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Paper

Detection and Genotyping of Chlamydia abortus by qPCR in Aborted Dairy Cows in Algeria: Epidemiological Insights and Risk Factor Assessment

Roufaida Fares[1], Omar Salhi[2], Safia Yousfi[3], Nadjet Amina Khelifi Touhami[2*], Nassim Ouchene[2]

[1]*Departement of Biology, Faculty of Natural and Life Sciences, University of Blida 1, Algeria - DZ*
[2]*Institute of Veterinary Sciences, University of Blida 1, BP 270, Street Soumaa, 09000, Blida, Algeria - DZ*
[3]*Department of Biology, Faculty of Biological and Agronomical Sciences, Mouloud Mammeri University of Tizi Ouzou, Tizi Ouzou 15000, Algeria; Laboratory of Ecology and Biology of Terrestrial Ecosystems (LEBET), Faculty of Biological and Agronomical - DZ*

[*]*Corresponding author at: Institute of Veterinary Sciences, University of Blida 1, BP 270, Street Soumaa, 09000, Blida, Algeria - DZ*
E-mail: khelifi_nadjet@univ-blida.dz

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Abstract

Chlamydiaceae are Gram-negative obligate intracellular bacteria responsible for reproductive disorders in ruminants, notably *Chlamydia abortus*, the aetiological agent of enzootic abortion. Despite its veterinary importance, chlamydiosis remains poorly documented in Algeria. This study aimed to detect and genotype *Chlamydia* spp. in placental tissues from aborted and normally calving dairy cows using quantitative PCR (qPCR), and to identify associated risk factors. A total of 98 placental samples (84 aborted, 14 normally calving) were collected from four Algerian provinces between 2022 and 2024. qPCR targeting the 23S rRNA gene revealed 11 positive samples (11.22%), all from aborted cows. Sequencing of amplified fragments showed 97.22% similarity to *Chlamydia abortus* strain NL2335-4C, confirming its presence in Algerian herds. Statistical analysis identified the occurrence of abortion, the spring season, poor hygiene, and tick infestation as significant risk factors for *Chlamydia* positivity ($p < 0.05$). These findings demonstrate the active circulation of *C. abortus* in Algerian dairy cattle and underline the importance of improving herd hygiene, biosecurity, and tick control to reduce infection pressure. Further molecular surveillance and sero-epidemiological studies are warranted to assess better the zoonotic potential of chlamydial infections in Algeria.

Keywords

Chlamydia abortus, dairy cows, qPCR, abortion, risk factors, Algeria

Introduction

The Chlamydiaceae family comprises Gram-negative, obligate intracellular bacterial pathogens belonging to the single reclassified genus *Chlamydia*. These microorganisms are globally distributed and responsible for a wide range of infections affecting both animals and humans (Ball et al., 2015; Cheong et al., 2019). The family currently includes nine recognised species, several of which are pathogenic to livestock. In cattle, reproductive disorders are mainly associated with *Chlamydia abortus*, although *Chlamydia pecorum* and *Chlamydia psittaci* have also occasionally been implicated (Barati et al., 2017; Caspe and Hill, 2024). *Chlamydia* spp. infections are also common in small ruminants, where *C. abortus* is well known to cause enzootic abortion; however, in cattle, infections are often endemic and mostly subclinical, and the epidemiology of chlamydial abortion remains poorly understood (Walker et al., 2015; Struthers et al., 2021). Transmission occurs primarily via the oral route, with the respiratory and venereal routes playing lesser roles. Major sources of infection include aborted foetuses, placental tissues, uterine or vaginal discharges, faeces, and milk from infected animals (Wheelhouse and Longbottom, 2015; Kayesh et al., 2024). Although bacterial shedding is usually intermittent and limited in quantity, *Chlamydia* spp. are highly resistant in the external environment, and contaminated facilities can thus act as reservoirs of infection (Wheelhouse and Longbottom,

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2015; Kayesh et al., 2024). Host susceptibility appears to increase during the last third of gestation, and infection in non-pregnant females may occasionally cause abortion in subsequent pregnancies. Despite its veterinary relevance, the zoonotic potential of bovine *Chlamydia* species appears to be minor (Anstey et al., 2019; Wu et al., 2023). Infectious abortion represents a major economic constraint in dairy farming due to the associated reproductive losses (Rahal et al., 2018). In Algeria, apart from brucellosis which is a notifiable disease abortions are frequently reported by veterinarians but rarely investigated aetiologically. Epidemiological data on bovine chlamydiosis remain limited in the Maghreb region, despite previous reports confirming its occurrence (Merdja et al., 2025). Therefore, the objective of the present study was to investigate the presence of *Chlamydia* spp. in dairy cattle, identify circulating genotypes, and assess potential risk factors associated with chlamydial infection in placental tissues collected from aborted and naturally calving cows in Algeria, in order to provide new insights into the epidemiological situation of this infection in the country. To our knowledge, this is the first study combining qPCR-based detection and genotyping of Chlamydia spp. in bovine placental tissues in Algeria, coupled with a comprehensive risk factor analysis.

Materials and methods

Study site and sample collection

The study was conducted between 2022 and 2024 in four wilayas (provinces) of central and eastern Algeria: Blida, Médéa, Bouira, and Bordj Bou Arréridj. These regions (Figure 1) constitute one of the country's main dairy production areas, with an estimated 100,000 dairy cows.

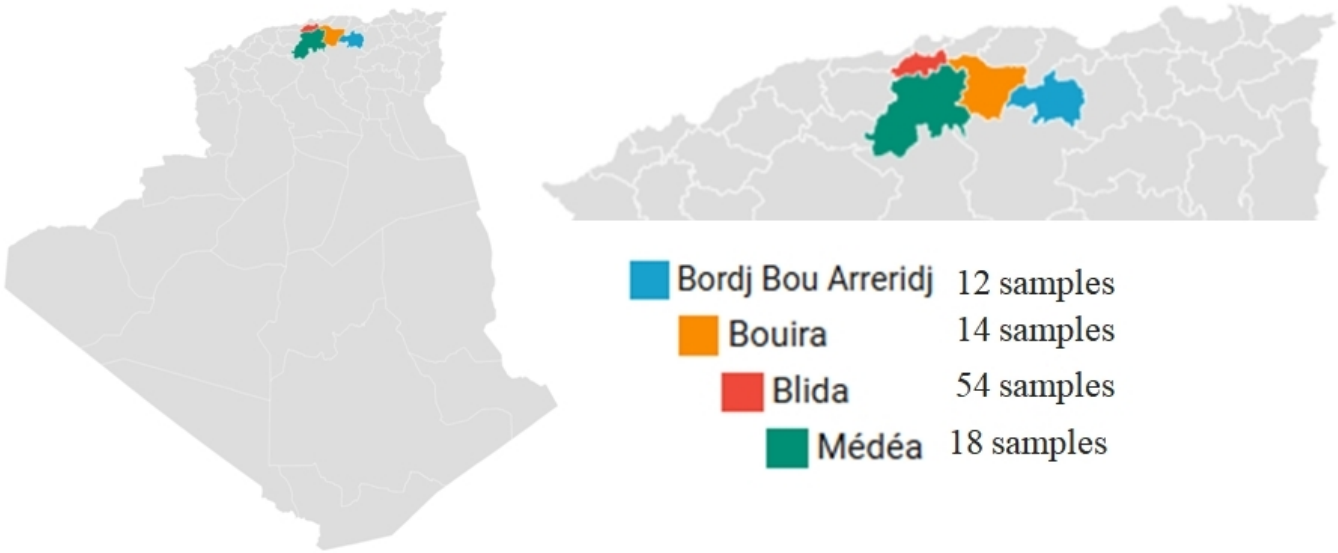

Figure 1. Map of Algeria showing the geographical distribution of the samples.

A total of 98 placental fragments were collected, including 14 from cows that calved normally and 84 from cows that had aborted within 12 to 24 hours of foetal expulsion.

Abortion was defined as the loss of a foetus between 42 and approximately 260 days of gestation.

To minimise contamination risk, samples were collected concurrently with the manual removal of the placenta, before any contact with soil, bedding, or excreta. In cases where calving occurred prior to the veterinarian's arrival, placental tissues were sampled immediately upon expulsion.

Each placental fragment was rinsed at least twice with distilled water to remove adherent debris, and then approximately 2.5 cm^3 of tissue was aseptically excised from the cotyledonary region of the placenta and transferred into individual 10 mL sterile vials containing 70% ethanol.

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Sample processing

All samples were transported at 4°C to the laboratory within 6 h for molecular analysis. Prior to DNA extraction, each placental fragment was rinsed twice with sterile distilled water to remove residual ethanol and surface contaminants. The tissues were then dissected into small pieces using sterile scalpel blades and manually homogenised with a sterile pestle. Approximately 40 mg of each homogenate was digested with 25 µL of proteinase K and 180 µL of G2 buffer at 56°C for 16 hours to ensure complete lysis of host cells and bacterial structures (Pantchev et al., 2009).

Total DNA was extracted using the EZ1® DNA Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol, yielding a final eluate volume of 100 µL per sample. Extracted DNA was stored at −20°C under sterile conditions until further molecular analysis to prevent degradation or contamination.

The specific primers and probe sequences used were those described by Ehricht et al. (2006) (Table I).

Specificity	Primer / Probe	Sequence (5′–3′)	Amplicon size (bp)	Target gene	Reference
Chlamydiaceae	Ch23S-F	CTG AAA CCA GTA GCT TAT AAG CGG T	111	23S rRNA	Ehricht et al., 2006
	Ch23S-R	ACC TCG CCG TTT AAC TTA ACT CC	111	23S rRNA	
	Ch23S-P	FAM–CTC ATC ATG CAA AAG GCA CGC CG–TAMRA	—	23S rRNA	

Table I. Primers and probe used for real-time PCR amplification of the Chlamydiaceae 23S rRNA gene.

Real-time PCR detection

All DNA extracts were individually screened for the presence of *Chlamydia* spp. using a real-time PCR assay targeting the 23S rRNA gene, which is conserved across all members of the *Chlamydiaceae* family. *Chlamydia trachomatis* DNA extracted from a reference bacterial culture was used as a positive control, while DNA-free water was included in each reaction as a negative control to monitor potential contamination during reaction setup. All qPCR reactions were performed in duplicate, and a sample was considered positive when consistent amplification was obtained in both reactions and the cycle threshold (Ct) value was below 38, according to Mattmann et al. (2019). This threshold was selected to ensure reliable detection of *Chlamydia* spp. DNA while minimising the inclusion of amplification signals close to the assay detection limit.

In addition to *Chlamydia* detection, all placental tissue samples were also screened for two other bacterial pathogens commonly associated with bovine abortion, namely *Coxiella burnetii* and *Leptospira* spp. Detection was performed by real-time PCR targeting the IS1111 and IS30A rRNA elements for *C. burnetii*, and the rrs (16S rRNA) gene for *Leptospira* spp., following the method described by Rahal et al. (2018).

Conventional PCR amplification

All samples that tested positive by real-time PCR were subjected to conventional PCR amplification prior to sequencing. Amplification targeted fragments of the Chlamydia 16S rRNA gene using the primer sets described by Rostami et al. (2017) and Kebbi-Beghdadi et al. (2022) (Table II).

PCR reactions were performed in a final volume of 25 µL containing 12.5 µL of PCR Master Mix, 0.5 µM of each primer, 2 µL of template DNA, and nuclease-free water to volume. Amplification was carried out under the following conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min.

PCR products were separated by electrophoresis on a 1.5% agarose gel stained with GelRed® and visualised under ultraviolet illumination. A 100 bp DNA ladder was included to estimate amplicon sizes. Positive (*Chlamydia* DNA) and negative (nuclease-free water) controls were included in each PCR run.

Sequencing and sequence analysis

Following successful conventional PCR amplification, all qPCR-positive samples were submitted for Sanger sequencing analysis.

For genotyping purposes, all *Chlamydia*-positive samples identified by qPCR were subjected to genotyping based on

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multiple ribosomal RNA gene fragments, including 16S1, 16S2, rRNA1a, rRNA1b, rRNA01, rRNA02, rRNA03, rRNA04, rRNA4a, and rRNA4b (Table II).

DNA amplification was performed according to the standard PCR program described by Rostami et al. (2017) and Kebbi-Beghdadi et al. (2022). The thermal cycling conditions were as follows: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 40°C for 1 minute, and extension at 72°C for 2 minutes, with a final elongation step at 72°C for 10 minutes.

All PCR amplicons were purified using the Millipore NucleoFast® 96 PCR filter plate kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Sequencing reactions were performed according to the protocol described by Kebbi-Beghdadi et al. (2022).

The resulting nucleotide sequences were assembled, edited, and quality-checked using ChromasPro version 1.7 (Technelysium Pty Ltd., Tewantin, Australia). Consensus sequences were then compared with reference sequences available in the NCBI GenBank database using the BLASTn algorithm to determine sequence similarity and identify *Chlamydia* genotypes.

Primer name	Nucleotide position (5′→3′)	Sequence (5′–3′)	Target region	Reference
16S1	−2 to 18	5′-CGG ATC CTG AGA ATT TGA TC-3′	16S rRNA (fragment 1)	Rostami et al., 2017
16S2	1554 to 1537	5′-TGT CGA CAA AGG AGG TGA TCC A-3′	16S rRNA (fragment 2)	Rostami et al., 2017
rRNA1a	−1 to 21	5′-GGA TCC TGA GAA TTT GAT CIT G-3′	16S rRNA variable region	Kebbi-Beghdadi et al., 2022
rRNA1b	547 to 523	5′-TCC GTA TTA CCG CAG CTG CTG GCA C-3′	16S rRNA variable region	Kebbi-Beghdadi et al., 2022
rRNA01	523 to 547	5′-GTG CCA GCA GCT GCG GTA ATA CGG A-3′	16S rRNA domain I	Kebbi-Beghdadi et al., 2022
rRNA02	742 to 718	5′-GCC TTC GCC ACT GGT GTT CTT CCA C-3′	16S rRNA domain II	Kebbi-Beghdadi et al., 2022
rRNA03	886 to 912	5′-TAT GCC GCC TGA GGA GTA CAC TCG C-3′	16S rRNA conserved region	Kebbi-Beghdadi et al., 2022
rRNA04	1076 to 1052	5′-TGA CGA CAG CCA TGC AGC ACC TGT G-3′	16S rRNA domain III	Kebbi-Beghdadi et al., 2022
rRNA4b	1077 to 1099	5′-GCT CGT GCC GTG AGG TGT TGG GGT TA-3′	16S rRNA hypervariable region	Kebbi-Beghdadi et al., 2022
rRNA4a	1554 to 1534	5′-TGT CGA CMG GAG GTG ATC CAG CC-3′	16S rRNA hypervariable region	Kebbi-Beghdadi et al., 2022

Table II. Primers used for rDNA amplification and direct sequencing of Chlamydia spp. 16S rRNA gene fragments.

Tabl Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarise the distribution of *Chlamydia* spp. positivity according to the investigated variables. Associations between *Chlamydia* spp. detection and categorical variables (region, parity, season, type of breeding, climate, hygiene conditions, tick infestation, and type of calving) were initially evaluated using Pearson's chi-square test. When expected cell counts were less than five, Fisher's exact test was applied as appropriate. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated to estimate the strength of associations between explanatory variables and Chlamydia spp. positivity. A p-value < 0.05 was considered statistically significant.

Results

Detection of *Chlamydia* spp. by quantitative real-time PCR (qPCR)

Out of the 98 placental samples collected from four Algerian provinces - Blida (n = 54), Médéa (n = 18), Bouira (n = 14), and Bordj Bou Arréridj (n = 12) - a total of 11 samples tested positive for *Chlamydia* spp. using the 23S rRNA real-time PCR assay, corresponding to an overall positivity rate of 11.22% (11/98). All positive samples originated from cows that had aborted, resulting in a positivity rate of 13.09% (11/84) among abortion cases. Seven positive cases were detected in Blida and four in Médéa, whereas all placental samples collected from cows following normal calving tested negative.

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The qPCR-positive samples exhibited relatively homogeneous bacterial loads, with cycle threshold (Ct) values ranging from 33.30 to 36.52. The mean Ct value was 35.13 ± 1.08, indicating a moderate concentration of *Chlamydia* DNA in the positive placental samples (Figure 2; Table III). In contrast, all placental samples collected from cows following normal calving tested negative for the presence of *Chlamydia* spp. DNA.

All qPCR-positive samples were subsequently subjected to conventional PCR amplification for sequencing purposes. Amplification products of the expected size were successfully obtained and processed for Sanger sequencing.

Although the apparent positivity rate varied among provinces, with the highest prevalence in Médéa (22.22%) followed by Blida (12.96%), no significant association was found between *Chlamydia* spp. detection and geographic origin (χ^2 = 6.21, *df* = 3, *p* = 0.10). This trend may reflect local differences in herd management or environmental exposure but requires confirmation in a larger sample size (Table IV).

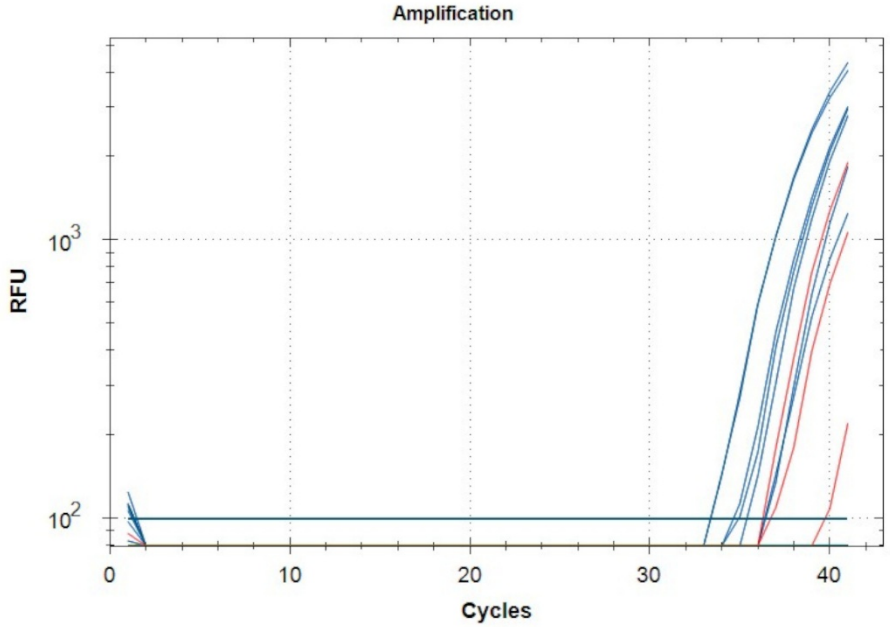

Figure 2. Real-time PCR amplification curves for the detection of *Chlamydia* spp. DNA from bovine placental samples.

Classification	Sample ID	Ct Value (Positive Samples)	Wilaya (Region)
1	14	33.30	Médéa
2	28	33.31	Blida
3	29	34.97	Médéa
4	30	36.25	Blida
5	33	35.33	Blida
6	48	35.15	Médéa
7	52	36.38	Blida
8	59	35.48	Blida
9	64	34.78	Blida
10	73	34.95	Blida
11	74	36.52	Médéa
Positive control 1	—	36.38	—
Positive control 2	—	36.89	—
Positive control 3	—	39.87	—

Table III. Detection of Chlamydia spp. by qPCR (the bacterial load with Ct of positive samples). Ct = Cycle threshold.

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Province (Wilaya)	Total samples (n)	Positive samples (n)	Negative samples (n)	Positivity rate (%)	Mean Ct value (range)	χ^2 (partial)	*p* -value	Interpretation
Blida	54	7	47	12.96	33.31–36.38	—	—	Moderate positivity
Médéa	18	4	14	22.22	33.30–36.52	—	—	Highest positivity
Bouira	14	0	14	0.00	—	—	—	Negative
Bordj Bou Arréridj	12	0	12	0.00	—	—	—	Negative
Total	**98**	**11**	**87**	**11.22**	33.30–36.52	**χ^2 = 6.21 (df = 3)**	***p* = 0.10**	Not significant

Table IV. Detection of Chlamydia spp. by qPCR and statistical analysis of positivity by province.

Sequencing

All eleven samples that tested positive by qPCR targeting the 23S rRNA gene were submitted for sequencing analysis. For genotyping purposes, multiple ribosomal RNA gene fragments, including 16S1, 16S2/rRNA1a, rRNA1b/rRNA01, rRNA02/rRNA03, and rRNA04/rRNA4a/4b were targeted for amplification and sequencing.

However, due to incomplete sequence data, only one sequence from the 16S ribosomal RNA gene could be reliably analysed. This sequence showed a 97.22% similarity to the Chlamydia abortus strain NL2335-4C chromosome (GenBank accession no. CP018296), suggesting a close relationship with this species.

Risk factors analysis

Table V presents the analysis of potential risk factors associated with *Chlamydia* spp. positivity by qPCR. Several variables showed a significant influence on the prevalence of *Chlamydia* spp. DNA detection.

Cows that aborted had a significantly higher prevalence compared to those that calved normally ($p = 0.01$). Regarding seasonal effects, infection was more frequent in spring (OR = 1.68, $p = 0.02$), suggesting that environmental conditions during this period favour pathogen transmission. Hygiene also played a crucial role, with poor hygiene associated with a higher prevalence (OR = 1.84, $p = 0.04$), while good sanitary practices appeared protective. Moreover, tick infestation significantly increased infection risk (OR = 1.98, $p = 0.01$), emphasising the impact of ectoparasites on disease dynamics.

Figure 3 (multivariate analysis bubble plot) illustrates the relationship between prevalence and odds ratios for the various risk and protective factors. The results confirm that season, tick infestation, and type of calving are significant risk factors (OR > 1), indicating that favourable environmental conditions, ectoparasite presence, and uncontrolled calving increase the likelihood of infection. Conversely, good hygiene, semi-arid climates, and extensive breeding systems are associated with lower odds (OR < 1), suggesting a protective effect.

Figure 4 (heatmap) visualises the prevalence of *Chlamydia* spp.infection across different categories. Spring (18.6%) and tick-infested animals (14.7%) show the highest prevalence, followed by multiparous cows (13.4%) and abortion type calving (13.1%). Lower prevalence values were observed for factors such as good hygiene, semi-arid climates, and extensive breeding, indicating a potential protective influence.

Figure 5 (forest plot of odds ratios) further illustrates the magnitude and direction of each factor's association with infection. Points to the left of the red dashed line (OR < 1) correspond to protective factors notably semi-arid climate, good hygiene, and extensive breeding. Conversely, points to the right (OR > 1) represent risk factors, including tick infestation, abortion-type calving, and poor hygiene.

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Variable	Category	No. sampled	Positive n (%)	95% CI (Prevalence)	OR	95% CI (OR)	*p*-value
Total	-	98	11 (11.22)	8.6–14.2	–	–	–
Region	Blida	54	7 (12.96)	9.3–15.6	–	0.31–0.97	0.06
	Médéa	18	4 (22.22)	19.8–25.3	1.48	1.05–3.27	–
	Bordj Bou Arréridj	14	0 (0.00)	–	–	–	–
	Bouira	12	0 (0.00)	–	–	–	–
Type of calving	Abortion	84	11 (13.09)	11.2–15.3	-		0.01
	Normal calving	14	0 (0.00)	0.0–0.0	-		–
Parity	Multiparous	67	9 (13.43)	10.7–16.5	0.48	0.31–0.73	0.07
	Nulliparous	31	2 (6.45)	5.1–7.8	0.98	0.54–1.69	–
Season	Spring	43	8 (18.60)	15.2–22.4	1.68	1.04–2.60	0.02
	Summer	29	3 (10.34)	8.4–12.8	–	0.34–0.92	–
	Autumn	14	0 (0.00)	–	–	–	–
	Winter	12	0 (0.00)	–	–	–	–
Type of breeding	Extensive	62	7 (10.14)	7.5–13.2	0.83	0.52–1.78	0.82
	Semi-extensive	36	4 (11.11)	8.9–14.3	0.98	0.58–2.08	–
Climate	Semi-arid	42	4 (9.52)	7.1–12.3	0.42	0.25–0.77	0.06
	Sub-humid	56	7 (12.50)	10.4–15.2	0.89	0.58–1.83	–
Hygiene	Good	44	2 (4.54)	3.5–7.2	0.56	0.35–0.82	0.04
	Moderate	33	4 (12.12)	10.2–14.8	0.78	0.52–2.03	–
	Poor	21	5 (23.80)	20.6–26.5	1.84	1.21–2.45	–
Tick infestation	Yes	68	10 (14.70)	11.2–17.6	1.98	0.92–3.60	0.01
	No	30	1 (3.33)	2.3–5.4	0.48	0.31–0.79	–

Table V. Analysis of risk factors associated with *Chlamydia* spp. PCR positivity. CI = Confidence Interval; OR = Odds Ratio; p-value = probability value.

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Figure 3. Multivariate analysis (bubble plot) illustrating the relationship between the prevalence (%) and odds ratios (OR) of various risk and protective factors associated with Chlamydia spp. infection in aborted dairy cows in Algeria.

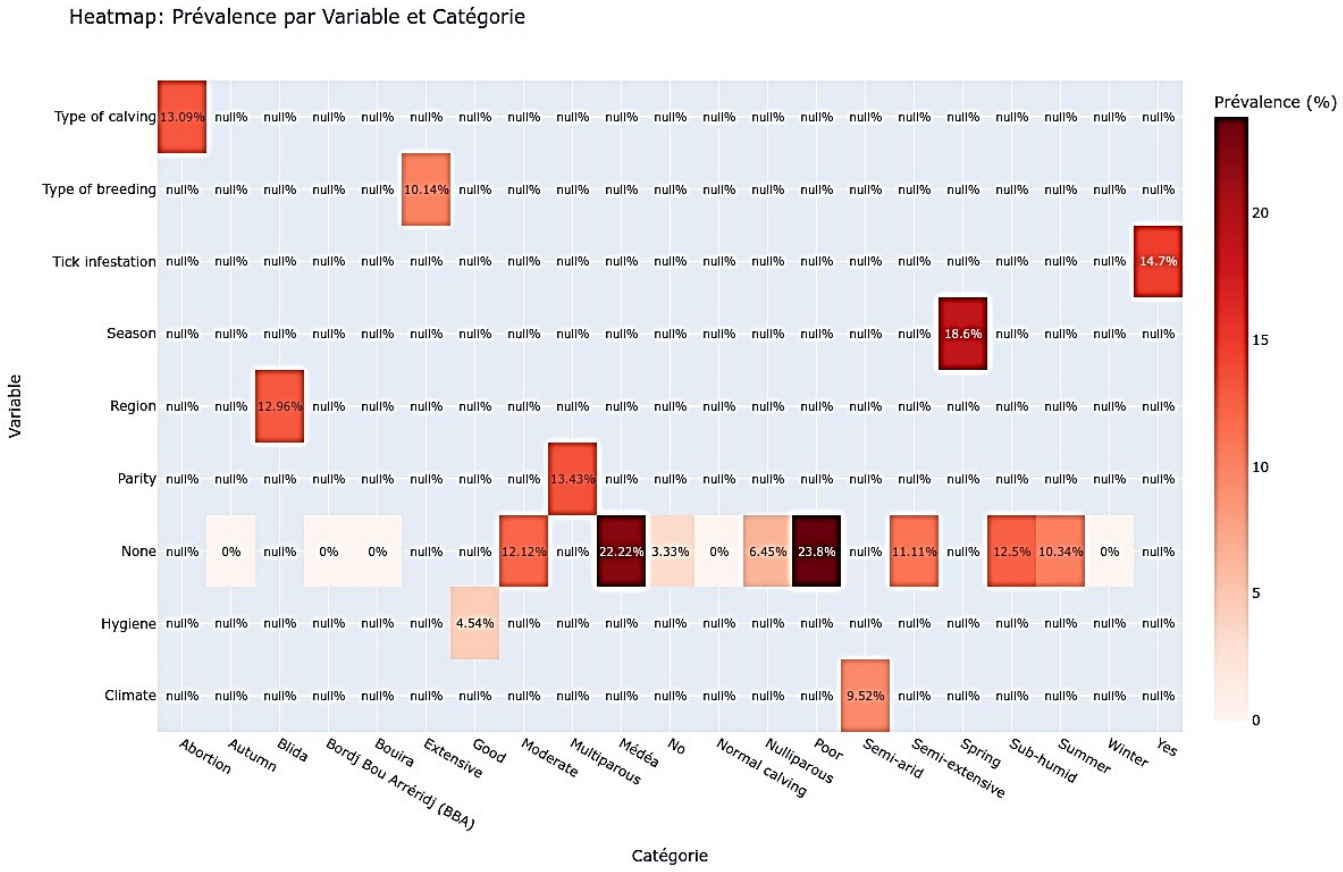

Figure 4. Heatmap showing the prevalence (%) of *Chlamydia* spp. positivity according to variable and category in aborted dairy cows in Algeria.

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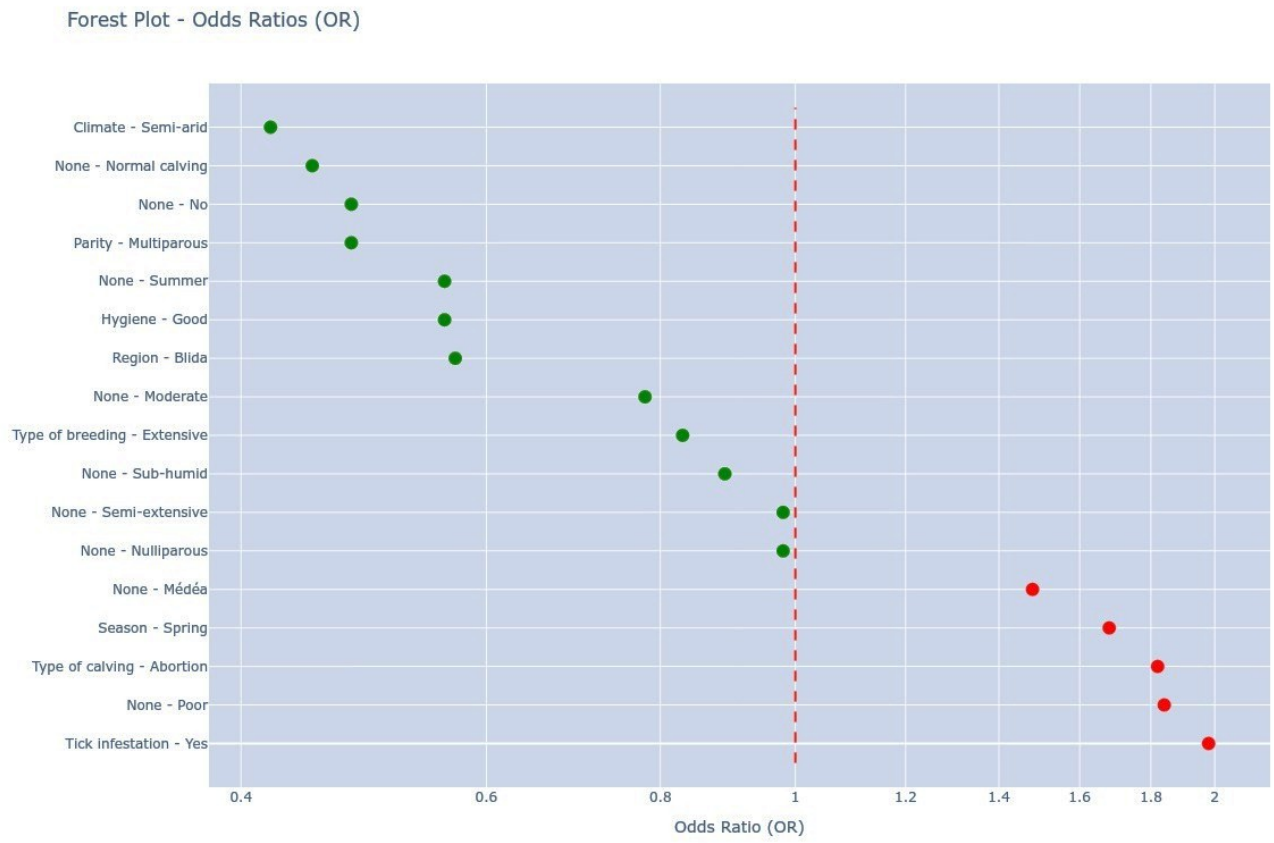

Figure 5. Forest plot of odds ratios (OR) for the studied risk factors.

Discussion

Epidemiological data on the extent of chlamydiosis in ruminants in Algeria are scarce. This zoonosis has long been neglected by veterinarians despite its potential economic and public health implications (Merdja et al., 2015). In our study, molecular screening by PCR revealed an overall prevalence of 11.22% (11/98) for *Chlamydia* spp., while the prevalence among aborted dairy cows was 13.1% (11/84). All positive cases originated from animals that had aborted, whereas all placental samples from normal calvings were negative. This finding strengthens the epidemiological association between *Chlamydia* infection and abortion in the studied herds (Jonker and Michel, 2023). Nevertheless, although qPCR provides reliable evidence of the presence of *Chlamydia* DNA in placental tissues, PCR detection alone is insufficient to definitively demonstrate that the detected organism was the direct cause of abortion. In abortion investigations, pathogen detection should ideally be supported by compatible histopathological lesions, demonstration of the microorganism within affected tissues, and exclusion of other abortifacient agents (Wolf-Jäckel et al., 2021; Hecker et al., 2023; Jonker and Michel, 2023). Similar trends have been reported in neighbouring countries; for instance, a Moroccan study found a 29% prevalence in aborted ruminants (El Jai et al., 2004), and comparable levels have been observed elsewhere in North Africa (Semango and Buza, 2024).

Clinical diagnosis of chlamydial abortion remains difficult because clinical signs and lesions are non-specific and can resemble those caused by other abortifacient pathogens such as *Brucella* spp. or *Coxiella burnetii* (Essig and Longbottom, 2015). In this context, molecular techniques such as PCR and qPCR represent the most reliable diagnostic tools, as they detect bacterial DNA without requiring viable organisms, thereby simplifying sample collection, transport, and storage (Barati et al., 2022). Numerous PCR assays have been developed for detecting and differentiating members of the family *Chlamydiaceae* (Pantchev et al., 2009). The optimisation and application of qPCR assays targeting *Chlamydiales* 23S or 16S rRNA genes have proven essential for detecting intracellular bacteria that cannot be cultured on cell-free media (Jonker and Michel, 2023). qPCR also allows quantification of the bacterial load, providing valuable information for interpreting diagnostic results (Sachse et al., 2009).

In our study, the relatively high Ct values (33–36) indicated moderate to low bacterial loads, possibly reflecting

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subclinical or chronic infections, DNA degradation, or late-stage contamination. This suggests that the true prevalence of infection could be underestimated, as samples with lower bacterial loads may fall below the detection threshold. Comparable observations have been reported in studies of abortion materials from domestic ruminants, where qPCR sensitivity was lower for broad Chlamydiales screening than for targeted *C. abortus/C. pecorum* assays, possibly due to the 16S rRNA target design (Jonker and Michel, 2023).

Environmental contamination plays a major role in transmission. The placenta and aborted foetuses are considered the main sources of *C. abortus* dissemination; animals can become infected through ingestion or inhalation of contaminated materials (Longbottom and Coulter, 2003). Following abortion, large quantities of *Chlamydiaceae* are shed in placental fluids, in aborted tissues, and even on the coat of newborns, promoting oral transmission to susceptible animals (Rekiki et al., 2002). In the Maghreb, local farming conditions characterised by nutritional deficiencies and parasitic infestations may further enhance *Chlamydia* virulence (Rekiki et al., 2002). Moreover, recent genomic analyses have identified genes such as ORF663 and IncA with variable repeat sequences potentially linked to pathogenicity (Babu Sait et al., 2025).

Genotyping in the present study revealed one complete 16S rRNA gene sequence showing 97.22% similarity to *Chlamydia abortus* strain NL2335-4C, suggesting the presence of this strain or a closely related clone in Algerian cattle herds. However, the lack of multilocus sequence typing (MLST), VNTR, or whole-genome data limits phylogenetic resolution and prevents the identification of regional or emerging variants (Rhawy et al., 2024). Future molecular epidemiological studies using these tools could clarify the genetic diversity and potential virulence markers of local strains.

Our statistical analysis identified several significant risk factors associated with *Chlamydia* positivity: abortion, spring season, poor hygiene, and the presence of ticks. The higher positivity observed during spring should be interpreted with caution. This pattern may reflect environmental conditions that favour pathogen persistence, such as moderate temperature and humidity, or physiological stress associated with seasonal management changes and the transition to pasture (Turin et al., 2022). However, it may also be influenced by the seasonal distribution of calving and abortion events, which could increase the probability of detecting infected placental samples during this period. Further longitudinal studies including detailed reproductive records are required to distinguish between these effects. Poor hygienic conditions facilitate environmental persistence of the pathogen in placentas, stalls, and equipment, enhancing the risk of oral or inhalation transmission (Borel and Greub, 2018). Although tick infestation was significantly associated with *Chlamydia* positivity in the present study, this finding should be interpreted with caution. *C. abortus* is not currently recognised as a tick-borne pathogen, and the observed association may reflect indirect effects related to animal health, management practices, or environmental factors rather than a direct transmission route. Further studies are needed to clarify this relationship. Herd size also influences infection dynamics, as larger herds increase animal contact and the likelihood of exposure to contaminated environments (Jiménez-Martín et al., 2025).

Comparative data from different regions highlight considerable variation in the prevalence of Chlamydia infections depending on host species, geographical conditions, herd management, and diagnostic methodology. For example, a Colombian study reported a seroprevalence of 47.1% for *C. abortus* in cattle, with significant associations with age and reproductive status (Orjuela et al., 2022). Recent investigations in Europe have further confirmed the widespread occurrence of Chlamydiaceae in ruminant reproductive disorders and emphasised the value of molecular techniques for their detection in abortion materials (Jonker and Michel, 2023; Szymańska-Czerwińska et al., 2024). Although*C. abortus* is recognised worldwide as an important cause of abortion in small ruminants and an emerging reproductive pathogen in cattle (Borel and Greub, 2018; Turin et al., 2022), its epidemiology remains insufficiently documented in North Africa, where molecular studies are still scarce (Ehricht et al., 2006; Vidal et al., 2017). The present findings therefore contribute valuable data to the understanding of bovine chlamydiosis in this region.

Finally, despite being a notifiable disease in Algeria, field screening and vaccination against chlamydial abortion are not practised. Given the detection of *Chlamydia spp.* in cattle and its zoonotic potential, particularly for pregnant women and farm workers exposed to abortion materials, a One Health-based surveillance strategy integrating veterinary and human health sectors is urgently needed (Borel and Greub, 2018; Turin et al., 2022). Future research should combine molecular, histopathological, serological, and cultural techniques to strengthen diagnostic reliability, improve epidemiological understanding, and support the development of targeted control and prevention measures.

Taken together, these findings suggest that the detection of Chlamydia spp. in aborted dairy cows is influenced by both environmental and management-related factors. The higher positivity observed during spring, in tick-infested animals, and under poor hygiene conditions highlights the potential role of these factors in pathogen transmission and persistence. Therefore, improving farm hygiene, strengthening tick control programmes, and implementing preventive measures during high-risk periods may contribute to reducing the occurrence of chlamydial infections in dairy herds.

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Limitations of the study

Several limitations should be considered when interpreting the results of the present study. First, although eleven placental samples tested positive for Chlamydia spp. by qPCR, only one complete sequence was successfully obtained for molecular characterisation. The relatively high Ct values observed in positive samples (33–36.52) suggest low bacterial DNA concentrations, which may have reduced the efficiency of conventional PCR amplification and sequencing. Consequently, the identification of Chlamydia abortus was based on a single informative sequence, limiting our ability to assess the genetic diversity of circulating strains and to draw broader conclusions regarding the molecular epidemiology of Chlamydia infections in Algerian dairy cattle.

Second, the detection of Chlamydia DNA in placental tissues demonstrates the presence of the pathogen but does not establish a causal relationship with abortion. Confirmation of causality would require complementary evidence, including histopathological examination, immunohistochemical detection of the organism within lesions, and the exclusion of other abortifacient pathogens.

Conclusion

This study provides the first molecular evidence of Chlamydia spp. circulation in dairy herds from central Algeria and documents its detection in placental tissues collected from aborted cows. Although the exclusive detection of Chlamydia DNA in abortion cases suggests a possible association with bovine abortion, molecular detection alone demonstrates the presence of the pathogen and should not be considered proof of a causal relationship.

Risk factor analysis identified abortion status, spring season, poor hygiene, and tick infestation as factors associated with Chlamydia positivity. These findings highlight the importance of improving farm biosecurity, strengthening reproductive health monitoring, and implementing effective tick control measures.

Further investigations integrating molecular, serological, bacteriological, and histopathological approaches are needed to clarify the epidemiological significance and pathogenic role of C. abortus in bovine abortion, as well as its potential zoonotic implications.

The establishment of a coordinated One Health surveillance strategy involving veterinary and public health sectors would contribute to a better understanding of chlamydial infections and support the development of effective prevention and control measures in Algeria.

Ethical approval

All animal sampling procedures were conducted in accordance with Algerian national legislation governing animal research and welfare. Authorisation for sampling was obtained from the Wilaya Veterinary Inspectorate of each province involved in the study. Experimental procedures were reviewed and approved by the Institutional Committee for Animal Protection of the National Administration of Higher Education and Scientific Research of Algeria, in compliance with Law No. 98-11 of 22 August 1998.

Conflict of interest

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

Author Contributions

Conceptualisation: RF, OS; Methodology: OS, MR; Formal analysis: SY,NAKT; Investigation: RF, OS, MR; Writing original draft preparation: OS,MR; Writing, review and editing: SY,NAKT, NO; Visualisation: NO; Supervision: NO

All authors have read and agreed to the published version of the manuscript.

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Data availability

Data will be made available on request.

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