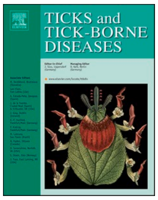
Ticks and Tick-borne Diseases 16 (2025) 102486

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis



Original article

Epidemiological study on the prevalence of *Anaplasma ovis* infection in Aragón, Spain

Héctor Ruiz[a,b], José María González[a,b,c], Marta Ruiz de Arcaute[a,b,*], Sergio Villanueva-Saz[a,b,*], Juan José Ramos[a,b], José Luis Arnal[d], Cristina Baselga[d], Pablo Quilez[a,b], Aurora Ortín[a,b], Delia Lacasta[a,b]

[a] *Department of Animal Pathology, Faculty of Veterinary Medicine, C/ Miguel Servet 177, 50013, Zaragoza, Spain*
[b] *Food Institute of Aragón-IA2 (Universidad de Zaragoza-Cita), C/ Miguel Servet 177, 50013, Zaragoza, Spain*
[c] *Gabinete Técnico Veterinario, SLU. G.V.de las Cortes Catalanas 932, 08014, Barcelona, Spain*
[d] *Exopol S.L, Pol. Río Gallego D-14, 50840, San Mateo de Gállego, Spain*

ARTICLE INFO

Keywords:
Anaplasma ovis
Climate
Epidemiology
Ovine anaplasmosis
Sheep
Spain
Tick-borne diseases

ABSTRACT

Ovine anaplasmosis is an emerging disease in Europe, primarily affecting the Mediterranean region. It is caused by *Anaplasma ovis*, a bacterium mainly transmitted by ticks. Despite its growing presence, comprehensive data on its distribution across Europe remain scarce. This epidemiological study aimed to assess the prevalence of *A. ovis* in sheep farms across Aragón, Spain, between March 2023 and April 2024. A total of 70 farms, covering a diverse range of climatic zones within Aragón, were sampled, representing 700 sheep. Two pooled blood samples, each comprising five sheep per farm, were tested using quantitative PCR (qPCR) to detect the presence of *A. ovis*. The results revealed a widespread presence of the bacterium, with 69 out of 70 farms testing positive. The only negative case was a farm located in a Steppe Dry climate area near Zaragoza, at approximately 240 m above sea level. Notably, no clinical signs of the disease were observed in any of the animals during the study period. In addition to the high prevalence, the study highlighted a concerning lack of awareness among farmers, with only 33 % reporting familiarity with the disease. These findings underscore the extensive distribution of *A. ovis* in Aragón and suggest that neither climatic conditions nor livestock management practices had a significant influence on transmission. The study also emphasizes the urgent need for enhanced control measures, increased farmer education, and further research into environmental and management factors that may contribute to outbreaks.

1. Introduction

Anaplasma ovis is the causative bacterium of ovine anaplasmosis, an emerging disease in Europe and a growing concern in the Mediterranean basin. In recent years, the disease has been reported in several European countries, especially those located in the Mediterranean region, such as France, Greece, Italy, Portugal, Turkey and Spain, but also in other northern countries, as Germany and Hungary (Hornok et al., 2007; Renneker et al., 2013; Stuen, 2016). The disease have also been diagnosed in several African and Asian countries, where it affects wide geographic areas and typically causes mild clinical signs (Dahmani et al., 2017; Aouadi et al., 2017; Song et al., 2018; Enkhtaivan et al., 2019; Said et al., 2022).

Bacteria of the *Anaplasma* genus belong to the order *Rickettsiales*, and comprise several species of obligate intracellular microorganisms (Rar et al., 2021). Currently, seven of these species have been described affecting ruminants: *Anaplasma marginale, Anaplasma bovis, Anaplasma centrale, Anaplasma caudatum, Anaplasma capra, Anaplasma ovis* and *Anaplasma phagocytophylum* (Dumler et al., 2001; Rar et al., 2021). Among these, only *A. ovis* and *A. phagocytophilum* are considered signigicant pathogens in sheep. *Anaplasma phagocytophilum* is the causative agent of tick-borne fever, while *A. ovis* is associated with ovine anaplasmosis (Stuen, 2016).

Anaplasma ovis is a non-motile, obligate intraerythrocytic, Gram-negative bacterium (Dumler et al., 2001; Rar et al., 2021). It is primarily transmitted by ticks of the Ixodidae family, particularly species within the genera *Dermacentor* and *Rhipicephalus*, which are considered biological vectors (Kocan et al., 2004). However, mechanical

---

[*] Corresponding authors at: Department of Animal Pathology, Faculty of Veterinary Medicine, C/ Miguel Servet 177, 50013, Zaragoza, Spain.
E-mail addresses: martarda@unizar.es (M. Ruiz de Arcaute), svs@unizar.es (S. Villanueva-Saz).

https://doi.org/10.1016/j.ttbdis.2025.102486
Received 3 December 2024; Received in revised form 22 April 2025; Accepted 28 April 2025

transmission is also considered possible, involving arthropods such as fleas, tabanid flies, keds (*Melophagus ovinus*), as well as through iatrogenic means (Mason et al., 2017; Zhao et al., 2018).

The bacterium was first described in Spain in 2008, in wild roe deer (*Capreolus capreolus*) (de la Fuente et al., 2008). However, it was not until 2014 when the disease was diagnosed for the first time in this country, causing a severe outbreak in sheep flocks in a southern region of Aragón. Notably, these flocks had been experiencing unexplained health issues since 2010 (Jiménez et al., 2019). Since then, several cases have been diagnosed in both the regional and national territory. Ovine anaplasmosis is characterised by nonspecific clinical signs primarily associated with severe haemolytic anaemia. The most commonly observed symptoms include weakness, pale mucous membranes, and significant weight loss. In some outbreaks, fever, depression, and even death have also been reported (Yasini et al., 2012; Jiménez et al., 2019). Furthermore, in 2020, *A. ovis* was diagnosed in lambs, causing a new clinical presentation characterised by the condemnation of a significant percentage of jaundiced carcasses at the abattoir (Lacasta et al., 2020). Similar outbreaks were subsequently reported in lambs from various farms processed at multiple abattoirs in the following years (SESC, 2020; Lacasta et al., 2022).

However, in recent years, there has been growing interest in this pathogen due to the trend observed in the region. This is attributed to the increased incidence of yellow carcasses observed at slaughterhouses (Ruiz et al., 2025) following the initial association of this cause of condemnation with A. ovis infection (Lacasta et al., 2020). This has led to a growing interest among both producers and researchers regarding the underlying causes. Based on this, one suggested hypothesis is that the disease, considered emergent, has been spreading throughout the territory and sheep flocks due to the climatic variations of recent years, leading to this situation.This situation underscores the need for further research in the field to better understand and control this disease.

Diagnosis has been historically relied on the direct observation of blood smears (WOAH 2015). However, this method is known for its low sensitivity, subjectivity, time consuming nature, and susceptibility to inter-observer variability. To overcome these limitations, several studies have employed enzyme-linked immunosorbent assay (ELISA) tests targeting the major surface protein MSP5. Nevertheless, this protein does not allow for differentiation between multiple *Anaplasma* species, limiting its diagnostic specificity (Hornok et al., 2008). Currently, polymerase chain reaction (PCR) targeting the *msp4* gene is widely accepted as the most reliable and specific diagnostic method for detecting *A. ovis* (de la Fuente et al., 2002; Torina et al., 2012). In addition to its specificity, PCR is capable of identifying subclinical or asymptomatic infections, which are common in animals that remain persistently infected following initial exposure to *A. ovis* (Ruiz et al., 2024a). These features highlight the suitability and diagnostic value of PCR in both clinical and epidemiological studies.

Although *A. ovis* has been reported in several areas worldwide, relatively few epidemiological studies have been published about the distribution of *A. ovis* infection in the territories where the disease has been diagnosed. Some investigations have been conducted in North African countries of the Mediterranean basin, as well as in the Persian Gulf and parts of Asia (Gharbi et al., 2015; Said et al., 2018, 2022; Atif et al., 2023). However, only two epidemiological studies have been published to date on the European continent. The first study was conducted in Spain in 2014, shortly after the initial diagnosis of the disease, in the Matarraña region (926.06 km^2) in shouthern Aragón —the same area where the outbreak occurred (Lacasta et al., 2021). This study revealed a wide distribution of A. ovis across the studied territory, along with clinical cases of ovine anaplasmosis in multiple flocks. Subsequently, in 2016, another epidemiological study was carried out in a typical Mediterranean environment on the island of Corsica, France (8680 km^2). However, unlike the first study, this investigation focused on goat herds rather than sheep (Cabezas-Cruz et al., 2019).

Given the lack of comprehensive epidemiological studies on ovine anaplasmosis and the significant gaps in regional data, the primary objective of this study was to assess the distribution and presence of Anaplasma ovis across the territory of Aragón, a typical Mediterranean environment. This region, characterised by diverse bioclimatic zones and a strong tradition of sheep farming, has reported periodic cases of ovine anaplasmosis since its initial identification in 2014. By including flocks from all areas of Aragón, this study aimed to provide an updated, region-wide overview of the current status of *A. ovis* infection. Particular emphasis was placed on detecting the presence or absence of the pathogen in sheep flocks, as well as exploring potential environmental and management-related factors that may be associated with its distribution.

2. Materials and methods

The research was conducted in the Aragón region, a large territory in northeastern Spain covering 47,719.2 km^2 between March 2023 and April 2024.

Due to its latitudinal position, Aragón is considered to have a typical continentalised Mediterranean climate. This climate is mainly characterised by scarce precipitation, mainly occurring in spring and autumn, and a marked annual thermal rhythm, with warm summers and cold winters, as described in the Climatic Atlas of Aragón (Cuadrat et al., 2007). Moreover, the climatic characteristics are strongly influenced by the topographical configuration of Aragón. As a result, Aragón offers a varied mosaic of climatic situations, contrasting the warm and arid flatlands of the Ebro valley with the cold and humid areas of the Pyrenean Mountains. This climatic variability, largely shaped by elevation and relief, provides a unique setting for studying the environmental factors associated with *Anaplasma ovis* distribution.

2.1. Division of the territory according to climatic conditions

The division of the territory according to climatic conditions was obtained from the Climatic Atlas of Aragón using the criteria proposed by Köppen based on the annual and monthly average temperature and precipitation values (Cuadrat et al., 2007). Aragón was classified into seven distinct climate types, with each type represented by a specific colour on the map of Aragón (Fig. 1). These colours, identified by their "Hex codes", were selected following recommendations from the Coblis "Colour Blindness Simulator" (Wickline, 2001), ensuring that individuals with colour blindness are able to distinguish the territorial divisions. The total area and proportion occupied by each of the different climates are shown in Table 1.

The Steppe Dry Climate ("Strong pink"; #d64161) is primarily confined to the axis of the Ebro River. Annual precipitation is minimal, averaging around 300 mm per year, with marked interannual variability, peaking during spring and autumn. Temperatures are extreme in both summer and winter, with an absolute thermal amplitude exceeding 40 °C.

Adjacent to this area is the Continental Mediterranean Climate ("Yellow Green"; #f0e442). This climate occupies areas of higher average altitude within the Ebro Valley and the northern and southern foothills of the region. Although it exhibits significant thermal contrasts, precipitation remains scarce but somewhat higher than in the steppe, ranging from 400 to 600 mm annually, with a peak in autumn.

The Cold Continental Sub-Mediterranean Climate ("Olive Green"; #7f7f00), is predominantly found in the plateau regions of the southern territory, situated at approximately 1000 m above sea level. It is characterised by significant thermal amplitude, with warm summers and very cold winters. This zone also experiences low annual precipitation, averaging around 400–500 mm.

Near this area, there is also another climate with very similar characteristics. This climate, known as the **Warm Continental Sub-Mediterranean Climate ("Purple; #5e3c99),** is influenced by the Mediterranean Sea, which notably reduces the annual thermal amplitude while increasing the average summer temperature. Annual

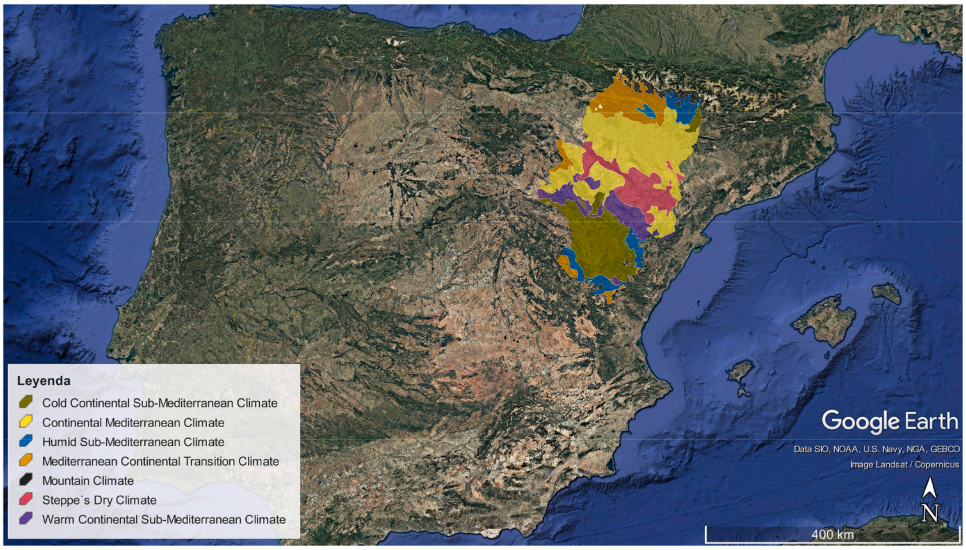


Fig. 1. View of Spain from Google Earth Pro at an altitude of 1200 km, with the territory of Aragón shaded according to the different climate zones, as indicated in the legend in the bottom left-hand corner of the figure.

Table 1
Total area (km^2) and proportion (%) occupied by each of the different climate types in the territory of Aragón, alongside the number of flocks sampled in each climate.

Type of Climate	Area (km^2)	Area (%)	Flocks sampled
Steppés Dry	5488.19	11.50	12
Continental Mediterranean	17,339.80	36.34	23
Cold Continental Sub-Mediterranean	9168.67	19.21	11
Warm Continental Sub-Mediterranean	4387.88	9.20	7
Mediterranean Continental Transition	6046.98	12.67	10
Humid Sub-Mediterranean	3741.74	7.84	7
Mountain	1545.94	3.24	0
	47,719.2	100	70

precipitation in this climate is similarly low, ranging from 400 to 450 mm, with two distinct peaks: one at the end of spring and another in autumn.

The transitional zones between the mountainous areas and the Ebro Valley, particularly to the north, exhibit two distinct climates, primarily characterized by the higher annual precipitation in these regions. The Mediterranean-Continental Transition Climate (“Golden Yellow, #e69f00) is mainly located in the northwestern areas, including the pre-Pyrenean and Pyrenean mountains. In these regions, annual precipitation is abundant, ranging from 800 to 1200 mm, with mild summer temperatures and cold winters.

The remaining territory, situated in the northeastern and southern areas, is classified as a Humid Sub-Mediterranean Climate (“Vivid Blue”¸ #0072b2), again clearly influenced by the Mediterranean Sea. This influence results in increased annual precipitation, exceeding 900 mm, with two notable peaks occurring in early spring and late summer. Temperatures in this zone are mild in summer and moderately cold in winter.

Additionally, there are areas above 1500 m above sea level, characterised by the presence of a Mountain Climate (“Charcoal”; #222222). This climate features very abundant precipitation and an average annual temperature below 5 °C. However, there are no permanent human or animal settlements in these areas, which are primarily used during the summer by grazing flocks.

2.2. Selection of flocks

Sheep farms participating in the present survey were selected on a convenient basis based on geographical location, climate, and census. The selection of flocks to be included in the study considered the Agrarian District organisation of Aragón, which divides the territory into 21 agrarian areas. Additionally, livestock distribution data for the year 2022, provided by the Government of Aragón were considered (Gobierno de Aragón, 2023). This data includes the overall census of adult sheep and fattening places, as well as the number of sheep flocks. The area occupied by each Agrarian District was also noted after being facilitated by the Delegation of Agrarian Districts of Aragón (Comarcalización de Aragón, 2022). Finally, the different types and number of climates present in each Agrarian District were considered, ensuring an adequate selection of flocks in each area. Setting a maximum number of 70 flocks to be sampled across the entire territory, the number of flocks to be sampled in each of the 21 divided areas divided estimated according to overall number of sheep flocks into them (Table 2).

Once the number of flocks was estimated, their locations were selected based on the climates of each Agrarian District. A simple stratified sampling was conducted based on the climatic areas of each veterinary zone. The selection of herds was made according to their position on the list for each veterinary area, and, following this selection, the herds were contacted to request their consent, which all agreed to provide. Finally, 70 flocks were sampled across the entire territory, representing all climatic areas, as indicated above.

2.3. Sampling and animal selection

To determine the presence of *Anaplasma ovis* infection in sheep flocks, whole blood samples were collected in EDTA tubes from 10 randomly selected ewes over 2.5 years old. The only selection criterion was that animals had to be female. This sampling strategy enables the detection of *A. ovis* with a 95 % confidence interval, assuming intra-herd prevalences lower than 37 % (Barandika et al., 2013). Prior to sampling, the health of the sheep was assessed. Blood samples were obtained by puncturing the jugular vein using a vacutainer system with BD Vacutainer® PrecisionGlide™ 18 G x 1″ (1.2 × 25 mm) needles and VACUTEST® Kima 4 ml K3 EDTA tubes (7.2 mg). Subsequently, the samples were duplicated and stored individually at −20 °C in 1.5 ml Eppendorf tubes until molecular tests were conducted. In total, whole blood samples were collected from 700 adult ewes belonging to the 70 sampled flocks.

Table 2
Number of flocks, sheep census, area, and climate types in each of the 21 Agrarian Districts into which the territory was divided. The final column indicates the number of flocks selected for sampling.

Agrarian District Organization of Aragón	N° Flocks (%)	Census (%)	Area in km2 (%)	N° climates	N° flocks to be sampled
Cinco Villas	112 (4.64 %)	113,918 (9.53 %)	3062.5 (6.42 %)	3	**7**
Comarca Central – Ribera Alta – Ribera Baja-Belchite	180 (7.45 %)	110,697 (9.26 %)	4738.5 (9.93 %)	3	**6**
Ribagorza	151 (6.25 %)	77,445 (6.48 %)	2459.8 (5.15 %)	3	**5**
Sierra Montalbán – Andorra - Bajo Martín	143 (5.92 %)	58,380 (4.88 %)	2877.9 (6.03 %)	3	**5**
Jacetania – Alto Gállego	170 (7.04 %)	73,870 (6.18 %)	3217.7 (6.74 %)	3	**4**
Hoya de Huesca	110 (4.55 %)	51,355 (4.30 %)	2525.6 (5.29 %)	2	**4**
Campo Borja – Comarca Aranda – Tarazona	49 (2.03 %)	32,079 (2.68 %)	1703.9 (3.57 %)	2	**4**
Jiloca	153 (6.33 %)	83,403 (6.98 %)	1932.1 (4.05 %)	2	**4**
Bajo Aragón – Matarraña	229 (9.48 %)	108,628 (9.09 %)	2237.2 (4.69 %)	3	**4**
La Litera – Cinca Medio	49 (2.03 %)	18,039 (1.51 %)	1310.6 (2.75 %)	2	**3**
Monegros	57 (2.36 %)	37,630 (3.15 %)	2764.4 (5.79 %)	2	**3**
Calatayud	97 (4.01 %)	53,438 (4.47 %)	2518.1 (5.28 %)	2	**3**
Sierra Albarracín	106 (4.39 %)	52,452 (4.39 %)	1329.1 (2.79 %)	2	**3**
Sobrarbe	138 (5.71 %)	42,159 (3.53 %)	2202.7 (4.62 %)	1	**2**
Bajo Cinca	46 (1.90 %)	21,813 (1.82 %)	1419.6 (2.97 %)	2	**2**
Bajo Aragón – Caspe	79 (3.27 %)	32,553 (2.72 %)	997.3 (2.09 %)	2	**2**
Campo Daroca	36 (1.49 %)	20,652 (1.73 %)	1117.9 (2.34 %)	2	**2**
Valdejalón – Cariñena	86 (3.56 %)	51,201 (4.28 %)	1705.3 (3.57 %)	2	**2**
Hoya de Teruel	213 (8.82 %)	87,213 (7.29 %)	2876.5 (6.03 %)	2	**2**
Maestrazgo – Gúdar/ Javalambre	149 (6.17 %)	43,203 (3.61 %)	3555.9 (7.45 %)	2	**2**
Somontano	63 (2.61 %)	25,492 (2.13 %)	1166.6 (2.44 %)	1	**1**
	2416	**1195,620**	**47,719.2**		**70**

2.4. Molecular analysis

Prior to molecular testing, blood samples were thawed and subsequently pooled with each pool comprising five randomly selected animals from the same farm. Two pooled samples were prepared per farm for the detection of *A. ovis* using quantitative PCR (qPCR). In the single farm that initially tested negative, individual PCR analyses were conducted on all ten sampled animals to confirm the absence of infection.

Nucleic acid extraction prior to qPCR testing was performed using the commercial kit MagMAX Core™ (Thermo Fisher Scientific, Austin, TX, USA) with an automated magnetic particle processor (KingFisher Flex System, Thermo Fisher Scientific, Vantaa, Finland), according to the manufacturer's instructions. Amplification was carried out on a QuantStudio 5 Real-Time PCR machine (Applied Biosystems, Marsiling, Singapore), with results analysed using the corresponding software (QuantStudio Design & Analysis software v1.5.1).

Anaplasma ovis was analysed using the commercial kit EXOone *Anaplasma ovis* (EXOPOL S.L., San Mateo de Gállego, Spain), following the manufacturer's instructions. This qPCR assay has an analytical sensitivity of 50 genomic equivalents per reaction and includes a quantified synthetic positive control. The assay targets the single-copy msp4 gene, which has been described by Torina et al. (2012) as an appropriate marker for differentiating A. ovis from the closely related A. marginale. An internal control was also included to prevent false-negative results. Bacterial load was expressed in terms of the quantification cycle (Cq), the cycle number at which the PCR amplification curve intersects the threshold line (Bustin et al., 2009). All Cq values below 38 were considered positive results.

2.5. Epidemiological questionnaire

Immediately following sample collection, each participating farmer completed an epidemiological questionnaire. The survey gathered information on various aspects of flock management and health, including breed, flock size and its evolution in recent years, geographical location, reproductive management, grazing practices, feeding strategies, health plans, and diseases currently affecting the flock. Additional questions addressed the presence of vectors, the farmer's awareness of ovine anaplasmosis, and any observed clinical signs consistent with the disease.

2.6. Statistical analysis

Statistical comparisons could not be performed on the data obtained from the molecular tests or the epidemiological questionnaires due to the high positivity rate, which resulted in insufficient variability across the dataset. Although preliminary statistical analyses were attempted, the lack of variation precluded meaningful or robust comparisons. As a result, the data are presented using basic descriptive statistics, focusing on variables such as the breeds present on each farm, farmers' knowledge of the disease, flock size, and the type of production system employed.

3. Results

3.1. Anaplasma ovis detection by qPCR

Sixty-nine out of the 70 farms analysed in this study tested positive for Anaplasma ovis in both pools (69/70: 98.57 %) (95 % CI: 92.47 %–99.92 % for the total population of 2416 farms). The Cq values for A. ovis in the pools ranged from 25 to 38. The only negative farm was located near Zaragoza, within a Steppe Dry Climate (Fig. 2).

This negative farm comprised a flock of 44 sheep belonging to an

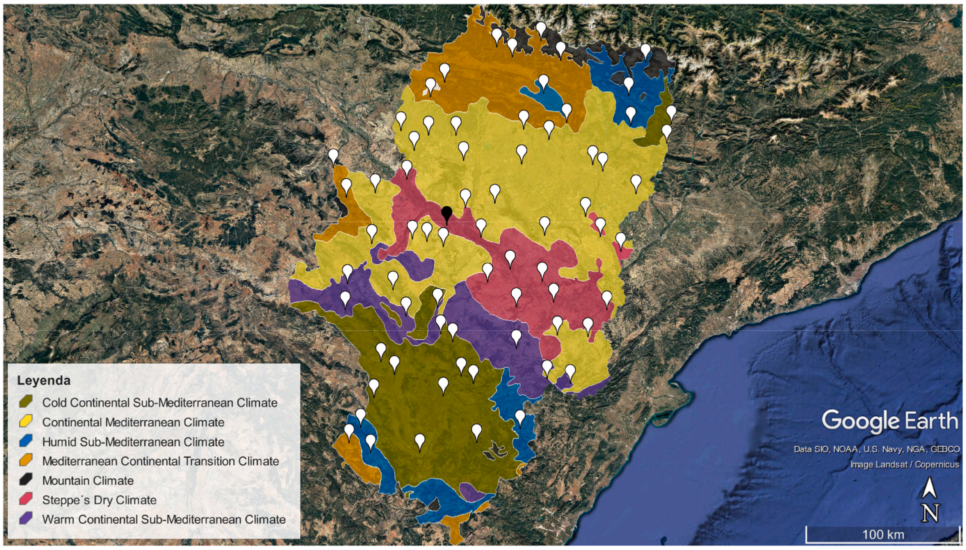


Fig. 2. View of Spain from Google Earth Pro at an altitude of 520 km, showing the territory of Aragón shaded according to the different climate zones, as indicated in the legend in the bottom left-hand corner of the figure. The white markers represent the locations of sheep herds testing positive for A. ovis (69) and its distribution across the studied territory, while the black markers indicate the locations of herds testing negative for A. ovis (1).

educational institution dedicated to environmental education for children. The animals grazed exclusively in the same fenced fields, which were utilized solely by various species on the farm (bovine, horses, donkeys, and goats) under a rotational grazing system.

3.2. *Epidemiological survey*

Herd sizes among the sampled farms varied considerably, ranging from 44 to 10,000 sheep (median: 665; average: 1044). All 70 farms followed production systems typical of the Aragón region, primarily based on pasture utilization. Of these, 63 operated under semi-intensive systems (63/70: 90 %), where animals are kept indoors for certain periods, while only 7 were managed extensively (7/70: 10 %), housing animals only a few days post-lambing. None of the analysed farms operated under intensive systems with animals kept indoors at all times.

Several breeds were represented in the study, with the Rasa Aragonesa being the most predominant (33/70: 47 %). Other included breeds, mostly indigenous, were Ojinegra (4/70: 6 %), Roya Bilbilitana (3/70: 4 %), Ripollesa (3/70: 4 %), Xisqueta (3/70: 4 %), Merina (3/70: 4 %), Ansotana (2/70: 3 %), and INRA (1/70: 1 %). The remaining 18 flocks were classified as mixed breeds, not registered as any particular breed, comprising animals of different breeds within the herd (18/70: 26 %).

In most of the sample flocks, goats were present and grazed alongside the sheep (50/70: 71 %), while the remaining farms (20/70: 29 %) reported no co-grazing with other sprecies. Grazing areas varied widely and included forests, dry and irrigated farmland, as well as production orchards such as olive, almond, and fruit trees. Additionally, many flocks made use of scrubland and mountain pastures, reflecting the diverse land use practices in the region.

Regarding ectoparasite presence, 38 farmers (54 %: 38/70) reported no notable increase in parasites in recent years. Conversely, 31 farmers (44 %: 31/70) did observe an increase in vectors, primarily noting a rise in ticks from the Ixodidae family during spring and black flies (*Simulium* spp.) during the summer, especially in flocks grazing near the Ebro River. Only one farmer (1 %: 1/70), located in the western part of Zaragoza province, reported a decrease in vectors, specifically ticks, in recent years.

Nonetheless, only 23 farmers reported being aware of ovine anaplasmosis (23/70: 33 %). The primary source of their awareness (14/23: 61 %) was information provided by their veterinarian during a visit, while in 6 sampled flocks, previous outbreaks of ovine anaplasmosis had been diagnosed in adult sheep or lambs (6/23: 26 %). These data shows that of the total farms analysed, 9 % (6/70) had previously been diagnosed with the disease, either due to clinical signs in the adult flock or due to problems observed in the carcasses after the lambs were slaughtered. Additionally, 3 farmers learned about the disease from discussions with other farmers (3/23: 13 %). The remaining 47 farmers were unaware of the disease prior to the sampling visit (47/70: 67 %).

Only 5 farms reported an increase in the carcass condemnation rate due to jaundice at the abattoir (5/70: 7 %). However, only two of these farms were aware of the disease, while the other three were uninformed about the cause and were only notified of the issue.

4. Discussion

The results of this study indicate a widespread distribution of *A. ovis* in areas with a typically continental Mediterranean climate. High herd-level prevalences has also been reported in similar studies, supporting the extensive presence of the pathogen observed here. In Corsica (France), *A. ovis* was detected also by PCR in 86.37 % of apparently healthy goat herds (Cabezas-Cruz, 2019). Similarly, studies conducted in parts of Asia, such as the Persian (Noaman and Sazmand, 2022) ans southern Kazakhstan (Ostrovskii et al., 2025), have reported infections rates nearing 90 % in ovine herds. These findings are consistent with the broad territorial and herd-level distribution observed in the present study. Other serological surveys have also revealed high herd-level prevalence, such as in Jordan and Hungary, where >94 % of herds were seropositive for *A. ovis* (Obaidat and Salman, 2019; Hornok et al., 2007). Prior to 2014, little was known about the distribution of *A. ovis* in Aragón. The first outbreak was reported in the Matarraña region, in southern Aragón, and was followed by a localized epidemiological study that found 91.1 % of farms tested positive by PCR (Lacasta et al., 2021). These findings closely align with the present study, which identified *A. ovis* in 98.57 % of farms sampled across the broader Aragón region.

Several authors have reported that A. ovis infection typically manifests as a mild or even subclinical condition under normal circumstances (Stuen, 2016; Cabezas-Cruz et al., 2019). This clinical sublety, combined with diagnostic challenges and limited awareness among farmers and veterinarians, may have contributed to the silent and undetected spread of the infection across flocks. The nonspecific nature of clinical signs further complicates diagnosis, often leading to misattribution of symptoms to other diseases. Moreover, Spain's climatic conditions provide an ideal environment for the establishment and proliferation of potential biological vectors of *A. ovis*, such as *Rhipicephalus sanguineus* (Balmori-de la Puente et al., 2024). Climate change, influenced by human activity, has also been shown to impact tick distribution and abundance, expanding their geographic range and seasonality (Dantas-Torres, 2015). In Aragón, the traditional management of sheep often involving extensive or semi-extensive grazing systems may further increase exposure to tick vectors. This combination of ecological, climatic, and management factors likely plays a key role in facilitating transmission and promoting the widespread distribution of *A. ovis* observed in this study.

Notably, in both studies, many farms where *A. ovis* was detected reported no clinical signs typically associated with the disease. This is a key observation, considering the widespread prevalence of the bacterium and the potential for severe outbreaks under certain conditions. In the 2021 study, differences between the northern and southern parts of the region were observed, leading to the hypothesis that *A. ovis* may have been more recently introduced into the northern areas. This possible introduction could be linked to more favourable climatic conditions for tick development in the north, as well as a greater presence of potential wild reservoirs. These factors may contribute to the establishment and spread of the pathogen in new areas, even in the absence of overt clinical disease.

As previously demonstrated in experimental studies (Yasini et al., 2012; Jiménez et al., 2019) and more recently confirmed by field data (Ruiz et al., 2024a), animals that survive the initial phase of *A. ovis* infection, often marked by notable anemia, can go on to harbor a persistent infection without exhibiting clinical signs. This chronic infection can persist throughout the animal's productive life, remaining asymptomatic under normal conditions (Ruiz et al., 2024a). However, variations in environmental conditions, livestock management practices, and regional climate may influence tick population dynamics and behavior. These factors can facilitate increased vector activity and, in turn, promote broader transmission of the pathogen across herds and regions.

Despite the widespread distribution of the bacterium across the Mediterranean Basin, clinical disease is rarely reported. As observed in this study, only 8.57 % of the farms had previously been diagnosed, either following the appearance of clinical signs in adult animals or due to pathological findings in slaughtered lambs. The underdiagnosis of the disease is likely related to the nonspecific nature of its clinical signs, which can complicate detection and often lead to misdiagnosis. In many cases, symptoms may be attributed to more common conditions included in the differential diagnosis for "thin ewe syndrome" or general ill-thrift within flocks (Busin, 2020). Additionally, certain authors suggest that severe clinical signs tend to manifest only when compounded by other conditions or diseases. These findings highlight the necessity for additional research to better understand the factors contributing to disease outbreaks, both in lambs and adult sheep. Stressors such as overcrowding, animal mixing, inadequate nutrition, and thermal stress have been linked to immunosuppression and the emergence of clinical signs (Yasini et al., 2012; Jiménez et al., 2019). However, further studies are required to confirm this hypothesis.

Recent studies using qPCR for the specific detection of A. ovis have consistenly demonstrated a high prevalence of infection in flocks across European countries, while clinical cases remain rarely reported (Hornok et al., 2007; Torina and Caracappa, 2012; Renneker et al., 2013; Cabezas-Cruz et al., 2019; Ruiz et al., 2024b). Similarly, the prior epidemiological study conducted in Spain following the 2014 outbreak provided data that challenge this trend. In that study, although 93.62 % (44/47) of the flocks tested positive for A. ovis, only 19 showed or had shown clinical signs of the disease (40.42 %: 19/47) (Lacasta et al., 2021). However, the higher proportion of clinically affected farms in that study is likely attributable to the concurrent outbreak in the region at the time of sampling. In contrast, the present study employed a blind, region-wide sampling strategy aimed at assessing the true prevalence of *A. ovis* across Aragón, independent of any known disease outbreak. This approach revealed a much lower proportion of farms reporting clinical signs (8.57 %), highlighting the largely subclinical nature of the infection under non-outbreak conditions.

Unlike the present study, Noamann and Sazmand (2022) reported significant differences in infection prevalences across Iran, depending on geographical area, climate type, and altitude. This is comparable to the differences observed during the 2014 outbreak in southern Aragón (Lacasta et al., 2021). Additionally, the Iranian study found higher prevalences in mountainous areas and in flocks with non-native breeds, factors that did not show relevance in our study. Similarly, a study in Sicily, Italy, found differences in the prevalence of *Anaplasma* species between eastern and western regions, which were attributed to the presence of different wild reservoir populations and habitat variability that favoured distinct tick species (Torina et al., 2009). In contrast, no such differences were observed in the present study, likely due to the widespread distribution of the disease in the analysed territory. The age-related risk factor commonly associated with anaplasmosis (Enkhtaivan et al., 2019; Atif et al., 2023) could not be evaluated in this study, as only adult animals were sampled.

No significant associations were found between climatic variables or questionnaire responses, primarily due to the lack of variability in the results caused by the high overall positivity rate. However, the single negative herd in the study displayed distinctive management characteristic that may be relevant for infection control. This herd, part of a school farm dedicated to environmental education, consisted of only 44 sheep and was closely monitored by its owners. The farm practiced rotational grazing within well-defined, fenced fields, effectively limiting contact with wild fauna and external animals. Rotational grazing has been proposed as a potential strategy for reducing tick infestations and related diseases (Walker, 2011). Furthermore, restricting the entry of wild animals—potential reservoirs or carriers of A. ovis and other pathogens (de la Fuente et al., 2008; García-Pérez et al., 2016)—can serve as a protective biosecurity measure to protect livestock (Martin et al., 2011; Gortázar et al., 2015).

In addition, there has been increasing interest in the distribution and population dynamics of tick species across Europe, which could be relevant in the distribution of the disease. The European Centre for Disease Prevention and Control (ECDC) has conducted significant studies to provide updated data on the distribution of ticks, with particular attention to Spain. Specifically in Aragon, the latest update of the ECDC of October 2023 reports the presence of permanent populations of several ticks from the Ixodidae family, such as *Ixodes ricinus, Hyalomma marginatum, Rhipicephalus sanguineus,* and *Dermacentor reticulatus*. All of these species are considered potential vectors for *Anaplasma ovis* (ECDC, 2023). However, most of the studies in the Mediterranean Basin reported ticks of the genus *Rhipicephalus* such as *R. bursa* (Aouadi et al., 2017; Cabezas-Cruz et al., 2019), *R. sanguineus* sensu lato (Lacasta et al., 2020, 2022), and *R. turanicus* (Aouadi et al., 2017; Jiménez et al., 2019) involved in the outbreaks, being considered a tick specie widely distributed across southern Europe (EFSA, 2010; Defaye et al., 2022; Millán et al., 2024).

Although tick samples were not collected from the flocks surveyed in this study, official data indicate the presence of tick populations capable of acting as vectors for disease transmission, as illustrated in the ECDC maps (ECDC, 2023). Recent research has highlighted the significant role of R. sanguineus tick in the transmission of Anaplasma spp., identifying areas of potentially transmission high risk within the studied region of the Iberian Peninsula (Balmori-de la Puente et al., 2024). Nevertheless, more comprehensive studies are needed to better understand the distribution of different tick species and potential transmission risk areas in Spain and Europe. The presence of these tick populations in the study area, combined with the fact that infected animals remain carriers of the disease for life and can act as reservoirs (Ruiz et al., 2024a), may be key factors contributing to the widespred prevalence and distribution of the disease. These observations also support previous hypotheses suggesting the development of enzootic stability in regions where ticks are abundant year-round (Corona et al., 2005). However, the diagnosis of multiple outbreaks of ovine anaplasmosis in Aragón over the past decade including clinical cases in lambs, demonstrates that while the infection may often circulate silently, it retains the potential to cause clinical disease under favorable conditions (Lacasta et al., 2020, 2022). These findings may help explain the spread of the disease across the region since the first diagnoses were made a decade ago.

Finally, the findings of this study suggest the presence of a potential state of enzootic stability for ovine anaplasmosis, characterized by widespread pathogen circulation with minimal clinical disease expression. While such a state may appear clinically benign, it poses significant epidemiological challenges, as infected animals can serve as long-term carriers and reservoirs, sustaining transmission cycles through tick vectors. Notably, only 33 % of surveyed farmers reported familiarity with ovine anaplasmosis, revealing a critical gap in disease awareness and control readiness. Targeted educational initiatives and training programs for farmers and veterinarians are urgently needed to improve early detection, promote appropriate treatment, and encourage the adoption of preventive measures. Currently, no standardized vector control measures are implemented at the regional level. There is a pressing need for public health and agricultural authorities to develop integrated vector management strategies tailored to local tick ecology, including systematic tick sampling and mapping to identify high-risk transmission zones. Although climate type was not significantly associated with disease prevalence in this study, future research should model the potential impacts of climate change and land use alterations on tick dynamics and pathogen transmission, particularly in light of increased tick activity reported by nearly half of the surveyed farmers. Finally, the limited recognition of carcass condemnations associated with subclinical Anaplasma ovis infection has economic implications. Enhanced training of abattoir personnel and veterinarians in the identification of post-mortem signs could improve surveillance efforts and facilitate back-tracing of infection sources.

5. Conclusions

The study confirms the widespread presence of Anaplasma ovis in Aragón, with infection detected in 98.57 % of the farms studied, despite the absence of clinical signs. Notably, the only negative farm in the study implemented infection control measures such as rotational grazing and restricted interaction with wildlife, suggesting the potential effectiveness of these practices. The results underscore the need for further investigation into environmental and management factors. Additionally, increased farmer awareness is essential to improve diagnosis and control efforts. Further studies are needed to determine wheter the high prevalence in Aragón reflects broader trends across Spain and Europe.

Funding

The work was partially supported by the Aragón Government (A15_20R and A15_23R) and the European Social Fund.

Data protection and ethics

Ethical approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Zaragoza (Project Licence PI 06/21, 2021) for studies involving animals.

CRediT authorship contribution statement

Héctor Ruiz: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **José María González:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Marta Ruiz de Arcaute:** Writing – review & editing, Visualization, Investigation. **Sergio Villanueva-Saz:** Writing – review & editing, Supervision, Resources. **Juan José Ramos:** Writing – review & editing, Visualization. **José Luis Arnal:** Resources, Methodology. **Cristina Baselga:** Resources, Methodology. **Pablo Quilez:** Writing – review & editing, Resources, Investigation. **Aurora Ortín:** Writing – review & editing, Supervision, Project administration. **Delia Lacasta:** Writing – original draft, Supervision, Conceptualization.

Acknowledgements

We would like to express our gratitude to all the veterinarians and farmers who contributed in various ways to this study, as it would not have been possible without their support. Among them, we would like to highlight Julián Sancho, María José Blasco, Cristina Hernández, and Carlos Soriano from the COTEVE group; Luis Figueras and Calasanz Jiménez from VACSA SL; Marcos Pons, Lucas Grasa, and María Ferrer from Rumex Grupo Veterinario SL; Víctor Miguel, Isabel Cuartielles, Ana Marquina, Elena Servera, Maru Milian, and Javier Espada from the Cooperativa Oviaragón; and the AGABA Veterinary Centre group. We must also thank David Forcano, Ángel Artigor, Paula Artigot, Santiago Sanz, José Antonio Collado, Manolo Gutierrez, Víctor Ara, Santiago Santa Pau, Miguel Vila, and Enrique Navarro, who, despite their busy work schedules, took the time to assist me.

Likewise, we would like to thank the laboratory technician Maria Ángeles Lostao and the intern students from the Ruminant Clinical Service (SCRUM) of the Faculty of Veterinary Medicine at the University of Zaragoza, who helped us with sampling, examinations, and sample processing, especially Lucía Rodríguez, Marina Pomar, Janira Magreñán, and Gabriella Rapetti.

The authors also would like to thank Janine Elizabeth Davies for her help with the English revision.

Data availability

Data will be made available on request.

References

Aouadi, A., Leulmi, H., Boucheikhchoukh, M., Benakhla, A., Raoult, D., Parola, P., 2017. 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. Dis. 50, 34–39. https://doi.org/10.1016/j.cimid.2017.04.002.

Atif, F.A., Ullah, S., Cossío-Bayúgar, R., Kashif, M., Khan, A.U., Wu, W.F., 2023. Molecular epidemiology, seasonality, and phylogenetic investigations of *Anaplasma ovis* in small ruminants from diverse agro-climatic regions of Punjab, Pakistan. Microorganisms 11 (10), 2430. https://doi.org/10.3390/microorganisms11102430.

Balmori-de la Puente, A., Rodríguez-Escolar, I., Collado-Cuadrado, M., González-Mohino, E.I., Vieira-Lista, M.C., Hernández-Lambraño, R.E., Sánchez-Agudo, J.A., Morchón, R., 2024. Transmission risk of vector-borne bacterial diseases (*Anaplasma* spp. and *Ehrlichia canis*) in Spain and Portugal. BMC Vet. Res. 20, 526. https://doi.org/10.1186/s12917-024-04383-3.

Barandika, J.F., Oporto, B., Ros-García, A., Povedano, I., Hurtado, A., García-Pérez, A.L., 2013. *Anaplasma* spp. and Piroplasms Infection in a Latxa Breed Sheep Flock During Mountain Grazing Season. Sociedad Española de Ovinotecnia y Caprinotecnia (SEOC).

Busin, V., 2020. Recognising and dealing with illthrift in ewes. In Pract. 42 (9), 498–509. https://doi.org/10.1136/inp.m3881.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Wittwer, C. T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622. https://doi.org/10.1373/clinchem.2008.097740.

Cabezas-Cruz, A., Gallois, M., Fontugne, M., Allain, E., Denoual, M., Moutailler, S., Devillers, E., Zientara, S., Memmi, M., Chauvin, A., Agoulon, A., Vayssier-Taussat, M., Chartier, C., 2019. Epidemiology and genetic diversity of *Anaplasma ovis* in goats in Corsica, France. Parasit. Vect. 12, 1–11. https://doi.org/10.1186/s13071-019-3706-0.

Comarcalización de Aragón, 2022. https://www.comarcas.es/index.php/mod.documentos/mem.listado/relcategoria.1507/chk.9029ed77f167e8ed11d2f9e6a3fae2bc.html (accesed 2 December 2024).

Corona, B., Rodríguez, M., Martínez, S., 2005. Anaplasmosis bovina. Revista Electrónica de Veterinaria 6, 1–27.

Cuadrat, J.M., Saz, M.A., Vicente-Serrano, S.M., 2007. Atlas Climático de Aragón. Gobierno de Aragón.

Dahmani, M., Marié, J.L., Scandola, P., Brah, S., Davoust, B., Mediannikov, O., 2017. *Anaplasma ovis* infects sheep in Niger. Small Rumin. Res. 151, 32–35. https://doi.org/10.1016/j.smallrumres.2017.04.007.

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: the butterfly effect. Int. J. Parasitol.: Parasit. Wildl. 4 (3), 452–461. https://doi.org/10.1016/j.ijppaw.2015.07.001.

De la Fuente, J., García-García, J.C., Blouin, E., Saliki, J.T., Kocan, K.M., 2002. Infection of tick cells and bovine erythrocytes with one genotype of the intracellular *Anaplasma marginale* excludes infection with other genotypes. Clin. Diagn. Lab. Immunol. 9 (3), 658–668. https://doi.org/10.1128/CDLI.9.3.658-668.2002.

De la Fuente, J., Ruiz-Fons, F., Naranjo, V., Torina, A., Rodríguez, O., Gortázar, C., 2008. Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. Res. Vet. Sci. 84 (3), 382–386. https://doi.org/10.1016/j.rvsc.2007.09.009.

Defaye, B., Moutailler, S., Pasqualini, V., Quilichini, Y., 2022. Distribution of tick-borne pathogens in domestic animals and their ticks in the countries of the Mediterranean Basin between 2000 and 2021: a systematic review. Microorganisms 10, 1236. https://doi.org/10.3390/microorganisms10061236.

Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia*, and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and *HGE* agent as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51 (6), 2145–2165. https://doi.org/10.1099/00207713-51-6-2145.

EFSA Panel on Animal Health and Welfare (AHAW), 2010. Scientific Opinion on Geographic Distribution of Tick-borne Infections and their Vectors in Europe and the other Regions of the Mediterranean Basin. EFSA J. 8 (9), 1723. https://doi.org/10.2903/j.efsa.2010.1723.

Enkhtaivan, B., Narantsatsral, S., Davaasuren, B., Otgonsuren, D., Amgalanbaatar, T., Uuganbayar, E., Zoljargal, M., Myagmarsuren, P., Suganume, K., Molefe, N.I., Sivakumar, T., Inoue, N., Battur, B., Battsetseg, B., Yokoyama, N., 2019. Molecular detection of *Anaplasma ovis* in small ruminants and ixodid ticks from Mongolia. Parasitol. Int. 69, 47–53. https://doi.org/10.1016/j.parint.2018.09.007.

Gharbi, M., Omri, H., Jedidi, M., Zorii, S., Darghouth, M.A., 2015. Epidemiological study of sheep anaplasmosis (*Anaplasma ovis* infection) in Kairouan, Central Tunisia. J. Adv. Parasitol. 2 (2), 30–33.

García-Pérez, A.L., Oporto, B., Espí, A., Del Cerro, A., Barral, M., Povedano, I., Barandika, J.F., Hurtado, A., 2016. Anaplasmataceae in wild ungulates and carnivores in northern Spain. Tick. Tick Born. Dis. 7 (2), 264–269. https://doi.org/10.1016/j.ttbdis.2015.12.001.

Gobierno de Aragón, 2023. Distribución de Ganadería. https://www.aragon.es/-/distribucion-de-ganaderia (accesed 2 December 2024).

Gortázar, C., Diez-Delgado, I., Barasona, J.A., Vicente, J., De La Fuente, J., Boadella, M., 2015. The wild side of disease control at the wildlife-livestock-human interface: a review. Front. Vet. Sci. 1, 27. https://doi.org/10.3389/fvets.2015.00027.

Hornok, S., Elek, V., De La Fuente, J., Naranjo, V., Farkas, R., Majoros, G., Földvári, G., 2007. First serological and molecular evidence on the endemicity of *Anaplasma ovis* and *A. marginale* in Hungary. Vet. Microbol. 122 (3–4), 316–322. https://doi.org/10.1016/j.vetmic.2007.02.005.

Hornok, S., Földvári, G., Elek, V., Naranjo, V., Farkas, R., De la Fuente, J., 2008. Molecular identification of *Anaplasma marginale* and rickettsial endosymbionts in blood-sucking flies (Diptera: tabanidae, Muscidae) and hard ticks (Acari: ixodidae). Vet. Parasitol. 154 (3–4), 354–359. https://doi.org/10.1016/j.vetpar.2008.03.019.

Jiménez, C., Benito, A., Arnal, J.L., Ortín, A., Gómez, M., López, A., Villanueva-Saz, S., Lacasta, D., 2019. *Anaplasma ovis* in sheep: experimental infection, vertical transmission, and colostral immunity. Small Rum. Res. 178, 7–14. https://doi.org/10.1016/j.smallrumres.2019.03.014.

Kocan, K.M., De La Fuente, J., Blouin, E.F., Garcia-Garcia, J.C., 2004. *Anaplasma marginale* (Rickettsiales: anaplasmataceae): recent advances in defining host–pathogen adaptations of a tick-borne rickettsia. Parasitology 129 (S1), S285–S300. https://doi.org/10.1017/S003118200400626X.

Lacasta, D., Ferrer, L.M., Sanz, S., Labanda, R., González, J.M., Benito, Á., Ruiz, H., Rodríguez-Largo, A., Ramos, J.J., 2020. Anaplasmosis outbreak in lambs: first report causing carcass condemnation. Animals 10 (10), 1851. https://doi.org/10.3390/ani10101851.

Lacasta, D., Lorenzo, M., González, J.M., Ruiz de Arcaute, M., Benito, Á., Baselga, C., Milian, M.E., Lorenzo, N., Jiménez, C., Villanuev-Saz, S., Ferrer, L.M., 2021. Epidemiological study related to the first outbreak of ovine anaplasmosis in Spain. Animals 11 (7), 2036. https://doi.org/10.3390/ani11072036.

Lacasta, D., Ruiz, H., Ortín, A., Villanueva-Saz, S., Estrada-Peña, A., González, J.M., Ramos, J.J., Ferrer, L.M., Benito, A.Á., Labanda, R., Malo, C., Verde, M.T., Fernández, A., Ruiz de Arcaute, M., 2022. Comparative study of the use of doxycycline and oxytetracycline to treat anaplasmosis in fattening lambs. Animals 12 (17), 2279. https://doi.org/10.3390/ani12172279.

Martin, C., Pastoret, P.P., Brochier, B., Humblet, M.F., Saegerman, C., 2011. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. Vet. Res. 42, 1–16. https://doi.org/10.1186/1297-9716-42-83.

Mason, K.L., Gonzalez, M.V., Chung, C., Mousel, M.R., White, S.N., Taylor, J.B., Scoles, G.A., 2017. Validation of an improved *Anaplasma* antibody competitive ELISA for detection of *Anaplasma ovis* antibody in domestic sheep. J. Vet. Diagn. Invest. 29 (5), 763–766. https://doi.org/10.1177/1040638717715640.

Millán, J., Rodríguez-Pastor, R., Estrada-Peña, A., 2024. Description of *Rhipicephalus hibericus* sp. nov. (Ixodoidea: ixodidae), a species of the *Rhipicephalus sanguineus* group in southwestern Europe. Tick. Tick Borne Dis. 15 (4), 102340. https://doi.org/10.1016/j.ttbdis.2024.102340.

Noaman, V., Sazmand, A., 2022. *Anaplasma ovis* infection in sheep from Iran: molecular prevalence, associated risk factors, and spatial clustering. Trop. Anim. Health Prod. 54 (1), 6. https://doi.org/10.1007/s11250-021-02801-x.

Obaidat, M.M., Salman, A.E.B., 2019. *Anaplasma* spp. in dairy ruminants in Jordan: high individual and herd-level seroprevalence and association with abortions. J. Vet. Diagn. Invest. 31 (3), 481–484. https://doi.org/10.1177/1040638719843171.

Ostrovskii, A., Kadyrova, M., Yerzhanova, N., Kamalova, D., Kassen, A., Tursunbay, N., Shevtsov, A., Bauer, C., Mukanov, K., 2025. First study on molecular identification of *Anaplasma ovis* in sheep in southern Kazakhstan. Vet. World 18 (1). https://doi.org/10.14202/vetworld.2025.67-75.

Renneker, S., Abdo, J., Salih, D.E.A., Karagenç, T., Bilgiç, H., Torina, A., Oliva, A.G., Campos, J., Kullmann, B., Ahmed, J., Seitzer, U., 2013. Can *Anaplasma ovis* in small ruminants be neglected any longer? Transbound. Emerg. Dis. 60, 105–112. https://doi.org/10.1111/j.1865-1682.2012.01310.x.

Rar, V., Tkachev, S., Tikunova, N., 2021. Genetic diversity of *Anaplasma* bacteria: twenty years later. Infect. Genet. Evol. 91, 104833. https://doi.org/10.1016/j.meegid.2021.104833.

Ruiz, H., de Arcaute, M.R., Benito, Á., Villanueva-Saz, S., Jiménez, J.C., Lacasta, D., 2024a. Long-lasting infection with *Anaplasma ovis* in sheep. Vet. Res. Commun. 48 (1), 521–525. https://doi.org/10.1007/s11259-024-09935-3.

Ruiz, H., Lacasta, D., Villanueva-Saz, S., González, J.M., Ortín, A., Ramos, J.J., Benito, A.Á., Estrada-Peña, A., Fernández, A., Pomar, M., Ruiz de Arcaute, M., 2024b. Tick control prevents carcass condemnations in lambs caused by *Anaplasma ovis*. Vet. Res. Commun. https://doi.org/10.1007/s11259-024-10562-2.

Ruiz, H., González, J.M., Bello, J.M., Villanueva-Saz, S., de Arcaute, M.R., Ortín, A., Lacasta, D., 2025. Distribution of lamb carcass condemnations due to jaundice over six years in a slaughterhouse in the Northeast of Spain. Prev. Vet. Med. 238, 106473. https://doi.org/10.1016/j.prevetmed.2025.106473.

Said, M.B., Belkahia, H., Messadi, L., 2018. *Anaplasma* spp. in North Africa: a review on molecular epidemiology, associated risk factors, and genetic characteristics. Tick. Tick Born. Dis. 9 (3), 543–555. https://doi.org/10.1016/j.ttbdis.2018.01.001.

Said, M.B., Attia, K.A., Alsubki, R.A., Mohamed, A.A., Kimiko, I., Selim, A., 2022. Molecular epidemiological survey, genetic characterization, and phylogenetic analysis of *Anaplasma ovis* infecting sheep in Northern Egypt. Acta Trop. 229, 106370. https://doi.org/10.1016/j.actatropica.2021.106370.

Song, R., Wang, Q., Guo, F., Liu, X., Song, S., Chen, C., Tu, C., Wureli, H., Wang, Y., 2018. Detection of *Babesia* spp., *Theileria* spp., and *Anaplasma ovis* in border regions of northwestern China. Transbound. Emerg. Dis. 65 (6), 1537–1544. https://doi.org/10.1111/tbed.13065.

Stuen, S., 2016. Haemoparasites in small ruminants in European countries: challenges and clinical relevance. Small Rum. Res. 142, 22–27. https://doi.org/10.1016/j.smallrumres.2016.07.018.

Suport a Escorxadors (SESC),, 2020. Anaplasmosis en Canales de Cordero con Ictericia y Esplenomegalia. https://sesc.cat/es/anaplasmosis-en-canales-de-cordero-con-ictericia-y-esplenomegalia/. accesed 2 December 2024.

The European Centre for Disease Prevention and Control (ECDC), 2023. Tick maps. https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps (accesed 2 December 2024).

Torina, A., Alongi, A., Naranjo, V., Estrada-Peña, A., Vicente, J., Scimeca, S., Marino, A. M.F., Monteverde, V., Caracappa, S., De la Fuente, J., 2009. Prevalence of *Anaplasma* species and habitat suitability for ticks in Sicily. Clin. Microbiol. Infect. 15 (Suppl. 2), 57–58. https://doi.org/10.1111/j.1469-0691.2009.02943.x.

Torina, A., Agnone, A., Blanda, V., Alongi, A., D'Agostino, R., Caracappa, S., Marino, A., Di Marco, V., De la Fuente, J., 2012. Development and validation of two PCR tests for the detection of and differentiation between *Anaplasma ovis* and *Anaplasma marginale*. Tick. Tick Born. Dis. 3, 282–286. https://doi.org/10.1016/j.ttbdis.2012.04.001.

Walker, A.R., 2011. Eradication and control of livestock ticks: biological, economic, and social perspectives. Parasitology 138, 945–959. https://doi.org/10.1017/S0031182011000199.

Wickline, M., 2001. Coblis—Color Blindness Simulator. https://www.color-blindness.com (accesed 2 December 2024).

World Organisation for Animal Health, 2015. Bovine Anaplasmosis - WOAH - World Organisation for Animal Health. https://www.woah.org/en/disease/bovine-anaplasmosis/#:~:text=Bovine20anaplasmosis20is20a20rare20disease20typically20transmitted. accesed 2 December 2024.

Yasini, S.P., Khaki, Z., Rahbari, S., Kazemi, B., Amoli, J.S., Gharabaghi, A., Jalali, S.M., 2012. Hematologic and clinical aspects of experimental ovine anaplasmosis caused by *Anaplasma ovis* in Iran. Iran. J. Parasitol. 7 (4), 91.

Zhao, L., He, B., Li, K.R., Li, F., Zhang, L.Y., Li, X.Q., Liu, Y.H., 2018. First report of *Anaplasma ovis* in pupal and adult *Melophagus ovinus* (sheep ked) collected in South Xinjiang, China. Parasit. Vect. 11, 1–6. https://doi.org/10.1186/s13071-018-2841-2.