https://doi.org/10.52973/rcfcv-e35681 Revista Científica, FCV-LUZ / Vol. XXXV

Seroprevalence survey of bovine Brucellosis in Eastern Algeria: spatial distribution and risk factor analysis. Technical note

Seroprevalencia de brucelosis bovina en el este de Argelia: distribución espacial y análisis de factores de riesgo. Nota técnica

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ABSTRACT

This cross-sectional study assessed the prevalence of bovine brucellosis and its associated risk factors in the Sétif region of Algeria, which spans diverse agro-climatic zones. Between March and December 2023, blood samples were collected from 391 dairy cows across 32 farms and analyzed using the ID Screen® Brucellosis Serum Indirect Multi-species immunoassay (featuring protein G-HRP conjugate) manufactured by IDVet Innovative Diagnostics (Grabels, France). The study revealed an individual seroprevalence of 30.18% and a herd prevalence of 84.37%, with significant variation across bioclimatic zones. The highest prevalence was observed in the southern zone (41.2%), followed by the highland (30.9%) and northern zones (23.7%). Logistic regression analysis identified external animal introduction (P = 0.0001; OR = 20.3; 95% CI: [5.33-77.19]), infertility (P = 0.0001; OR = 8.6; 95% CI: [3.71-19.89]), and abortion history (P = 0.0001; OR = 8.9; 95% CI: [3.78-20.49]) as primary risk factors. Geographic location was found to significantly influence disease risk, with animals in the northern bioclimatic zone exhibiting a markedly higher likelihood of infection (P = 0.017; OR = 3.0; 95% CI: [1.22-7.54]) compared to those in the central region. Throughout the study period, five brucellosis outbreaks were identified, prompting the implementation of targeted control measures, including the culling of 144 seropositive animals and the imposition of restrictions on the distribution of unpasteurized dairy products. These findings highlight the necessity for enhanced control measures for affected herds. The study provides essential epidemiological data to inform evidence-based policies for sustainable brucellosis control in Algeria.

Key words: Bovine brucellosis; seroprevalence; risk factors; epidemiology; Algeria

RESUMEN

Este estudio transversal evaluó la prevalencia de brucelosis bovina y sus factores de riesgo asociados en la región de Sétif de Argelia, que abarca diversas zonas agroclimáticas. Entre marzo y diciembre de 2023, se recolectaron muestras de sangre de 391 vacas lecheras en 32 explotaciones y se analizaron utilizando un kit comercial ELISA ID Screen® Brucellosis Serum Indirect Multi-species (conjugado proteína G-HRP), IDVet Innovative Diagnostics, Grabels, Francia. El estudio reveló una seroprevalencia individual del 30,18% y una prevalencia de rebaño del 84,37%, con variaciones significativas entre zonas bioclimáticas. La mayor prevalencia se observó en la zona sur (41,2%), seguida por las zonas de meseta (30,9%) y norte (23,7%). El análisis de regresión logística identificó la introducción externa de animales (P = 0,0001; OR = 20,3; IC 95%: [5,33-77,19]), la infertilidad (P = 0,0001; OR = 8,6; IC 95%: [3,71-19,89]) y el historial de abortos (P = 0,0001; OR = 8,9; IC 95%: [3,78-20,49]) como factores de riesgo principales. La ubicación geográfica también influyó en el riesgo de enfermedad, con animales en la zona bioclimática norte mostrando probabilidades significativamente más altas de infección (P = 0,017; OR = 3,0; IC 95%: [1,22-7,54]) que en la región central. Durante el período de estudio, se identificaron cinco brotes de brucelosis, lo que llevó a la implementación de medidas de control específicas, incluyendo el sacrificio de 144 animales seropositivos y restricciones en la distribución de productos lácteos no pasteurizados. Estos hallazgos subrayan la necesidad de mejorar las medidas de control para los rebaños afectados. El estudio proporciona datos epidemiológicos cruciales para informar políticas basadas en evidencia para el control sostenible de la brucelosis en Argelia.

Palabras clave: Brucelosis bovina; seroprevalencia; factores de riesgo; epidemiología; Argelia

Recibido:22/04/2025 Aceptado:24/07/2025 Publicado: 03/09/2025

INTRODUCTION

Bovine brucellosis, caused by *Brucella abortus*, remains a major zoonotic disease of global concern, with substantial prevalence reported in the Middle East, Mediterranean Basin, sub-Saharan Africa, Asia, and Latin America [1 , 2 , 3]. The economic and public health burdens of the disease are particularly pronounced in developing regions, where traditional animal husbandry practices continue to facilitate its persistence [4].

Bovine brucellosis is an endemic disease in Algeria, with significant regional variations in its prevalence. Studies have documented diverse rates across regions, including the eastern [5 , 6] and western areas [7 , 8]. This geographical heterogeneity appears to be linked to local cultural practices, limitations in diagnostic capabilities, and inadequate vaccination programs. This varied distribution suggests that effective control requires region-specific strategies that consider both local epidemiological patterns and socioeconomic factors [9].

Algeria's post-independence brucellosis control strategy has evolved through various phases. The 1970 program introduced surveillance, culling, decontamination, and B19 calf vaccination [10]. In 1976, vaccination was discontinued as surveillance efforts intensified in the affected districts [11]. National coverage was achieved by 1984, with the establishment of regional diagnostic laboratories and risk-based farm classification. The 1995 program further strengthened control measures by implementing standardised protocols for diagnosis, certification, culling compensation, quarantine, and movement restrictions [12].

Despite these interventions, bovine brucellosis remains endemic in Algeria, causing substantial economic losses [13]. Between 2002 and 2004, the mandatory culling of 2,235 cattle and 5,140 small ruminants resulted in compensation payments of 83 million Algerian dinars [14]. Brucellosis maintains its enzootic status in Algeria and poses significant public health risks, primarily through the consumption of unpasteurized dairy products and occupational exposure to infected animals [13]. This persistence highlights the need for enhanced epidemiological surveillance and risk factor identification to optimise the control strategies.

The Sétif district, located in Algeria's eastern High Plains region, supports approximately 10% of the national bovine population [15]. The distinctive tripartite bioclimatic zonation and diverse agricultural practices of this region exemplify the challenges of brucellosis control in Algeria. This study aimed to quantify the prevalence of bovine brucellosis in Sétif Wilaya and its associated risk factors, thereby contributing to development of evidence-based control strategies tailored to the region.

MATERIALS AND METHODS

Ethical statement

The animals were studied according to the ethical principles of animal experimentation and international guidelines for animal welfare (Terrestrial Animal Health Code 2018, section 7. Art 7.5.1) and national executive decree No. 95-363 of November 11, 1995 of Algeria [16].

Study area

The investigation was conducted in the Sétif district, located in the high plains of eastern Algeria (36° - 36°30'N, 5° - 5°30'E), encompassing 6,549 km² (FIG. 1). The region is characterized by a semi-arid Mediterranean climate with distinct seasonal variations. Based on mean annual precipitation, the area is stratified into three bioclimatic zones [17 , 18]:

- Northern zone: >450 mm
- Central zone: 300-450 mm
- Southern zone: <300 mm

Study population

Algeria's semi-arid high plains constitute a homogeneous agroecological zone containing 63% of the national bovine population [19], with Sétif accounting for approximately 10% of Algeria's cattle population [19]. The Sétif region, characterized by heterogeneous farming systems and bioclimatic gradients, hosts approximately 121,000 cattle, including 66,700 dairy cows, distributed across 10,758 farms [20]

Study design and sample size determination

The sample size was calculated using Thrusfield's formula for infinite population [21]:

$$n = z^2p(1-p)/m^2$$

Where: n = required sample size, z = confidence level coefficient [1.96 for 95% confidence], p = estimated population proportion [0.5 when no prior data exists], and m = tolerated margin of error [0.05 for 5% accuracy].

Given the absence of previous regional studies, an expected individual prevalence of 50% was assumed, yielding a required sample size of 384 individuals. This cross-sectional study included 391 dairy cows sampled between March and December 2023 across 32 farms in 17 communes, stratified by bioclimatic zone. Sampling was conducted by random selection from a list of eligible livestock producers, with a reserve list maintained for non-participants. Farm selection employed telephone-based consent with replacement sampling for declining participants. Inclusion criteria specified cows aged ≥2 years, regardless of reproductive history. The sampling protocol implemented proportional allocation:

- 25% sampling intensity for herds >11 cows
- Complete enumeration for herds ≤11 cows

This non-probabilistic approach, suitable for assessing herd-level disease prevalence, aligns with established protocols for smallholder systems [22].

Data collection and serological analysis

Standardized questionnaires were distributed to farm owners to evaluate reproductive indicators and potential risk factors,

yielding a thorough assessment of farm health. The risk variables are categorized into environmental, husbandry practices, and animal health. The attributes of the biogeographical region represent a primary level of investigation. Farming system and co-breeding, external acquisitions constitute a secondary level. The health history of the animals, encompassing breed, age, abortions, stillbirths, and infertility, informs a comprehensive evaluation of the farm's health concerns.

Blood samples were collected via jugular venipuncture using sterile Vacutainer® tubes following physical examination. Samples were maintained at ≤4°C for 24 hours (h) (Réfrigérateur CONDOR CRDN560W, RF0962, Algeria) before centrifugation (1 006 g, 10 minutes –(min)) (TDZ4-WS, Bioridge, Shanghai, China). Sera were aliquoted into sterile Eppendorf® tubes and stored at -20° (Congélateur CFH-T13GM03, Algerie) until analysis.

For serological analysis, we employed the ID Screen® Brucellosis Serum Indirect Multi-species kit (with protein G-HRP conjugate) manufactured by IDVet Innovative Diagnostics in Grabels, France. This assay can identify antibodies against three *Brucella* species: *B. suis*, *B. melitensis*, and *B. abortus*. Following the protocol provided by the manufacturer, we processed each serum sample individually. A Multiskan ELISA reader (Biotek Instruments Inc, USA) was utilized to determine the optical density (OD) at 450 nm wavelength. The interpretation criteria established that samples with an S/P percentage exceeding 120% were considered positive, while those falling between 110-120% were deemed inconclusive, and readings below 110% were classified as negative. To calculate the S/P percentage, we applied the following equation: S/P % = [(OD sample - OD negative control) / (OD positive control - OD negative control)] × 100.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics software (Version 23.0, IBM Corp., Armonk, NY, USA). Statistical evaluation of brucellosis prevalence and associated risk determinants was conducted employing chi-square and Fisher's exact test. The threshold for statistical significance was established at $P \leq 0.05$. Chi-square analysis was utilized to examine relationships between risk factors and brucellosis seropositivity. Variables demonstrating statistical significance ($p \leq 0.05$) underwent subsequent evaluation through binary logistic regression modeling. The model used was: $Logit(Y)=\alpha+\beta1X1+\beta2X2+\cdots+\beta nXn$, where Y represents the binary outcome, α the intercept, and β1, β2, …, βn the regression coefficients for risk factors X1, X2, …, Xn. The model's goodness of fit was assessed using the Hosmer-Lemeshow test. The strength of associations between risk factors and seropositivity was quantified using odds ratios (OR) with 95% confidence intervals (CI).

RESULTS AND DISCUSSION

This work represents the first comprehensive epidemiological assessment of bovine brucellosis in the Sétif Wilaya, it documents substantial disease burden with significant risk factors. The findings offer crucial insights to inform evidence-based control strategies and enhance disease management efforts.

Seroprevalence distribution

Individual-level seroprevalence in the Sétif district was 30.18% (118/391), with positive cases detected in 94.11% (16/17) of surveyed communes. Seroprevalence varied considerably across bioclimatic zones, with the southern zone (41.2%), followed by highland zone (30.9%) and the northern (23.7%) zones (TABLE I).

Herd-level analysis revealed a prevalence of 84.37% (27/32), with seropositivity rates exhibiting marked spatial heterogeneity, ranging from 3.85% in Ouled Tabane to 100% in Bazer Sakhra (TABLE I). FIG. 1 illustrates the spatial distribution of infected farms across Setif provinces.

The observed seroprevalence of 30.18% demonstrates concordance with previous studies in Batna, Algeria (28.6%) [23] and is comparable regional studies conducted in Morocco (33.48%) [24] and Senegal (36.36%) [25]. However, this prevalence markedly exceeds reported rates from central Algeria (0.81%) [14], Algiers (3.06%) [26], and several other African nations, including Ethiopia (7.17%) [27], Kenya (16.7%) [28], and Egypt (16.7%) [29], and certain Latin American countries, such as Venezuela (9.1%) [30] Higher seroprevalence has been reported in Uganda (46.8%) [31] and Togo (41%) [32], suggesting regional heterogeneity. This variability may be influenced by difference in ecological conditions, husbandry practices, biosecurity measures, and surveillance methodologies.

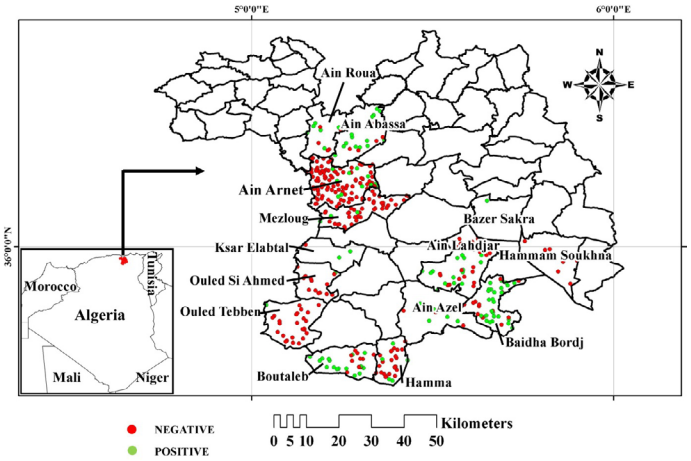

FIGURE 1. Spatial distribution of positive and negative brucellosis farms across Setif provinces

TABLE I
Brucellosis Prevalence in Cattle Across Bioclimatic Regions of Sétif District, Algeria

Bioclimate Region	Commune	Animals Tested [n]	Positive Cases [n]	Prevalence [%]	Regional Total
Northen region	Ain roua	11	9	81.82%	n = 140 Positive = 34 Prevalence = 24.29%
	Ain abbassa	19	14	73.68%	
	El bouhira	67	9	13.43%	
	Ai arnat	30	2	6.67%	
	El mehdia	13	0	0.00%	
Central region	Mezlougue	44	3	6.82%	n = 188 Positive = 58 Prevalence = 30.85%
	Bazersekhra	7	7	100.00%	
	Rmada	10	2	20.00%	
	Ainlehdjer	29	14	48,28%	
	Kasr-elabtal	10	2	20.00%	
	Hammamsoukhna	8	1	12.50%	
	Bidhaborj	40	27	67.50%	
	Ouledtabban	26	1	3.85%	
	Ouledsihmed	14	1	7.14%	
Southern region	Ain azel	9	5	55.56%	n = 63 Positive = 26 Prevalence = 41.27%
	Boutaleb	26	16	61.54%	
	El hamma	28	5	17.86%	
Total		391	118	30.18%	

Risk factor analysis

Multivariable logistic regression analysis demonstrated high model fit (χ^2 = 210.937, P = 0.0001; R^2 = 0·590; TABLE III). Among variables evaluated, five emerged as significant predictors of seropositivity (TABLE II). The primary risk factor was external animal introduction constituted (P = 0.0001; OR = 20.3; 95% CI: [5.33-77.19]), followed by history of infertility [P = 0.0001; OR = 8.6; 95% CI: [3.71-19.89]) and previous abortion (P = 0.0001; OR = 8.9; 95% CI: [3.78-20.49]) (TABLE III).

Multivariate analysis identified external animal introduction as the primary risk factor (OR = 20.3), corroborating with meta-analytical findings from Brazil [33] and previous epidemiological studies emphasizing the risks associated with uncontrolled animal introduction [34 , 35] and The seasonal movement for grazing and commerce between pastoral regions [36].

The strong association between seropositivity and abortion history (OR = 8.6) aligns with *Brucella abortus* documented tropism for reproductive tissues and classical pathogenic mechanisms via placentitis [37]. This finding is consistent with previous Algerian studies documenting an increased abortion risk in seropositive cattle [38 , 6] and is further supported by other regional epidemiological studies [39 , 40 , 41].

Similarly, a history stillbirth showed a positive association with seropositivity (OR = 1.8; 95% CI: [0.71-4.60]), but failed to achieve statistical significance (P = 0.216) (TABLE III). While the odds ratio suggests cows with a history of stillbirth may be 1.8 times more likely to be seropositive, the wide confidence interval crossing 1.0 and non-significant P-value indicate a high degree of uncertainty in this relationship.

This observation, consistent with historical data demonstrating comparable stillbirth rates between infected and uninfected cattle (0.41% vs. 0.24%) [42], suggests the potential influence of indirect effects or unmeasured confounding variables. To clarify these mechanisms, future research should incorporate prospective study designs and enhanced reproductive monitoring.

Geographic location significantly influenced infection risk, with animals in the northern zone exhibiting higher odds of seropositivity (P = 0.017; OR = 3.0; 95% CI: [1.22-7.54]) (TABLE III). Compared to the central region (reference category), cattle in the southern region showed a trend toward higher risk of brucellosis (OR = 2.0; 95% CI: [0.85-4.59]), though this association did not reach statistical significance (P = 0.112). This suggests a potential increased risk in the southern region, but the current data does not provide strong enough evidence to confirm this relationship.

This pattern correlates with regional characteristics such as high cattle density, smallholder management systems, and comm unal grazing practices, which facilitate pathogen transmission through enhanced host contact [43 , 6]. In contrast, the southern region's arid environment and intensive farming systems exhibit a significantly lower infection risks (OR = 1.978, P = 0.112), attributed to reduced inter-herd contact. These epidemiological findings align with previous research. Studies in Cameroon and France have documented transmission through communal grazing areas [44 , 45], while studies in Burkina Faso identified environmental transmission via contaminated water sources [46]. Similarly, research in Pakistan highlighted the role of wildlife reservoirs as significant disease vectors [47]. Environmental water sources [48] and wildlife populations [49] represent additional risk factors that further explain the spatial epidemiology of the disease.

TABLE II
Univariate analysis for risk factors associated with the prevalence Bovine Brucellosis in Sétif District, Algeria

Risk Factors	Modalities	Brucellosis Result		*P*
		Negative	Positive	
Bioclimatic Region	Central	130	58	**0.036**
	Northern	103	32	
	Southern	40	28	
Farming system	Semi-extensive	45	23	0.471
	Semi-intensive	228	95	
External Animal Introduction	No	172	13	**0.0001**
	Yes	101	105	
Age	≤3	69	22	0.154
	>3	204	96	
Breed	Native	60	32	0.083
	Fleckvieh	13	8	
	Montbéliard	157	70	
	Holstein	43	8	
Infertility	No	167	33	**0.0001**
	Yes	106	85	
Stillbirth History	No	226	23	**0.0001**
	Yes	47	95	
Abortion History	No	257	65	**0.0001**
	Yes	16	53	

TABLE III
Multiple Logistic Regression Analysis of Risk Factors Associated with Bovine Brucellosis in Sétif District, Algeria

Risk Factors	B	S.E.	Wald	df	Sig.	OR	95% C.I.for OR	
							Lower	Upper
Bioclimatic Region [Central]			6,568	2	0.037			
Northern	1.111	0.464	5.736	1	0.017	3.037	1.224	7.539
Southern	0.682	0.429	2.524	1	0.112	1.978	0.853	4.590
Abortion History [Yes]	2.175	0.431	25.415	1	0.0001	8.799	3.778	20.494
Stillbirth History [Yes]	0.591	0.477	1.532	1	0.216	1.805	0,708	4.600
External Animal Introduction [Yes]	3.010	0.682	19.502	1	0.0001	20.293	5.335	77.190
Infertility History [Yes]	2.151	0.428	25.210	1	0.0001	8.592	3.711	19.894
Constant	-5.204	0.667	60.816	1	0.0001	0.005		
Model fit: $\chi^2 = 210.937$, df = 6, $p < 0.001$, Nagelkerke $R^2 = 0.590$								

Diverse socio-economic and cultural factors significantly influence the transmission dynamics of brucellosis across regions. Mixed farming systems, predominantly characterized by smallholder operations where cattle co-graze with small ruminants, increase the risk of cross-species transmission [50]. Cultural practices, such as the consumption of raw dairy products, combined with inadequate public health awareness programs, lead to substantial resistance to regulatory compliance [43]. Furthermore, the prevalence of smallholder farming systems contributes to marked regional disparities in the efficacy of infection control measures [51]. These interconnected socio-cultural factors pose considerable challenges to the implementation of standardized disease control protocols.

Control measures implementation

During the study period, five distinct brucellosis outbreaks were identified; prompting immediate intervention by local authorities. Control measures included the culling of 144 seropositive animals and the implementation of restrictions on

the distribution of unpasteurized dairy product within affected areas. These interventions were executed concurrently with the ongoing epidemiological investigation.

Historically, intervention strategies have primarily included mass vaccination programs in small ruminants and intermittently implemented test-and-slaughter protocols in cattle populations [52 , 51 , 7]. These control measures impose substantial economic burdens through mandatory compensation to livestock owners and herd losses. This underscores the need to investigate alternative infection sources, such as wildlife reservoirs and contaminated water, and to advocate for integrated One Health approaches to optimize disease control strategies.

CONCLUSION

This comprehensive epidemiological investigation of bovine brucellosis in Algeria's Sétif region reveals substantial disease burden at both individual and herd levels, as evidenced by the identification of five distinct outbreaks requiring targeted interventions. Multivariate analysis identified external animal introduction, infertility and abortion history as primary risk factors, indicating significant deficiencies in current biosecurity protocols and herd health management practices. The spatial heterogeneity in disease distribution, particularly elevated risk in northern pastoral zones, indicates an environmental effect which underscores the necessity for regionally-adapted control strategies.

These findings emphasize the need for enhanced control measures, including mandatory surveillance, improved biosecurity guidelines, producer education, and optimized compensation policies. Future research should focus on wildlife reservoirs, socioeconomic factors, and alternative prevention strategies. Integrating these insights into national one health approach could advance sustainable brucellosis control, protecting both animal and public health in Algeria.

Conflict of interest

No potential conflict of interest relevant to this article was reported

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