.

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc. Chicago, USA). Data were categorized according to the region and used statistical methods such as chi-square and odds ratios. The calculated prevalence was estimated at a 95 % confidence interval (95 % CI). The impact of the defined risk factors on absence or presence of disease was examined using the Chi2 test, or in some cases the exact Fisher test (age, gender, breed, livestock production system, presence of ticks, history of abortion, pregnant status, climate condition, and hygienic quality). The differences were considered statistically significant when $P < 0.05$. The kappa statistic was undertaken to assess concordance between blood smear microscopic examinations and the serologic method with the calculation of specificity, sensitivity, accuracy, positive predictive value, negative predictive value, McNemar and Cohen's Kappa values. The agreement with kappa values of 0.00 to 0.20 was considered light, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial and 0.81 to 1.00 almost perfect.

RESULTS

Tick species identification and associated risk factors

A total of 102 ticks were collected from 198 goats in the provinces of Guelma and El-Taref. 89 ticks were identified as adults (29 male and 60 female) and 13 ticks in the nymphal stage. Two tick species were identified among the overall collected ticks into one genus: *Rhipicephalus*. Seventy-six specimens 76 (85.39 %) were classified as *Rhipicephalus sanguineus*, and 13 (14.60 %) as *Rhipicephalus bursa*. All of the nymphs collected were identified as *R. bursa*. Of the 198 goats examined, 55 were infested with at least one tick species, an overall prevalence of 27.77 % (15.9—39.6 %, 95 %

Table 1. Risk factors analysis of exposure to a goat infestation by ticks

Variables	Number sampled	Positive number [%]	95 % CI on prevalence	○	95 % CI on OR	P-value
Age						
≤ 6 months	8	0 (0)	00.00—37.22			
6—12 months	61	9 (14.75)	07.73—25.95	0.73	0.24—4.42	0.610
>12 months	129	17 (13.18)	08.30—20.19			
Sex						
Male	71	11 (42.3)	23.3—61.3	1.36	0.53—3.41	0.463
Female	127	15 (57.7)	38.7—76.7			
Season						
Autumn	26	0 (0)	0.00—11.53			
Spring	91	8 (8.79)	04.30—16.62	0.13	0.07—2.07	0.009**
Summer	81	18 (22.22)	14.46—32.50			
Dress Colour						
Black	88	13 (14.77)	8.70—23.79			
White	42	5 (11.9)	4.73—25.46			
Black and White[a]	29	2 (6.90)	0.85—23.03			
White and Black[a]	21	4 (19.05)	7.08—40.59	1.13	0.06—2.02	0.402
Black and Brown	1	0 (0)	0.00—100.00			
Grey	1	0 (0)	0.00—100.00			
Wheel	16	0 (0)	0.00—18.75			
Type of breeding						
Extensive	126	11 (8.73)	4.80—15.10			
Semi-extensive	62	15 (24.19)	15.14—36.25	3.57	0.25—62.27	0.008**
Intensive	10	0 (0)	0.00—30.00			
Hygienic quality						
Good	112	12 (10.71)	06.10—17.94			
Moderate	69	14 (20.29)	12.37—31.35	0.47	0.20—1.09	0.047*
Bad/ poor	17	0 (0)	00.00—17.64			
Climate						
Humid	71	13 (18.31)	10.89—28.99			
Sub-humid	83	7 (08.43)	03.89—16.66	1.50	0.54—4.17	0.019**
Semi-arid	44	6 (13.64)	06.02—27.09			

OR—Odds Ratio; CI—Confidence Interval at 95 %; *—significant at P ≤ 0.05; **—significant at P ≤ 0.01; [a]—the difference between the terms of coat color «Black and White» and «White and Black» lies in the intensity and dominance of the colour of which the first colour is the most dominant

CI). The evaluation of the infestation rate by tick species identified showed a large predominance of *R. sanguineus*, of which 15 goats were infested, followed by *R. bursa* with an infestation rate of 7.27 % (4 goats). Thus, 65.46 % of the goats were infested with ticks in the larval stage.

We have studied some risk factors that may influence the rate of goat tick infestation. The factors considered were: sex, age, breed, farm hygiene, season and climate of the area. The results recorded in Table 1 revealed that factors such as: the season, the type of farming, the state of the hygiene of the farm, as well as the climatic conditions of the studied areas were found to be significantly associated with tick infestation of the goats ($P < 0.05$). For the tick harvest season, the prevalence of infestation was very high in the summer (69.2 %) compared to the spring (30.8 %), compared to 0 % in the fall.

If we now take into account the climatic conditions of the study areas, the prevalence of tick infestation was higher in the humid areas (50.0 %), followed by the sub-humid and semi-arid areas (26.9 % and 22.2 % respectively). Factors such as gender, age, and breed or coat colour do not significantly affect the tick infestation of goats.

Microscopic examination using MGG

A total of 128 blood smears were examined for the presence of intraerythrocytic inclusion bodies (considered as positive for *A. ovis* and/or *A. marginale*) using the Giemsa stain. *Anaplasma* spp. appeared as small spherical

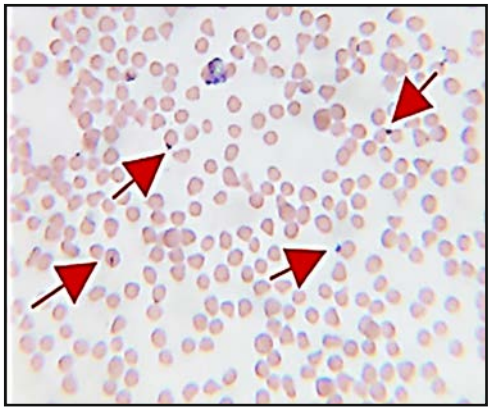


Fig. 1. *Anaplasma* spp. identified on blood smears stained with Giemsa, × 8 eyepiece and × 100 objective

Table 2. Risk factors analysis of exposure to *Anaplasma* spp. infection by cELISA

Variables	Number sampled	Positive number (%)	95 % CI on prevalence	Odds Ratio (OR)	95 % CI on OR	P—value
Age						
≤ 6 months	8	4 (50.00)	15.35—84.64			
6—12 months	55	40 (72.73)	59.68—82.81	0.57	0.26—1.21	0.146
> 12 months	119	98 (82.35)	74.45—88.23			
Sex						
Male	66	46 (69.70)	57.73—79.50	1.10	0.58—2.07	0.041*
Female	116	96 (82.76)	74.79—88.63			
Season						
Autumn	26	21 (80.77)	61.67—91.95			
Spring	38	13 (34.21)	21.15—50.17	1.35	0.45—4.06	0.588
Summer	82	62 (75.61)	65.24—83.69			
Dress Colour						
Black	87	66 (75.86)	65.84—83.70			
White	38	32 (84.21)	69.20—92.94			
Black and White[a]	25	17 (68.0)	48.27—82.94	0.96	0.53—1.34	0.298
White and Black[a]	18	15 (83.33)	59.95—94.99			
Black and Brown	1	1 (100)	0.00—100.00			
Grey	13	10 (76.92)	49.06—92.50			
Type of breeding						
Extensive	115	98 (85.22)	77.50—90.66			
Semi-extensive	58	42 (72.41)	59.71—82.33	9.18	1.72—48.98	0.000**
Intensive	09	02 (22.22)	26.90—80.27			
Hygienic quality						
Good	103	81 (78.64)	69.71—85.52			
Moderate	65	49 (75.38)	63.60—84.33	0.51	0.10—2.52	0.406
Bad/poor	14	12 (85.71)	58.81—97.24			
Climate						
Humid	74	56 (75.68)	64.71—84.10			
Sub-humid	66	54 (81.82)	70.70—89.44	1.40	0.54—3.62	0.480
Semi-arid	42	32 (76.19)	61.30—86.69			
Pregnant status						
Yes	26	23 (88.46)	70.20—96.82	1.70	0.45—6.37	0.426
No	88	72 (81.82)	72.38—88.59			
Tick infestation						
Yes	158	122 (77.22)	70.04—83.09	2.37	1.09—5.16	0.501
No	24	20 (83.33)	63.53—93.93			

OR—Odds Ratio; CI—Confidence Interval at 95 %; *—significant at P ≤ 0.05; **—significant at P ≤ 0.01; [a]—the difference between the terms of coat color «Black and White» and «White and Black» lies in the intensity and dominance of the colour of which the first colour is the most dominant

deep purple intraerythrocytic inclusions, measuring about 0.2 um to 0.5 µm (Fig. 1). Among the specimens, 54 were found positive with an overall prevalence of 42.18 %.

Prevalence of *Anaplasma* spp. antibodies using cELISA

The overall prevalence of *Anaplasma* spp. antibodies in the goats were determined using the MSP5 cELISA for *Anaplasma* spp. Of the 182 animals tested, 142 (78.02 %) were found to be positive for antibodies against the *Anaplasma* spp. antigen (*A. ovis* et/ou *A. marginale*). The prevalence of *Anaplasma* spp. infection in the herd goats was 47.4 % (n = 148). However, the infection rates were higher among females (67.6 %), animals of the age group superior to 12 months (61.1 %), and in infested animals by ticks (85.9 %) with no significant correlation (P > 0.05) (Table 2).

There was a highly significant association (P = 0.000) between the seroprevalence of *Anaplasma* spp. and the type of breeding and herd management (Table 2). However, no significant association was found with other factors such as: sex, age, pregnant status, abortion, tick infestation, season, coat colour, hygienic and climatic conditions (P > 0.05) (Table 2). The highest seropositivity in goats was observed in the extensive herd type (50 %). The infection rate was higher but not significantly among the humid climate (39.4 %) compared to the sub-humid and semi-arid climate (P > 0.05). Indeed, no significant relationship was observed between the *Anaplasma* spp. seroprevalence and the presence of *R. Sanguineus* and *R. bursa* (P > 0.05).

Comparative study of the two methods (MGG and cELISA)

The performance of the blood smear method was evaluated using the cELISA as a reference test (Table 3). For the blood smear method, sensitivity, specificity, relative accuracy, Cohen's kappa coefficient, Mc Nemar test, positive predictive value (VPP) and negative predictive value (VPN) were calculated.

Comparing the data obtained from the microscopic examination with cELISA, it was observed that the blood smear method had a lower sensitivity (45.16 %) and specificity (60.71 %) than cELISA. The results showed an accuracy of 48.46 %, a VPP of 79.24 % and a VPN of 75 %. The value of Kappa as 0.038, which means that there was a very weak agreement. The Mc Nemar test result showed that the two methods gave significantly different values (P < 0.05).

Table 3. Comparison of the MGG with the cELISA test as a reference test for the diagnosis of *Anaplasma* spp.

Anaplasma spp.	cELISA		MGG	
			+	–
Goats	+	93	42	51
	–	28	11	17
	Total	**121**	**53**	**68**
Intrinsic values	Se = 45.16 %		k = 0.038	
	Sp = 60.71 %		Mc Nemar test (P < 0.05)	
	Ra = 48.46 %			

Se—sensitivity; Sp—specificity; Ra—relative accuracy
K—Kappa value

The Gold standard of truth for the diagnosis of *Anaplasma* spp. relies on the combination of the microscopic examination and cELISA.

DISCUSSION

No previous study on anaplasmosis had ever been conducted in the Guelma and El-Taref regions before. Therefore, the present study was part of a large survey initiated to address the lack of data on the epidemiological features of anaplasmosis in the Guelma and El-Taref regions. The plan was to study all of the epidemiological links, i.e. goats and ticks; along with the risk factors associated with anaplasmosis. In Algeria, there is a lack of information about the *Anaplasmataceae* infections in animals and mainly in ruminants, where only *A. phagocytophilum*, *A. platys* and *Anaplasma* spp. were found in cattle [23].

Our study described a survey of ticks and tick-borne bacteria such as *Anaplasma* spp. circulating in different areas of North-eastern Algeria. *R. sanguineus* and *R. bursa* were the only tick species detected in the studied regions and they can serve as vectors of *A. marginale* and/or *A. ovis* as reviewed by K o c a n et al. [33].

Accordingly to our findings, *Rhipicephalus* spp., the most incriminated tick in the transmission of ovine and caprine anaplasmosis [30], has frequently been detected and abundant in the Gharb and the Middle Atlas regions of Morocco [35]. Nevertheless, the role of other biting hematophagous insects, such as the *Hippoboscidae*, flies (e. g. horseflies) and

some species of flea, should not be underestimated with regard to the transmission of anaplasmosis [32].

The North African ecosystem offers a favourable ecology for several tick species that can be competent vectors of several pathogens [17]. In Tunisia, the most dominant tick species collected from sheep was *R. turanicus* followed by *R. sanguineus* and then *R. annulatus* [24]. *R. anguineus* have been proposed previously as vectors of *A. ovis* in Mediterranean countries [4, 58]. Recently, A k t a s et al. [4] reported the presence of *A. ovis* DNA in the salivary glands of *R. sanguineus* collected from Turkey. L u et al. [38] showed that *Dermacentor nuttalli*, *Hyalomma asiaticum* and *Rhipicephalus pumilio* were the main vectors of *A. ovis* in China and *R. turanicus* was one of the main vectors of *A. ovis* in Sicily (Italy) [58]. Recently, using quantitative real-time PCR, S a d e d i n e et al. [53] has detected *A. ovis* in the blood of small ruminants, *Rh. sanguineus* s.l. and *Rh. bursa*. A previous study had already reported the presence of *A. ovis* in these ticks [10]. In Tunisia, B e l k a h i a et al. [14] indicated that ticks collected from goats were infected by *A. ovis*, such as *R. turanicus* and *R. sanguineus* s.l. with an absence of infection in *R. bursa* and *R. annulatus*. In addition, *Anaplasma ovis* has previously been reported in small ruminants and ticks from Algeria and Tunisia [9, 13, 15] and in ticks from Ethiopia [55]. However, further studies are needed to be done to identify the main vectors of *A. ovis* in our country.

Moreover, in our current study, we did not detect any case of *A. phagocytophilum* infection and no hard tick vector such as *Ixodes ricinus* was identified. Other tick species like those of the genus *Rhipicephalus* and *Hyalomma* have been suspected of transmitting this bacteria [21] and have been known to be widely distributed in Morocco [35] and probably in Algeria.

In contrast to our finding, B e l k a h i a et al. [12] and B e n S a i d et al. [15] showed that ruminants (cattle, goats, and sheep) infested by ticks were statistically more infected with *Anaplasma* spp. than those free from ticks. No statistically significant differences were found in the prevalence of *Anaplasma* spp. in animals or humans exposed or not to ticks [28, 29]; the same results were found in the current study. According to A i t L b a c h a et al. [3], it seems that the prevalence of *Anaplasma* spp. was very significantly influenced by the tick infestation and by tick-borne diseases prophylactic measures. Our results are in disagreement with the results of recent studies showing the strong relationship between the density of tick infestations and the occurrence of anaplasmosis [39].

In various previous serosurveys on the occurrence of *Anaplasma* spp. in animals, the serologic methods, the cut-off points, the study sample, and the study group population have been variable. Although a comparison of the present data with those reported in previous seroepidemiological studies may be difficult, it is worthwhile comparing our data with the seropositivity rates reported from other Mediterranean countries. In this longitudinal investigation, results clearly indicated evidence of *A. marginale/A. ovis* infection in goats sampled in all investigated bioclimatic areas and during the three seasons (autumn, spring, and summer). The average prevalence rate of *A. ovis/A. marginale* infection in goats was 78.02 % (minimum 30.7 % in spring and maximum 43.6 % in autumn). It was similar to those reported in Iraq (75.22 %) [44], higher than that observed in Turkey (66.4 %) [6], in Tunisia (65.3 %) [15], in Egypt (48 %) [31] and in Italy (31.7 %) [57], and lower than that estimated in Angola (100 %) [34], in Kenya (89 %) [40] and in China (27.5 %) [63], and it was much higher than the level observed in Korea (6.6 %) [36].

In this study, goats of all ages were susceptible to anaplasmosis, but the severity of the infection was directly related to the age, but the relationship was not statistically significant. The relatively high seroprevalence was observed in the age group above 1 year old as compared to other age groups. It has been reported that goats of all age groups are susceptible to *A. ovis/A. marginale* infection, but older animals may suffer from a greater reduction in haematocrit values [51]. The results of our study were completely in line with the findings of N a q i d, Z a n g a n a [44], and R a z m i et al. [52], who reported that adult animals were more susceptible to anaplasmosis infection than younger animals. This finding indicated that adult goat may have more opportunities for exposure to ticks carrying the pathogen than younger animals. This could be explained by the fact that adult goats were more exposed to tick infestation carrying *Anaplasma* spp. because they went through more tick seasons.

A significant relationship in the prevalence was revealed between male and female goats. This is in contrast with what has been reported elsewhere concerning goat anaplasmosis [15, 50]. Similar to our current findings, N a q i d and Z a n g a n a [44] have reported that females were more susceptible to *A. ovis/A. marginale* infec-

tion than males, but Zhou et al. [64] showed contrasting results. This could be related to the proportion of the populations sampled. Furthermore, most of the farmers keep a larger number of females than males especially for breeding purposes which affected the proportion of the sex ratio. In others, females were kept for a comparatively longer period within the breeding herd and this increased their chance of exposure to infections, but male animals were usually sold off at younger ages than females.

The current study showed that goat farmers use open lands for grazing with minimal grazing in the highlands regions. Similarly to the findings by Jordan [47], the difference in herd management could expose small ruminants to greater tick numbers and could explain the higher seroprevalence in goats. Grazing goats showed higher seropositivity compared to those who remain in permanent housing and this can be explained by the fact that grazing systems may increase the risk of getting vector ticks and enhance the risk of anaplasma infections [31]. A higher seroprevalence was demonstrated for the extensive system (69.0 %) when compared to the semi-extensive and intensive systems (29.6 % and 1.4 % respectively). Consistent with our current results, Stoltsz [54], Torina and Caracappa [57] showed that the grazing system and the type of breeding influenced potentially the seroprevalence of *Anaplasma* spp. infections in goats. The relatively high seroprevalence of *Anaplasma* spp. observed in our study could be due to the favourable environmental conditions, especially in spring, for the survival and proliferation of the tick vectors, since the goats are reared under extensive and semi-intensive systems.

Goats are usually vaccinated for certain diseases like brucellosis as part of the national vaccination campaigns in Algeria. These vaccination campaigns, already applied at the same period of the tick infestations, might explain the high seroprevalence as there is the possibility of iatrogenic transmission among goats by contaminated needles. Such stressful conditions, scarcity of water and pasture during summer, might exacerbate clinical diseases associated with *Anaplasma* spp. infections. In addition, the high prevalence of other diseases in dairy ruminants in Algeria, such as mastitis and respiratory diseases, might predispose animals to clinical disease associated with *Anaplasma* spp. infection such as described in Jordan [47, 48]. *Anaplasma* spp. prevalence was not affected by farm locations or the hygienic statute of farms. These findings corroborate with a previous study that did not find a significant difference in Iran [61].

In our study, the microscopic examination of goat blood smears obtained from Guelma and El-Taref provinces, demonstrated that 42.18 % of the goats were infected with *A. ovis/A. marginale*. This method has been commonly used in previous studies on ovine and caprine anaplasmosis surveys [2, 52]. The highest prevalence rate was found in the Ain La-assel region (50 %), followed by Ain-Makhlouf (44.8 %) and Ain-Arbi (37.9 %), but no statistically significant differences among regions were found ($P > 0.05$). Our prevalence is close to that found in the northeast region of Iran (Mashhad) (47.53 %) [52]. A lower positivity rates were reported in Tamil Nadu in India (26.15 %) [50], in Iraq (Baghdad) (28.8 %) [5], and in Iran (Ahvaz) (19 %) [31]. A previous study in the Duhok region of Iraq [44] reported a higher prevalence of *A. ovis* in local breeding goats (55.86 %) than that observed in our study.

One of the previous studies performed in Sicily (Italy) came to the conclusion that animals under poor health conditions may express a higher infection rate and also contribute to multiple *Anaplasma* infections [58]. The distribution and abundance of tick vectors can be affected by climatic conditions, including humidity and temperature [37]. These climatic conditions may be favourable for the survival of the ticks that transmit *Anaplasma* spp. and could increase the possibility of other tick-borne disease being spread to livestock and wild animals; therefore resulting in a higher prevalence rate as was observed in our study. The prevalence rate of *Anaplasma* spp. varied significantly in different agro-ecological areas considered in this study. However, it was noticed that goats in the sub-humid climate were more affected than in the humid and semi-arid climate. In fact, *Anaplasma* infections exhibited a certain seasonality linked in particular to the abundance of vectors; however, the preferential seasons vary according to region and bioclimatic conditions during the year [8, 37]. The humid and sub-humid stages constitute a very favourable biotopes for *R. Sanguineus* and *R. Bursa* [18] which are species recognized as vectors of *A. ovis* in goat in the Mediterranean region [13]. Our results do not correspond to those mentioned in the literature and this deference can be linked to the origin of the animals, which are in commercial exchange (sale and purchase) almost all year and they are transported between the different agro-ecological areas. Velusamy et al. [59] indicated

that there was no seasonal effect on goat anaplasmosis in India. Friedhoff [30] has reported that many factors affect the prevalence of *Anaplasma* spp. infections including: age, sex, breed, and the general conditions of the animals.

We had shown that *Anaplasma* infections were related to pregnancy disorders, such as abortion in goats. A history of abortion was associated with *Anaplasma* spp. seropositivity in small ruminants from Jordan [47]. It is interesting to declare that animals or flocks showing high seropositivity and/or tested positive by MGG against the pathogen did not show any clinical signs of the disease; other surveys have reported similar findings [51].

Nevertheless, serological diagnostic assay tests could be more practical for the diagnosis of a large number of animals than the conventional microscopic test including Giemsa stained blood smears. Also, serological diagnostics are more sensitive and specific diagnostic tools to detect and differentiate *Anaplasma* spp. in carrier animals. Several studies have specified that cELISA test has a very high sensitivity and specificity in the diagnosis of antibodies against *Anaplasma* species such as *A. marginale*, as well as *A. centrale*, *A. ovis* and *A. phagocytophilum* [26].

In our study, cELISA detected a higher number of infected animals with *A. ovis/A. marginale* (142/182; 78.02 %) than Giemsa stained blood smear (54/128; 42.18 %). Overall, the blood smear method showed average specificity, low sensitivity and accuracy with high VPP and VPN values. Calculating the concordance between the two methods using Cohen's Kappa test showed values from 0.00—0.20, which corresponds to a very weak concordance. The result of McNemar's test showed that the method gave significantly different values ($P < 0.05$). The result obtained in this study is comparable with those found in Egypt by Abou-Elnaga et al. [1] where they recorded a prevalence of 37 % and 67 % in cattle using the Giemsa staining and cELISA respectively. Mouloudi [43] obtained a frequency of 25.4 % and 63.9 % by MGG and cELISA respectively. These discrepancies could be explained by differences in the stage of infection and the antibody responses. During the chronic infections, antibodies remained in the blood circulation for a longer period (up to 10 years after the infection) even with low levels of bacteraemia [50]. The low sensitivity of MGG compared to serological tests could be explained by the low bacteraemia which characterizes the chronic carriage of this infection and that *Anaplasma* cannot be easily detected in blood smears after clinical manifestation [49].

However, a limitation of the microscopic examinations (Giemsa stain blood smear) has been their inability to differentiate the *Anaplasma* spp. organism and other similar structures like Heinz bodies, Howell-Jolly bodies, or staining artefacts. An additional complaint is the fact is that this method need special experiences, particularly in animals with a very low level of bacteraemia [45]. Giemsa stained blood smears can be used as a suitable method to detect *Anaplasma* spp. in clinically suspected animals for the acute diseases [22]. This makes microscopic analysis unreliable for the detection of persistent *Anaplasma* spp. infections in animals [46]. To circumvent this problem, an alternative diagnostic technique, such as serological diagnostic tests [45] and nucleic acid-based assays [46] can be used for the detection of tick-borne parasites.

The observed prevalence was lower when estimated by cELISA tests than with MGG method. Similar differences have been found in other studies and may reflect the absence of detectable levels of bacteraemia in some animals [25]. The use of serological tests for antibodies detection against these pathogens is the most suitable tool for epidemiological studies to assess the importance of these infections in the goat populations [7]. The "Gold standard" method for the diagnosis of *Anaplasma* spp. relies on the combination of the microscopic examination and the cELISA [27].

The difference observed in the prevalence rates of *Anaplasma* species among different countries and areas of the same country may be due to several factors including: environmental and climatic conditions (such as weather, soil, and types of vegetation), genetic (the susceptibility of each host species and breeds), ecological (such as composition and interactions of the tick populations, the presence of other animal species including wildlife reservoirs), and husbandry practices [13, 15, 58]. In our study, the high prevalence of *Anaplasma* spp. infections in goats is attributable to environmental and climatic factors that make the northern regions a very suitable one for tick vector life.

CONCLUSIONS

In this study, we reported a high prevalence of *Anaplasma* spp. infections in goats using two different methods

such as the Giemsa blood smear and serologic screening in North-eastern Algeria. Although we did contribute towards broadening the epidemiological knowledge about *Anaplasma* spp. infection even though it is impaired by the absence of a correct diagnosis, often based solely on clinical manifestations rather than on a laboratory investigation. Our results do not show the infection status of the region due to the small amount of material collected. Also, vector biology, host preference and zoonotic potential of different *Anaplasma* species need to be further studied. The results of our work did allow us to glimpse a series of studies in perspective aimed at further improving the knowledge of caprine anaplasmosis in Algeria.

The methods used in this investigation made it possible to detect with certainty this infection which threaten the reproduction/production capabilities of goats. The choice between these two techniques will depend on their performance, as well as the availability of the equipment, laboratory conditions and the number of samples to be tested. The evaluation of such techniques are becoming more and more important to be able to use sensitive and specific tests dedicated to epidemiological surveys, which conditions the measures to be taken for good control of such infections unnoticed in breeding. However, further molecular screening of different *Anaplasma* species in blood and ticks collected from goats with molecular characterization are needed to estimate the real prevalence and clarify the genetic variability of *Anaplasma* spp. in goats from North-eastern Algeria.

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