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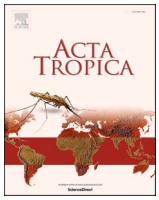

Molecular detection and associated risk factors of *Anaplasma marginale, A. ovis* and *A. platys* in sheep from Algeria with evidence of the absence of *A. phagocytophilum*

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ABSTRACT

Anaplasma species are obligate intracellular rickettsial pathogens that cause significant diseases in animals and humans. Despite their importance, limited information on *Anaplasma* infections in Algeria has been published thus far. This study aimed to assess the infection rate, characterize *Anaplasma* species, and identify associated risk factors in selected sheep farms across Oum El Bouaghi region in Algeria. In 2018, we collected 417 blood samples from sheep (*Ovis aries*) and performed molecular characterization of *Anaplasma* species infecting these animals. This characterization involved the use of 16S rRNA, *msp2, rpoB*, and *msp5* genes, which were analyzed through nested PCR, qPCR, cPCR, DNA sequencing, and subsequent phylogenetic analysis. Our findings revealed infection rates of 12.7 % for *Anaplasma* species detected, with *Anaplasma ovis* at 10.8 %, *Anaplasma marginale* at 1.7 %, and *Anaplasma platys* at 0.2 %. Interestingly, all tested animals were found negative for *Anaplasma phagocytophilum*. Statistical analyses, including the Chi-square test and Fisher exact test, failed to establish any significant relationships ($p > 0.05$) between *A. ovis* and *A. platys* infections and variables such as age, sex, sampling season, and tick infestation level. However, *A. marginale* infection exhibited a significant association with age ($p < 0.05$), with a higher incidence observed in lambs (5.2 %) compared to other age groups. Remarkably, this study represents the first molecular detection of *A. platys* and *A. marginale* in Algerian sheep. These findings suggest that Algerian sheep may serve as potential reservoirs for these pathogens. This research contributes valuable insights into the prevalence and characteristics of *Anaplasma* infections in Algerian sheep populations, emphasizing the need for further investigation and enhanced surveillance to better understand and manage these diseases.

1. Introduction

Bacterial species of the genus *Anaplasma* are obligate intracellular (Ben Said et al., 2015a), Gram-negative bacteria of the family Anaplasmataceae, the rickettsiales order and the alpha-proteobacteria class (Ait Lbacha et al., 2017) that cause animal and human anaplasmosis (Ben Said et al., 2018). This last causes important economic losses to animal breeders (Kaewmongkol et al., 2017; Soosaraei et al., 2020) due to decreased production, mortality and lowered work efficiency of affected animals (Shabana et al., 2018).

Anaplasmosis is a tick-borne rickettsial disease caused by: *A. ovis, A. marginale, A. centrale, A. platys, A. bovis and A. phagocytophilum* (Dumler et al., 2001; Rar and Golovljova, 2011; Atif, 2016). Currently, the genus also contains three new species, provisionally named *A. odocoilei, A.*

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capra (Tate et al., 2013, Li et al., 2015a) and *Anaplasma* sp. 'Omatjenne'. However, these potential species have not yet been formally described (Kola, 2023). Among *Anaplasma* species, *A. ovis* which infects sheep, goat and deer, is an obligate intraerythrocytic bacterium (Friedhoff, 1997; Yabsley et al., 2005; de la Fuente et al., 2006, 2007), the infection is frequently subclinical but can also cause disease which is more critical among goats than sheep, particularly in stressed or debilitated animals (Friedhoff, 1997). It can be severe with hemolytic anaemia, hemoglobinuria and fever (Barry and Van Niekerk, 1990, Stoltsz, 2004, Hornok et al., 2007). In addition, *A. ovis* infection may predispose to other infectious and/or parasitic diseases that aggravate the animal's condition, occasionally leading to death (Kocan et al., 2004). In North Africa, infection with *A. ovis* has been investigated in small ruminants in Sudan, Tunisia and Algeria (Renneker et al., 2013; Belkahia et al., 2014; Ben Said et al., 2015b; Aouadi et al., 2017).

Anapalsma marginale, the most common etiologic agent of bovine anaplasmosis, causes a variety of clinical signs, including fever, weight loss, abortion, lethargy, icterus and often death of the animals older than 2 years (Kocan et al., 2003). In North African countries, infection by this species has been investigated only in cattle and dromedaries (Belkahia et al., 2015b; Ben Said et al., 2018). *Anaplasma centrale* is an intraerythrocytic tick-borne rickettsia of cattle that has a different morphology and virulence compared to *A. marginale*. It can cause asymptomatic infection, or only a mild anemia in most cases (Rar and Golovljova, 2011). *A. centrale* is used for extensive vaccination of cattle against *A. marginale* infection in endemic areas (Rar and Golovljova, 2011). Until now, there have been no investigations on *A. centrale* in North Africa except in cattle and camels from Tunisia (Belkahia et al., 2015a, 2015b, 2017a, 2017b) and in cattle from Algeria (Rjeibi et al., 2018). *Anaplasma platys* is the causative agent of infectious canine cyclic thrombocytopenia in dogs (Dumler et al., 2001) but several reports described the infection in some ruminants such as sheep, goats, camels, buffalo and red deer (Allsopp et al., 1997; Chochlakis et al., 2009; Djiba et al., 2013; Li et al., 2015a, 2015b, 2016; Lorusso et al., 2016a, 2016b; Machado et al., 2016). *Anaplasma bovis* infects circulating monocytes (Liu et al., 2012) and tissue macrophages of domesticated and wild ruminants (Worthington and Bigalke, 2001). In general, it has been commonly reported in cattle, goats and wild deer (Liu et al., 2012; Ceci et al., 2014; Yang et al., 2014). *Anaplasma phagocytophilum* is a zoonotic bacterium that infects neutrophils of many host species such as cattle, sheep, goats, dogs, horses, deer and humans (Stuen et al., 2003; Franzén et al., 2005, 2007), transmitted mainly by *Ixodes* ticks. Also, this agent differs from other Anaplasmataceae bacteria by the high genetic diversity (Rar et al., 2021). *A. phagocytophilum* has been detected throughout Europe, America (North and South), Asia and Africa (Atif, 2015). In Algeria, the search of *A. phagocytophilum* has been performed in dogs and cattle (Azzag et al., 2015; Dahmani et al., 2015). Moreover, in ruminants, it is the causative agent of pasture fever or tick-borne fever (Woldehiwet, 2010) mostly characterized by high fever, anorexia, dullness and reduced milk production (Tuomi, 1967; Woldehiwet, 2010). It is responsible for important economic loss to cattle and sheep industry (Stuen et al., 2003). These latter are efficient reservoirs for *A. phagocytophilum* during the acute and post-acute phases of infection (Ogden et al., 2003). Sheep are also able to transmit the bacteria to ticks during chronic persistence (Almazán et al., 2020).

Finally, very few studies have been conducted on molecular epidemiology of *Anaplasma* species from Algeria. Therefore, the present study aimed to estimate the sheep prevalence of infections by *Anaplasma* spp. from North-eastern Algeria, the analysis of some risk factors for the *Anaplasma* infections and the genetic variability of *Anaplasma* species identified.

The current study significantly contributes to the existing literature on *Anaplasma* infections in North Africa in several unique ways. First and foremost, it represents one of the very few investigations into the molecular epidemiology of *Anaplasma* species within the region. While previous studies have primarily focused on specific *Anaplasma* species or limited geographic areas, our research takes a more comprehensive approach, encompassing multiple *Anaplasma* species and expanding our understanding of their prevalence and genetic variability. Furthermore, this study explores the presence of *Anaplasma* species in Algerian sheep, a livestock population of significant economic importance. We extend the knowledge base by providing crucial insights into the infection rates and associated risk factors for *Anaplasma* species, shedding light on the factors that may contribute to the spread of these pathogens among sheep populations. By providing valuable insights into the prevalence, risk factors, and genetic diversity of *Anaplasma* species in NE Algeria, our research fills critical gaps in the existing literature and offers a substantial contribution to the field of veterinary medicine and epidemiology.

2. Materials and methods

2.1. Study region

The current study was conducted between June and October 2018 in the province of Oum El Bouaghi, located in northeastern Algeria (Fig. 1). This province is situated within the Constantine highlands region, covering an area of approximately 7638.13 km^2. Oum El Bouaghi is geographically positioned at 35° 52′ 39" N latitude and 07° 06′ 49" E longitude, with an elevation of 890 m above sea level. The region exhibits a semi-arid continental Mediterranean climate, characterized by cold winters occasionally featuring snowfall, as well as hot and dry summers (Kabbout et al., 2016).

2.2. Study animals

The study was approved by the Scientific Committee of the Higher National Veterinary School (ENSV) in Algiers. All procedures were carried out in compliance with the ethical committee of the School's standards and regulations. The study was performed following ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and U. K. Animals Act 1986 and associated guidelines, and it was conducted according to the guidelines of the Ethics Committee of ENSV. The owners of the surveyed sheep provided their verbal agreement for the blood samples to be collected.

The small ruminants from all the farms in the study belong to the Oueled Djellel breed and were kept under a semi-intensive system. Moreover, forty farms throughout the province of Oum El Bouaghi (Fig. 1) were included in this study, which the sample size was determined by the following formula given by Thrusfield (2007):

$$n = \left[1.96^2 \mathrm{Pexp}(1-\mathrm{Pexp})\right]/d^2,$$

where n = required sample size; Pexp = expected prevalence; d = desired absolute precision; 1.96 was the Z value for the selected confidence level (95 %). According to this formula, the minimum sample size for an infinite population was 365 sheep using an expected individual prevalence of 61.7 % - according previous studies in this region - (Aouadi et al., 2017), a desired absolute precision of 5 % and a confidence level of 95 %. The sample size was increased to 417 in order to increase the absolute precision and compensate for 5 % attrition. The minimum number of sheep to be tested on each farm was established as 10 (Cannon and Roe, 1982), which were randomly selected.

2.3. Study protocol

2.3.1. Blood sampling

Blood samples were taken from the jugular vein of 417 sheep and collected in EDTA tubes, then they were stored at -20°C until DNA extraction. Similarly, the date, age, sex, presence or absence of ticks have been noted.

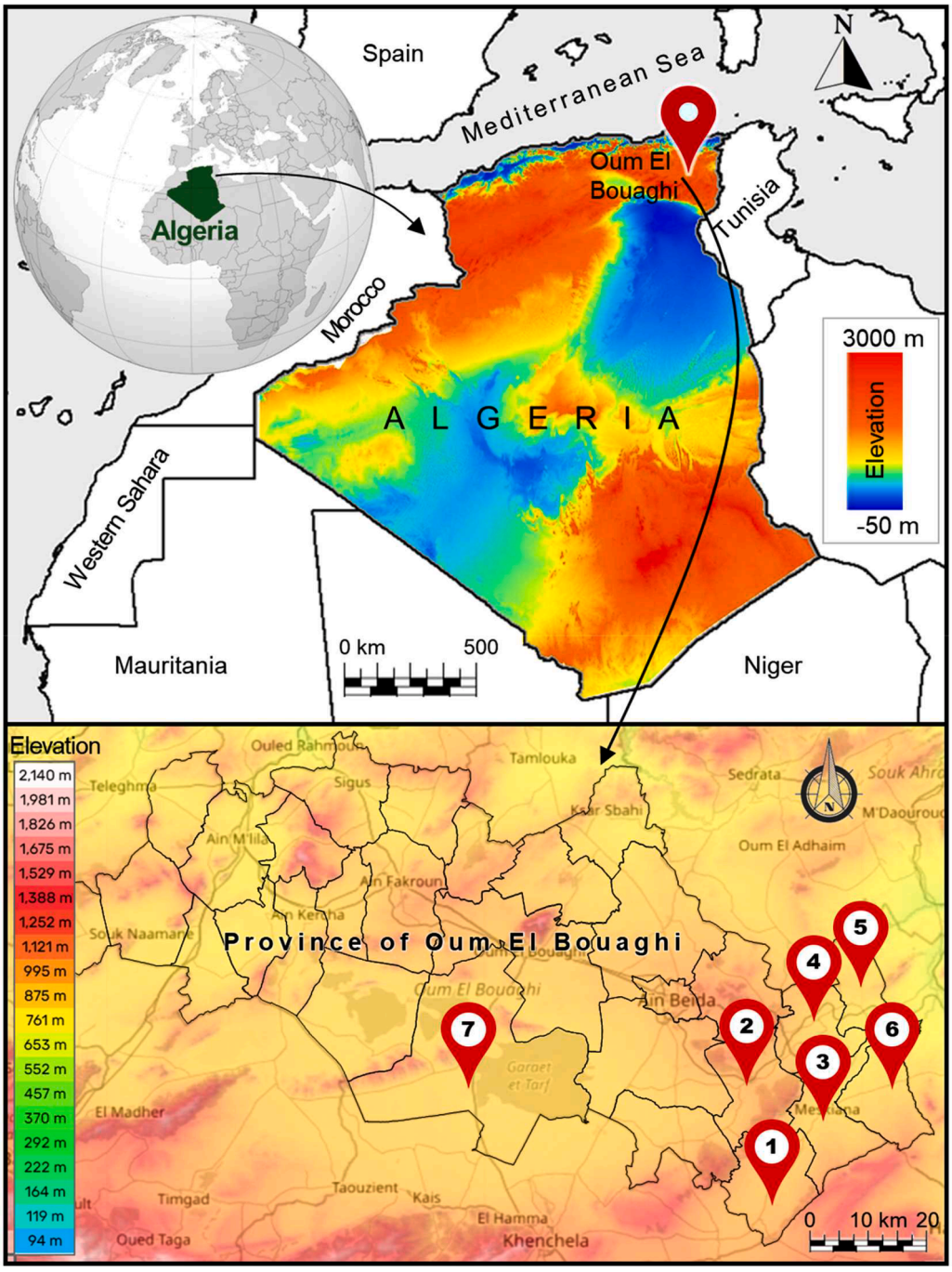

Fig. 1. Elevation maps of Algeria (top) and the province of Oum El Bouaghi (bottom) showing the location sampling sites (1: Dhalaa, 2: El Djazia, 3: Meskiana, 4: Rehia, 5: Behir Chergui, 6: El Belala, 7: Ain Zitoun).

2.3.2. DNA extraction

We used two methods: (i) Blood samples of each farm were thawed at room temperature, then they were pooled and 200 μL was used for extraction. DNA was extracted with the kit NucleoMag® VET (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. The eluted DNA samples were stored at -80°C until later used in the screening PCR and the qPCR; and (ii) DNA was extracted from 50 μL of blood sample from each animal tested using TRI Reagent as per the manufacturer's instructions (TRI Reagent®, catalogue number T9424, Sigma, USA) and the eluted DNA samples were stored at -80 °C until later used in the conventional PCR.

2.3.3. Molecular tests

2.3.3.1. nPCR assay. All pooling samples of each farm were primarily screened by Nested PCR using amplification of 16S rRNA gene to determine the presence of *Anaplasma* spp. DNA in the blood samples collected from the tested animals. The 16S rRNA gene of *Anaplasma/Ehrlichia* species was amplified with two rounds of nPCR. The primers for the first round were:

Ehr1 (5′-GAACGAACGCTGGCGGCAAGC-3′) and
Ehr2 (5′-AGTA[T/C]CG[A/G]ACCAGATAGCCGC-3′)

For the second round, they were:

Ehr3 (5′-TGCATAGGAATCTACCTAGTAG-3′) and
Ehr4 (5′-CTAGGAATTCCGCTATCCTCT-3′) (Jafar-bekloo et al., 2018).

The reaction mix for each amplification contains the primer pair (previously mentioned), the master mix (New England Biolabs, France) and water, which were then mixed with the DNA sample. All the thermal stages were performed using a thermocycler (Eppendorf AG, Humburg, Germany). The thermocycler was set to 5 min at 95 °C, followed by 35 cycles, each consisting of 1 min at 95 °C for further denaturation, 1 min at 57 °C for annealing and 1 min at 72 °C for extension, and then a final extension for 5 min at 72 °C. The products of the first round of PCR were used as the template for the second round of PCR, which was carried out under the same conditions and reaction mixture as the first round except that Ehr3 and Ehr4 were used as the primers (Jafar-bekloo et al., 2018). The PCR products were electrophoresed on 1.5 % agarose gel.

2.3.3.2. qPCR assay. Pooling samples founding positive for nPCR were used to detecting *Anaplasma phagocytophilum*. Therefore, *A. phagocytophilum* DNA was searched by qPCR for the amplification of the *msp2* gene. Briefly, the reaction mix per sample consisted of forward primer ApMSP2f (ATG GAA GGT AGT GTT GGT TAT GGT ATT), reverse primer ApMSP2r (TTG GTC TTG AAG CGC TCG TA), probe ApMSP2p (TGG TGC CAG GGT TGA GCT TGA GAT TG) (Hornok et al., 2018), RNAse free H_2O and Master mix (New England Biolabs, France). The extracted DNA samples and reaction mix were loaded onto a 96-well plate.

The qPCR amplification reactions were performed on a thermal-cycler (Applied Biosystems StepOnePlus Real-Time PCR System, Singapore), with initial denaturation temperature of 95 °C for 10 min, followed by 45 cycles of denaturation at 95°C for 30 s, hybridation at 50 °C for 20 s and elongation at 72 °C duration 1 min.

2.3.3.3. Conventional PCR assays. Positive pooling samples of nPCR were used to screen the infected herds. After all samples of each farm were tested in conventional PCRs using *Anaplasma* genus-specific primers targeting the 577 bp fragment of the RNA polymerase subunit beta (*rpoB*) gene. Briefly, the reaction was performed using the forward primer Ana-rpoBF:

GCTGTTCCTAGGCTYTCTTACGCGA
and the reverse primer Ana-rpoBR:
AATCRAGCCAVGAGCCCCTRTAWGG (Dahmani et al., 2016).

The amplification reactions were executed in an automated DNA thermal cycler (Eppendorf Mastercycler® personal, Westbury, NY, USA) under the following conditions: an initial denaturation step at 94 °C for 2 min, followed by 40 cycles consisting of 30 s denaturation at 95 °C, 45 s annealing at 55 °C and a 1 min extension at 72 °C. A final extension cycle at 72 °C for 7 min.

Moreover, the *msp5* gene from *Anaplasma marginale* was amplified by PCR using the following primers (Singh et al., 2012):

Amar-msp5 eR: 5' TCC TCG CCT TGG CCC TCA GA 3'
Amar-msp5 iF: 5' TAC ACG TGC CCT ACC GAG TTA 3'

The PCR was carried out according to the following steps an initial denaturation at 94 °C for 1 min, followed by 30 cycles of: denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 45 s, with a final extension step at 72 °C for 7 min. After, PCR products were electrophoresed on 1,5 % agarose gels to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 kb Plus DNA Ladder, Fermentas).

2.3.4. DNA sequencing

Positive PCR products from primers Ana-rpoBF / Ana-rpoBR and from primers Amar msp5 iF / Amar msp5 eR were purified with QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions and sequenced (SECUGEN, Madrid, Spain). The obtained sequences were analyzed and aligned to the sequences of the reference strains reported in the GenBank using the Basic Local Alignment Search Tool (BLAST).

2.3.5. Phylogenetic analysis

The phylogenetic analysis was carried out using ClustalW to align sequences obtained for the *msp5* (371 bp) and *rpoB* (465 bp) fragments, using as references sequences to different species (*Anaplasma ovis, A. marginale, A. centrale, A. platys* and *A. phagocytophilum*) available for these fragments were obtained from GenBank. Not all species were included in both fragments; based on Corrected Akaike Information Criterion (cAIC) and Bayesian Information Criterion (BIC) implemented in Molecular Evolutionary Genetics Analysis (MEGA X) (Kumar et al., 2018) The best-fit model of the evolution sequences was selected.

The phylogenetic trees were generated by using MEGA version 10 (http://www.megasoftware.net) with the Maximum Likelihood method. Bootstrap confidence limits were calculated on the basis of 1000 replicates; the numbers on branches in the tree indicate bootstrap results.

2.4. Statistical analyses

Comparisons of the prevalence of *Anaplasma* species in sheep according to risk factors were performed with SPSS version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp), using the Chi-squared test and Fisher exact test. A probability less than 0.05 was used as a threshold for statistical significance. Confidence intervals (CI) for prevalence rates at the 95 % level were calculated.

3. Results

3.1. Molecular survey of Anaplasma species

The overall prevalence of *Anaplasma* spp. was 12.7 % (53/417), *A. ovis* was the most prevalent species (45/417, 10.8 %), followed by *A. marginale* (7/417, 1.7 %) and *A. platys* (1/417, 0.2 %). In a qPCR, none of the tested pooled blood samples showed positive results for *A. phagocytophilum* (Tables 1 and 3).

Statistical analysis revealed a significant association ($p < 0.05$) between the molecular prevalence of *Anaplasma marginale* and the age of animals. In particular, animals in the age range of less than 1 year had a significantly higher prevalence (5.2 %) compared to other age groups (1–2 years and >2 years with prevalence rates of 3.3 % and 0.7 %, respectively) (Table 2). The Chi-square test and fisher's exact test showed that there was no statistically significant association between the prevalence of *Anaplasma ovis* and *Anaplasma platys* infections and risk factors in sheep.

3.2. Anaplasma spp. sequencing

Anaplasma ovis, A. marginale and *A. platys* infections were confirmed through sequencing of the *msp5* and *rpoB* genes (Table 3). In addition, our results showed that among the 40 herds of sheep tested, 30 were found positive for the presence of *Anaplasma* spp. with an overall prevalence of 75 %. *Anaplasma ovis* infection was detected in 28 out of 40 (70 %) of the herds, indicating the highest infection rate of *Anaplasma* spp. in this region. Low rates were observed in *A. marginale* that was found in 6 out of 40 (15 %) herds, while *A. platys* was detected in 1 out of 40 (2.5 %) herds

Table 1
Results of molecular tests.

Farms	Pooled sample results		Individual sample results		
	Anaplasma/Ehrlichia species (Amplification of the 16S rRNA gene) nPCR	*Anaplasma phagocytophilum* (Amplification of the *msp2* gene) qPCR	*Anaplasma* spp. prevalence (Amplification of the *rpoB* and *msp5* genes) cPCR		
			rpoB (n. of positive samples)	*msp5* (n. of positive samples)	Prevalence
F1	Positive	Negative	2	2	2/11
F2	Negative	Negative	0	0	0/10
F3	Negative	Negative	0	0	0/12
F4	Positive	Negative	1	2	3/10
F5	Positive	Negative	2	2	2/10
F6	Positive	Negative	2	1	3/10
F7	Positive	Negative	0	0	0/10
F8	Positive	Negative	0	0	0/10
F9	Positive	Negative	0	2	2/10
F10	Positive	Negative	0	1	1/10
F11	Positive	Negative	2	2	2/11
F12	Positive	Negative	2	2	3/10
F13	Positive	Negative	1	1	2/10
F14	Positive	Negative	0	0	0/10
F15	Positive	Negative	0	0	0/10
F16	Positive	Negative	2	0	2/10
F17	Positive	Negative	1	2	2/10
F18	Positive	Negative	1	0	1/10
F19	Positive	Negative	0	1	1/10
F20	Negative	Negative	0	0	0/10
F21	Positive	Negative	1	0	1/10
F22	Positive	Negative	2	0	2/20
F23	Positive	Negative	0	0	1/10
F24	Positive	Negative	1	1	1/10
F25	Positive	Negative	2	1	2/10
F26	Positive	Negative	2	1	2/10
F27	Positive	Negative	0	0	0/10
F28	Negative	Negative	2	2	2/10
F29	NA	Negative	2	2	2/11
F30	NA	Negative	1	1	1/10
F31	Positive	Negative	1	2	2/10
F32	Positive	Negative	2	2	2/10
F33	Negative	Negative	2	0	2/10
F34	Positive	Negative	1	0	1/10
F35	Negative	Negative	0	0	0/10
F36	Positive	Negative	2	0	2/10
F37	Positive	Negative	2	1	2/37
F38	Positive	Negative	0	1	1/10
F39	Negative	Negative	0	0	0/10
F40	Positive	Negative	1	1	1/10
Total		53/417			

3.3. Phylogenetic analyses

All sequences obtained in this study were submitted to GenBank under the following accession numbers: 1. For the *msp5* gene: OR405548 to OR405584; 2. For the *rpoB* gene: OR405585 to OR405599 and OR405600 to OR405623.

The phylogenetic analysis of *msp5* revealed that strains identified in this study were classified into two main clades (Fig. 2). The first clade defined by the reference sequences of *A. ovis* isolated from sheep in China contained thirty-one sequence types. The second clade composed by two sequences of *msp5* were very close with variant of *A. marginale* derived from Havana-Cuba with high bootstrap values (99 %). Also, other four sequences of *msp5* grouped in subclade with *A. marginale* strains isolated from cattle and buffalo in China and Seri-Lanka respectively.

On the other side, the phylogenetic analysis of *rpoB* showed that thirty-seven sequences of *rpoB* clustered with three variants of *A. ovis*. These latter were isolated from sheep in Senegal and France (Fig. 3). However, two sequences of *rpoB* clustered separately to the first clade. The first one clustered with *A. marginale* sequences amplified from cattle and tick in France, and the second one clustered with three variants of *A. platys* derived from dog in Senegal, France and Saint-Kitts-et-Nevis. Finally, none of the *rpoB* or *msp5* sequences isolated in this study appeared to be related to *A. phagocytophilum*.

4. Discussion

Ticks pose a significant threat to public health and veterinary well-being on a global scale (Rodríguez et al., 2011). Their impact extends beyond the direct physical harm they inflict on animals to encompass broader repercussions, leading to substantial losses in meat, milk, egg, leather production, and, tragically, even the loss of livestock. These adverse effects stem from both direct harm inflicted by ticks during blood-feeding (Hurtado and Giraldo-Rios, 2018) and indirect harm resulting from tick-borne pathogens (De Castro, 1997; Alim et al., 2012), which include viruses, bacteria, rickettsiae, and protozoa (Taylor et al., 2016). Ticks stand as the second most significant vectors of pathogens following mosquitoes (Parola and Raoult, 2001) and represent the primary vectors of disease-causing pathogens in animals (Mediannikov and Fenollar, 2014). Shockingly, annual losses attributable to tick-borne diseases are estimated at a staggering 17.33 billion dollars worldwide, with approximately 80 % of the global livestock population exposed to ticks and the diseases they transmit (Djakaridja et al., 2014).

Small ruminants, including sheep and goats, play a pivotal role as

Table 2
Risk factors in sheep for *Anaplasma ovis, Anaplasma marginale* and *Anaplasma platys* infections.

Parameters	Number of positives/total examined (prevalence, %) [95% confidence intervals of prevalence]		
	Anaplasma ovis	*Anaplasma marginale*	*Anaplasma platys*
Animal sex			
Female	40/330 (12.1) [8.8–16.1]	5/330 (1.5) [0.5–3.5]	1/330 (0.3) [0–1.7]
Male	5/87 (5.7) [1.9–12.9]	2/87 (2.3) [0.3–8.1]	0/87 (0)
p-value	0.08	0.64[a]	1.00[a]
Age group (years)			
< 1	3/58 (5.2) [1.1–14.4]	3/58 (5.2) [1.1–14.4]	0/58 (0)
1–2	6/61 (9.8) [3.7–20.2]	2/61 (3.3) [0.4–11.3]	1/61 (1.6) [0–8.8]
> 2	36/298 (12.1) [8.6–16.3]	2/298 (0.7) [0.1–2.4]	0/298 (0)
p-value	0.34[a]	0.02[a*]	0.29[a]
Saison			
Summer	25/264 (9.5) [6.2–13.7]	7/264 (2.7) [1.1–5.4]	1/264 (0.4) [0–2.1]
Autumn	20/153 (13.1) [8.2–19.5]	0/153 (0]	0/153 (0)
p-value	0.25	0.05[a]	1.00[a]
Tick infestation			
Infested	7/48 (14.6) [6.1–27.8]	1/48 (2.1) [0.1–11.1]	0/48 (0)
Not infested	38/369 (10.3) [7.4–13.9]	6/369 (1.6) [0.6–3.5]	1/369 (0.3) [0–1.5]
p-value	0.36	0.57[a]	1.00[a]
Total	45/417 (10.8) [8–14.2]	7/417 (1.7) [0.7–3.4]	1/417 (0.2) [0–1.3]

[a] Fisher's exact test.
[*] Statistically significant test ($p < 0.05$). Sheep prevalence of infections by *Anaplasma* spp. was computed as ratio between number of positively infected hosts (+ve) and the number of examined sheep.

reservoirs and hosts for various tick species, amplifying the impact of these vectors and perpetuating the life cycles of tick-borne pathogens (Pereira et al., 2016). Tick-borne diseases exert their influence on both domestic and wild ruminants, significantly affecting the health and well-being of species such as sheep, goats, cattle, and deer (Ghai et al., 2016).

The North African ecosystem provides an ideal environment for the proliferation of several tick species capable of transmitting a multitude of pathogens (Gharbi and Darghouth, 2014; Chaibi et al., 2023). Among these pathogens, anaplasmosis, a tick-borne rickettsial disease, has emerged as a notable concern. In Algeria, there exists a glaring gap in knowledge regarding Anaplasmataceae infections in animals (Ben Said et al., 2018), particularly in small ruminants. Prior studies have detected only *A. ovis* in sheep, utilizing techniques such as qPCR, standard PCR, and sequencing (Aouadi et al., 2017; Sadeddine et al., 2020). Additionally, molecular identification has confirmed the presence of *A. ovis* in ticks of the *Rhipicephalus* genus, including *Rhipicephalus turanicus* and *R. bursa*, collected from sheep (Aouadi et al., 2017).

The overall prevalence of *A. ovis* observed in our study was 10.8 %, which is lower than the rates reported in Souk Ahras, northern Algeria (61.7 %) (Aouadi et al., 2017), Tunisia (93.8 %) (Ben Said et al., 2015a), (70.1 %) (Belkahia et al., 2014), Sudan (83.9 %) (Lee et al., 2018), Uganda (26.1 %) (Kasozi et al., 2021), Iran (20.8 %) (Yousefi et al., 2017), Saudi Arabia (25.9 %) (Shabana et al., 2018), and Turkey (67.35 %) (Altay et al., 2014). Moreover, our results are comparable with those reported in Egypt (9.1 %) (Tumwebaze et al., 2020). This discrepancy may be attributed to various factors, including climatic conditions and the presence of vector ticks (Altay et al., 2014). These findings underscore the need for continued surveillance and research to understand the geographical variations in *A. ovis* prevalence.

Our study also marks the first identification of *A. marginale* and *A. platys* in Algeria. Molecular analysis revealed that 1.7 % of the sampled sheep were infected with *A. marginale*. While our result aligns with previous reports from Iran (0.54 %) (Yousefi et al., 2017), it contrasts with studies in Pakistan (47.25 %) (Hussain et al., 2017) and Iran (43.7 %) (Jalali et al., 2013), where the prevalence of *A. marginale* was notably higher. Moreover, our research represents the first molecular documentation of *A. marginale* in sheep based on *msp5* gene sequence analysis, suggesting that sheep may serve as potential reservoirs of this pathogen (Yousefi et al., 2017). It is noteworthy to mention that *A. marginale* infection has been previously molecularly described in cattle from Algeria (Rjeibi et al., 2018). *A. marginale* is primarily transmitted by ticks (Shoaib et al., 2021) and invades erythrocytes, leading to clinical signs that include depression, debility, reduced milk production, weight loss, abortion, and severe anemia and jaundice in endemic areas (Yousefi et al., 2017).

Anaplasma platys is an obligate parasite of platelets (Battilani et al., 2017). It has been for a long time considered a specific pathogen of dogs (Ben Said et al., 2018) but several studies described the infection in cats from Brazil (Lima et al., 2010; Li et al., 2015b) which indicated that the source of infection was probably accidental from dog and no significant clinical and hematological changes associated to *A. platys* infection are been observed (Lima et al., 2010). Infections with this bacterium have also been detected in goat from Cyprus (Chochlakis et al., 2009), cattle in Nigeria (Lorusso et al., 2016b) and camels in China (Li et al., 2015b) and Sub Saharian Africa (Lorusso et al., 2016a) which revealed that these animals might be a naturel host for this pathogen (Li et al., 2015b). DNA from *A. platys* was also detected in wildlife like red fox in Portugal (Cardoso et al., 2015) and red deer in China (Li et al., 2016). Foxes may play a role in the epidemiology of infection with this bacterium and serve as a reservoir for domestic dogs (Cardoso et al., 2015). In addition, *A. platys* infections were reported in two women from Venezuela (Arraga-Alvarado et al., 2014) and in a veterinarian (Maggi et al., 2013). These data indicate that have a broad host range. However, the ability of *A. platys* to act as a zoonotic pathogen has not been established (Li et al., 2015b). Our findings showed that *A. platys* was identified in sheep (0.2 %, 1/417). This discovery was probably related by the proximity of these animals with dogs. Otherwise, our result is similar to previous reports from Senegal, which was one case of *A. platys* (1/120, 0.83 %) (Djiba et al., 2013). To our knowledge, this is the first report describing the presence of *A. platys* in sheep based on *rpoB* gene sequence analysis from Algeria. The identification of this primarily canine pathogen in the blood of sheep expands the known pool of hosts (Djiba et al., 2013).

The statistical analysis of this study did not show any significant relation between infection rates for *A. ovis and A. platys* and variables of season, tick infestation, age and sex ($p > 0.05$). Infections of *A. ovis* were also found to be the same for age and sex, contrary to finding in Tunisia in which *A. ovis* prevalence was higher in adults (>2 years) than in young (≤ 2 years). Moreover, it was significantly higher in ewes than rams (Ben Said et al., 2015a).

Indeed, analysis of epidemiological data revealed that the age analyzed during this survey was associated with the *A. marginale* infection in sheep. It was also reported that lambs were more affected than adult, contrary to finding by Tanveer et al. (2022) and Yousfi et al. (2017) which indicated that none relation between *A. marginale* infection and age. The high prevalence rates of *A. marginale* in lambs were probably due to their immature immune system which made them more susceptible than adult sheep to infectious diseases (Watson et al., 1994). Furthermore, statistical analysis revealed that the infections of *A. marginale* were similar for season, sex and tick infestation. This latter was in difference with those reported with Hussain et al. (2017) which indicated that prevalence of tick infestation was higher.

Phylogenetic trees performed by analysis of partial sequences obtained from *msp5* and *rpoB* genes confirmed the presence of *Anaplasma ovis, A. marginale* and *A. platys* species in sheep belonging to the different flocks sampled. In the *msp5* phylogenetic tree, up to 31 samples were

Table 3
Results of molecular identification of *Anaplasma* spp. in 53 samples.

Farm	A.Num	GenBank		Phylogenetic tree		Consensus
		Maximum identity of *rpoB* gene (Accession number)	Maximum identity of *msp5* gene (Accession number)	*rpoB*	*msp5*	
F1	2	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	11	*A. ovis* /*A. marginale*	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F4	1	*A. ovis* 99.20 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
	3	NA	*A. marginale* 99.75 % (MK715439.1)	–	*A. marginale*	*A. marginale*
	6	NA	*A. marginale* 100 % (MK715439.1)	–	*A. marginale*	*A. marginale*
F5	6	*A. ovis* 100 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	7	*A. ovis* 100 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F6	4	*A. ovis* 99.79 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
	7	*A. ovis* 99.61 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	10	NA	*A. ovis* /*A. marginale*	–	*A. ovis*	*A. ovis*
F9	7	NA	*A. ovis* 99.75 % (CP015994.2)	–	*A. ovis*	*A. ovis*
	9	NA	*A. marginale* 100 % (MK715439.1)	–	*A. marginale*	*A. marginale*
F10	6	NA	*A. marginale* 100 % (MK715439.1)	–	*A. marginale*	*A. marginale*
F11	2	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	5	*A. ovis* 99.80 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F12	1	*A. ovis* 99.80 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	2	NA	*A. ovis* 100 % (CP015994.2)	–	*A. ovis*	*A. ovis*
	4	NA	*A. marginale* 100 % (KM624520.1)	–	*A. marginale*	*A. marginale*
F13	4	*A. marginale* 100 % (CP001079.1)	NA	*A. marginale*	–	*A. marginale*
	6	NA	*A. marginale* 100 % (KM624520.1)	–	*A. marginale*	*A. marginale*
F16	1	*A. platys* 93.35 % (KX155493.1)	NA	*A. platys*	–	*A. platys*
	7	*A. ovis* 99.43 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
F17	4	NA	*A. ovis* 99.51 % (CP015994.2)	–	*A. ovis*	*A. ovis*
	10	*A. ovis* /*A. marginale*	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F18	9	*A. ovis* /*A. marginale*	NA	*A. ovis*	–	*A. ovis*
F19	13	NA	*A. ovis* 99.26 % (CP015994.2)	–	*A. ovis*	*A. ovis*
F21	9	*A. ovis* 99.24 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
F22	17	*A. ovis*/*A. marginale*	NA	*A. ovis*	–	*A. ovis*
	18	*A. ovis* 99.62 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
F23	7	NA	*A. ovis* /*A. marginale*	–	*A. ovis*	*A. ovis*
F24	17	*A. ovis* 99.61 % (CP015994.2)	*A. ovis* 99.51 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F25	1	*A. ovis* 99.62 % (CP015994.2)	*A. ovis* 99.75 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	6	*A. ovis* 99.62 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
F26	7	*A. ovis* 99.42 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
	10	*A. ovis* 99.62 % (CP015994.2)	*A. ovis* 99.51 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F28	4	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
	10	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* 100 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F29	15	*A. ovis* 99.41 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
	19	*A. ovis* 99.61 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
F30	5	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F31	5	NA	*A. ovis* /*A. marginale*	–	*A. ovis*	*A. ovis*
	6	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F32	3	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
	4	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* 99.51 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*
F33	1	*A. ovis* 99.80 % (CP015994.2)	NA	*A. ovis*	–	*A. ovis*
	4	*A. ovis* /*A. marginale*	NA	*A. ovis*	–	*A. ovis*
F34	15	*A. ovis* /*A. marginale*	NA	*A. ovis*	–	*A. ovis*
F36	2	*A. ovis* 99.81 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
	6	*A. ovis* 99.62 % (CP015994.2)	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F37	8	*A. ovis* /*A. marginale*	NA	*A. ovis*	*A. ovis*	*A. ovis*
	11	*A. ovis* /*A. marginale*	*A. ovis* /*A. marginale*	*A. ovis*	*A. ovis*	*A. ovis*
F38	12	NA	*A. ovis* /*A. marginale*	–	*A. ovis*	*A. ovis*
F40	19	*A. ovis* 99.62 % (CP015994.2)	*A. ovis* 99.75 % (CP015994.2)	*A. ovis*	*A. ovis*	*A. ovis*

A.Num: Animal Number, NA: Not Amplified.

grouped with the *A. ovis* cluster, while only 6 samples were grouped in the *A. marginale* cluster. On the other hand, in the *rpoB* phylogenetic tree, up to 37 samples were grouped with the *A. ovis* cluster, while only one sample was grouped in the *A. marginale* cluster and only one was grouped in the *A. platys* cluster. Samples from which sequences of both genes were obtained ($n = 24$) showed a consensus in both phylogenetic trees with *A. ovis* species (Figs. 2 and 3). These samples could be marked with a symbol on both trees. The sequences of both genes were not obtained from all the samples analyzed, so they could not be included in both analyses. These samples could only be identified to species level by analysis of one of the genes studied.

Additionally, this research uniquely highlights the absence of *A. phagocytophilum* in Algerian sheep, a finding of substantial significance given the zoonotic potential and impact of this bacterium. This absence not only contributes to our understanding of the local epidemiology but also underscores the importance of continued surveillance and management efforts to prevent zoonotic transmission.

The results obtained from the phylogenetic analysis of the partial sequences of the *msp5* and *rpoB* genes are in concordance with the results of the homologies obtained with the NCBI (National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) tool.

5. Conclusion

In conclusion, this study provides molecular evidence of the presence of *A. ovis, A. marginale*, and *A. platys* in sheep from semi-arid regions of Algeria, while no infections with *A. phagocytophilum* were observed.

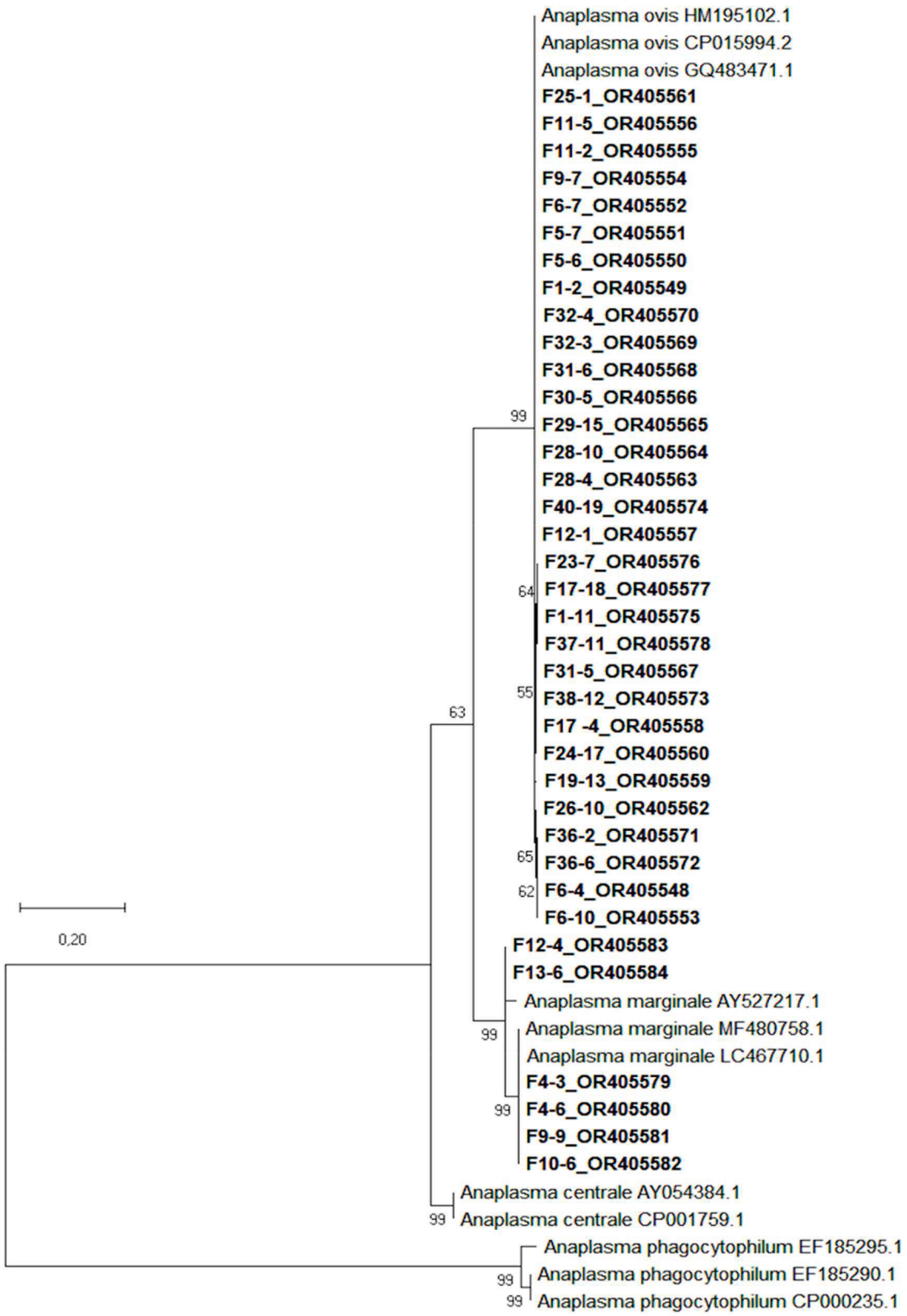

Fig. 2. Phylogenetic analysis for *msp5* (371 bp). The phylogenetic analysis was carried out using ClustalW to align 48 sequences of the *msp5* fragment, using different species as reference sequences (*Anaplasma ovis, A. marginale, A. centrale*, and *A. phagocytophilum*) available for this fragment and obtained from GenBank. Based on Corrected Akaike Information Criterion (cAIC) and Bayesian Information Criterion (BIC) implemented in Molecular Evolutionary Genetics Analysis (MEGA X) (Kumar et al., 2018) the best-fit model of the evolution sequences was selected. The phylogenetic tree was generated by using MEGA version 10 (http://www.megasoftware.net) with the Maximum Likelihood method with Kimura 2-parameter model (Kimura, 1980). Bootstrap confidence limits were calculated on the basis of 1000 replicates; the numbers on branches in the tree indicate bootstrap results.

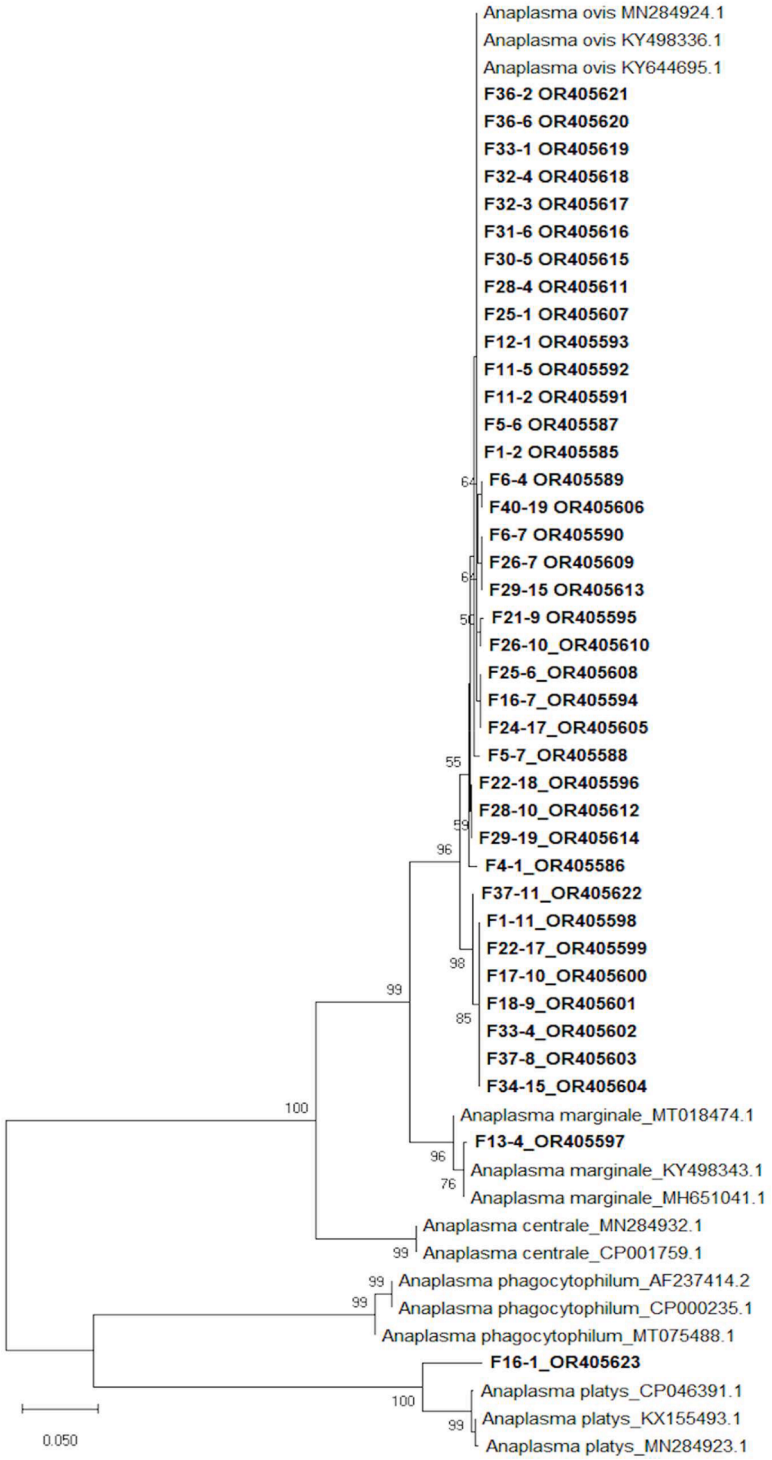

Fig. 3. Phylogenetic analysis for *rpoB* (465 bp). The phylogenetic analysis was carried out using ClustalW to align 53 sequences of the *rpoB* fragment, using different species as reference sequences (*Anaplasma ovis, A. marginale, A. centrale, A. phagocytophilum* and *A. platys*) available for this fragment from GenBank. Based on Corrected Akaike Information Criterion (cAIC) and Bayesian Information Criterion (BIC) implemented in Molecular Evolutionary Genetics Analysis (MEGA X) (Kumar et al., 2018) the best-fit model of the evolution sequences was selected. The phylogenetic tree was generated by using MEGA version 10 (http://www.megasoftware.net) with the Maximum Likelihood method with Kimura 2-parameter model (Kimura, 1980). Bootstrap confidence limits were calculated on the basis of 1000 replicates; the numbers on branches in the tree indicate bootstrap results.

These findings suggest that sheep can serve as potential reservoirs for certain *Anaplasma* species and a potential source of infection for other farm animals. The identification of *A. marginale* and *A. platys* in Algerian sheep highlights the need for comprehensive surveillance and management efforts to prevent zoonotic transmission and mitigate the risk of vector-borne disease outbreaks in dryland farming areas. This research contributes to our understanding of sheep epidemiology in North Africa and emphasizes the importance of continued research and monitoring to develop effective strategies for disease control and animal health management in the region. Further investigations are required to elucidate the underlying epidemiological factors influencing variations in infection rates and to facilitate the development of targeted control measures.

Author statement

All authors (Hafidha Chadi, Alberto Moraga-Fernández, Marta Sánchez-Sánchez, Haroun Chenchouni, Isabel G. Fernández de Mera, Mutien-Marie Garigliany, José de la Fuente, Safia Tennah, Tahar Sedrati, Farida Ghalmi) have read and approved the final submitted version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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