Spatial heterogeneity and factors associated with bovine brucellosis in Benin: a national epidemiological analysis

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Abstract

Bovine brucellosis remains insufficiently assessed in Benin. This study aims to estimate the seroprevalence of the disease, map its spatial distribution, and identify associated risk factors across all eight agro-ecological zones (AEZ). A multi-stage sampling design was conducted to include 520 cattle herds. Serum samples from breeding females were analyzed using an indirect ELISA. Individual apparent seroprevalence ranged from 2.7% to 13.8% depending on the zone. At the AEZ level, herd-level seroprevalence varied significantly from 38.46% in the south to 92.31% in the north ($p < 0.001$). Spatial autocorrelation analysis (Moran's I = 0.38, p = 0.036) and spatial scan statistics identified a highly significant cluster of infection in the northern and central AEZs. Risk factor profiles were explored using Correspondence Analysis, and their associations with herd seropositivity were quantified using ecological odds ratios (OR). The northern high-risk cluster was associated with a nearly sixfold increase in the odds of herd infection (OR = 5.96, 95% CI: 3.99–8.90). Significant ecological risk factors included the predominance of susceptible zebu breeds such as Goudali and White Fulani (OR = 3.89, 95% CI: 2.56–5.92), contact with wildlife near protected areas (OR = 2.37, 95% CI: 1.50–3.73), and cross-border transhumance (OR = 2.13, 95% CI: 1.36–3.33). Conversely, the use of hired professional labor emerged as a significant protective factor (OR = 0.37, 95% CI: 0.25–0.56). This study provides the first comprehensive national mapping of bovine brucellosis in Benin and highlights the urgent need for targeted, risk-based surveillance in pastoral systems.

Key words: Bovine brucellosis; spatial epidemiology; ecological odds ratio; pastoral systems; transhumance; Benin.

Résumé

La brucellose bovine reste insuffisamment évaluée au Bénin. Cette étude vise à estimer la séroprévalence de la maladie, cartographier sa distribution spatiale et identifier les facteurs de risque associés dans les huit zones agro-écologiques (ZAE) du pays. Un échantillonnage à plusieurs degrés a permis d'inclure 520 troupeaux bovins. Les sérums de femelles reproductrices ont été analysés par ELISA indirect. La séroprévalence apparente individuelle variait de 2,7 % à 13,8 % selon la zone. Au niveau des ZAE, la séroprévalence troupeau variait significativement de 38,46 % au sud à 92,31 % au nord ($p < 0{,}001$). L'analyse d'autocorrélation spatiale (I de Moran = 0,38, p = 0,036) et les statistiques de balayage spatial ont identifié un cluster d'infection hautement significatif dans les ZAE du nord et du centre. Les profils de facteurs de risque ont été explorés par Analyse Factorielle des Correspondances, et leurs associations avec la séropositivité des troupeaux ont été quantifiées par des odds ratios (OR) écologiques. Le cluster à haut risque du nord était associé à une multiplication par près de six du risque d'infection des troupeaux (OR = 5,96, IC 95 % : 3,99–8,90). Les facteurs de risque écologiques significatifs comprenaient la prédominance de races zébus sensibles comme le Goudali et le Peulh Blanc (OR = 3,89, IC 95 % : 2,56–5,92), le contact avec la faune sauvage près des aires protégées (OR = 2,37, IC 95 % : 1,50–3,73) et la transhumance transfrontalière (OR = 2,13, IC 95 % : 1,36–3,33). Inversement, le recours à une main-d'œuvre salariée s'est révélé être un facteur protecteur significatif (OR = 0,37, IC 95 % : 0,25–0,56). Cette étude fournit la première cartographie nationale exhaustive de la brucellose bovine au Bénin et souligne l'urgence d'une surveillance ciblée et basée sur les risques dans les systèmes pastoraux.

Mots clés : Brucellose bovine ; épidémiologie spatiale ; odds ratio écologique ; systèmes pastoraux ; transhumance ; Bénin.

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1 Introduction

Bovine brucellosis, caused by *Brucella abortus*, is a major zoonotic disease responsible for significant morbidity across multiple animal species and represents a persistent public health burden in low- and middle-income countries (Ducrotoy et al., 2017; Horefti, 2023). In Benin, despite the recognized economic and veterinary importance of the disease, epidemiological data remain scarce and geographically fragmented. The only available national-level evidence derives from two studies conducted in specific communes: Noudèkè et al. (2017) reported monthly fluctuations in individual seroprevalence, while Vikou et al. (2018) assessed concurrent bovine brucellosis and tuberculosis at slaughter. Both investigations were, however, limited in spatial scope and did not provide a nationally representative picture of disease distribution or associated risk factors.

This knowledge gap has become particularly critical in the context of the Sedentarization Project for Ruminant Herds in

Benin (ProSeR), a national initiative that seeks to intensify livestock production through the introduction of genetically improved cattle breeds. Any national genetic improvement strategy must be grounded in a rigorous sanitary baseline mapping of priority zoonoses, of which bovine brucellosis is foremost. Without comprehensive seroprevalence data at the national scale, it is impossible to adequately manage health risks associated with animal introductions, design appropriate prophylaxis protocols, or evaluate the effectiveness of planned zootechnical interventions.

This study was therefore designed to provide the first spatially comprehensive assessment of bovine brucellosis across all eight agro-ecological zones (AEZ) of Benin. Specifically, it seeks to *(1)* estimate the herd-level and individual-level seroprevalence of Brucella infection across the agro-ecological areas of Benin; *(2)* map the geographic distribution of bovine brucellosis and identify spatial clusters of elevated seroprevalence; and *(3)* identify and quantify risk factors associated with herd seropositivity across agroecological zones.

2 Methodology

2.1 Study area and design

A cross-sectional serological survey was conducted across all eight agro-ecological zones (AEZ) of Benin, where cattle farming systems are predominantly extensive, in accordance with the agroclimatic classification described by Dassou et al. (2015). This serological phase was preceded by a qualitative investigation into farmers' perceptions of bovine brucellosis (Djibril et al., 2024a), which informed the identification of at-risk herds.

2.2 Study population and strategy sampling

A three-stage sampling procedure was employed. The stage 1 relates to selection of reinforced pastoral camps. Within each AEZ, reinforced pastoral camps, defined as permanent or semi-permanent livestock encampments hosting multiple herds under shared grazing resources, were purposively selected based on criteria derived from the prior qualitative survey (Djibril *et al.,* 2024). Camps were retained when farmers reported a history of reproductive disorders (abortions, retained placentas), which are clinical hallmarks of brucellosis. Three camps were selected per AEZ. The second stage concern the selection of herds within camps. Within each selected camp, herds were enrolled through systematic random sampling. A total of 520 herds were included, with 65 herds per AEZ (approximately 20 herds per camp). This fixed allocation ensured balanced representation across all AEZ, facilitating inter-zonal comparisons. The last stage 3 related to selection of animals within herds. Within each selected herd, blood samples were collected from breeding females (cows and heifers of reproductive age), which represent the primary target population for Brucella serodiagnosis. The number of animals sampled per herd was proportional to the herd size. On average, approximately 50% of breeding females per herd were sampled.

2.3 Data collection procedures

Prior to data collection, the objectives and procedures of the study were explained to each livestock owner. Following these explanations, farmers provided their informed oral consent. Local social services agents and state veterinarians, who maintain regular contact with these pastoral communities, supervised the ethical process and validated the consent obtained. Data were collected using the KoboCollect mobile application. Structured questionnaires gathered information on the socio-demographic characteristics of the farmers (age, sex, ethnicity, education, religion, marital status, household size) and on potential risk factors related to livestock management (cattle breed, herd size, grazing and transhumance practices, veterinary care access, breeding management, contact with wildlife, use of hired labor, and proximity to protected areas).

2.4 Collection of samples and analyses in the laboratory

Blood samples were collected by jugular venipuncture from selected cattle using 5 ml VACUTEST KIMA dry tubes. Tubes were labeled, kept upright, and transported to the laboratory under cold chain conditions (4–8 °C). After clotting, sera were separated by centrifugation and stored at -20 °C until analysis. Antibodies against *Brucella spp.* were detected using an indirect enzyme-linked immunosorbent assay (iELISA) kit (ID Screen® Brucellosis Serum Indirect Multi-species, Innovative Diagnostics, Grabels, France). The test was performed and interpreted according to the manufacturer's instructions. Internal quality control was ensured by verifying that all positive and negative control panel samples (provided by Innovative Diagnostics) yielded expected results in each plate run.

2.5 Data epidemiological and risk factor data

For each herd, several variables were recorded and considered as potential risk factors for bovine brucellosis. These variables included cattle breed composition, which was identified based on standard phenotypic criteria such as coat colour, horn shape, and body conformation. The main genetic types recorded were the Goudali zebu, White Fulani (Peulh blanc) zebu, Borgou, and crossbreds including White Fulani × Somba and White Fulani × Lagunaire. Herd size was also documented and categorized as either small (fewer than 20 head) or large (20 head or more). The study accounted for grazing and transhumance practices by classifying them into sedentary farming, local transhumance, or cross-border transhumance. Furthermore, access to veterinary care was evaluated based on the presence or absence of regular veterinary interventions. Breeding management practices were recorded as controlled, indicating the use of selected bulls or artificial insemination, or uncontrolled, characterized by free mating. The assessment also included wildlife contact, specifically noting the proximity of herds to protected areas, such as the W-Arly-Pendjari biosphere reserve, alongside any reported contact with wild ruminants. Socioeconomic and operational factors were documented as well, including the type of labor utilized (family or hired labor) and the extent of agricultural activities, particularly involvement in cotton cultivation as a primary economic activity. Finally, the GPS coordinates for each sampling site were systematically recorded using KoboCollect.

2.6 Statistical and Geospatial Analysis

2.6.1 Statistical Analysis

Data were cleaned, coded, and organized in a Microsoft Excel database, then analyzed using R software version 4.1.1 (R Core Team, 2021). Herd-level seroprevalence was defined as the proportion of herds with at least one seropositive animal. Individual apparent prevalence was defined as the proportion of sampled animals testing seropositive within a given zone. Proportions were compared using the Chi-square test (χ^2) and pairwise two-proportion Z-tests (*prop.test*), with a significance threshold set at $\alpha = 0.05$. Confidence intervals (95% CI) for proportions were calculated using the Wald formula.

Correspondence Analysis (CA) was performed using the FactoMineR package to explore the multivariate structure of risk factor profiles across AEZs. This exploratory approach was specifically chosen because, in West African extensive pastoral systems, risk factors are strongly intercorrelated, for example, a given ethnic group typically rears a specific breed, practices a particular form of transhumance, and is associated with distinct veterinary care patterns. Under such collinearity, classical multivariable logistic regression may produce unstable coefficient estimates and fail to capture the synergistic risk profiles that characterize each zone (Greenacre, 2017). The CA thus served to identify coherent zone-risk factor clusters rather than to isolate individual variable effects.

Ecological Odds Ratios (OR) and 95% confidence intervals (CI) were subsequently calculated to quantify the strength of association between zone-level risk factor profiles and herd seropositivity. For each risk factor identified by the CA, AEZs were dichotomized into "exposed" zones (where the factor was characteristically present based on the CA classification) and "non-exposed" zones (all remaining AEZs). Herd-level outcomes (seropositive versus seronegative herds) were then cross-tabulated, and OR were computed as: $OR = (a \times d) / (b \times c)$. *where a = seropositive herds in exposed zones, b = seronegative herds in exposed zones, c = seropositive herds in non-exposed zones, d = seronegative herds in non-exposed zones.* The 95% CI was obtained using the Woolf logarithmic method: $95\%\ CI = \exp[\ln(OR) \pm 1.96 \times \sqrt{(1/a + 1/b + 1/c + 1/d)}]$. The Spearman rank correlation coefficient (ρ) was used to assess monotonic associations between zone-level risk factor prevalence and seroprevalence.

2.6.2 Geospatial Analysis

Geospatial analyses were conducted in R using the *sf* (Pebesma, 2018), *spdep* (Bivand & Wong, 2018), and *SpatialEpi* (Kim & Wakefield, 2018) packages. AEZ polygons were constructed by merging commune-level administrative boundaries (GADM v4.1) according to the national agro-ecological classification. Geometric validity was ensured using *st_make_valid*. Geographic centroids were extracted using *st_centroid* and converted to kilometre-based planar coordinates (UTM zone 31N). Global spatial autocorrelation of AEZ-level seroprevalence was assessed using Moran's Index (I), with row-standardized queen-contiguity spatial weights. Local indicators of spatial association (LISA) were computed using *localmoran_perm* with 999 Monte Carlo permutations to generate empirical p-values for each AEZ. The Kulldorff spatial scan statistic (*kulldorff* function) was applied to identify the most likely geographic cluster of elevated seroprevalence, using a Poisson-based log-likelihood ratio (Kulldorff, 1997).

3 Result

3.1 Demographic profile of livestock owners

The Table 1 summarizes the socio-demographic characteristics of the 520 herd owners enrolled across the eight agro-ecological zones. Livestock ownership was overwhelmingly male (97-100%), with women representing marginal minorities only in AEZ 2, 3, and 5 ($p < 0.05$). Monogamy and polygamy were observed in roughly equal proportions (45% each). The majority of owners (80%) were of Fulani ethnicity, practiced Islam, and had received no formal education. On average, household size was 11 members (including five working-age individuals), owner age was 44 years, and livestock farming experience was 24 years. The Bariba and Dendi ethnic groups were present mainly in AEZ 2, 3, and 5, while the Somba were confined to AEZ 1 and 4 (Table 1).

Table 1 : Identification of herd owners participating in the serological study across various agroecological zones.

Variables		AEZ 1 (N=65)		AEZ 2 (N=65)		AEZ 3 (N=65)		AEZ 4 (N=65)		AEZ 5 (N=65)		AEZ 6 (N=65)		AEZ 7 (N=65)		AEZ 8 (N=65)	
		P	CI (%)	P	CI (%)	P	CI (%)	P	CI (%)	P	CI (%)	P	CI (%)	P	CI (%)	P	CI (%)
Sex	Male	100a	0.00	97a	0.04468	98a	0.04	100a	0.00	98.4a	0.03	100a	0.00	100a	0.00	100a	0
	Female	0b	0.00	3b	0.04429	2b	0.04	0b	0.00	1.6b	0.03	0b	0.00	0b	0.00	0b	0
Marital status	Single	0c	0.00	10b	0.07721	17c	0.10	0c	0.00	20c	0.10	12c	0.08	21b	0.10	21b	0.10483
	Monogamous	55.88a	0.13	49a	0.12756	43a	0.13	45b	0.13	36b	0.12	53a	0.13	63a	0.12	63a	0.1232
	Polygamous	44.12b	0.13	41a	0.12445	40b	0.12	55a	0.13	44a	0.13	35b	0.12	16c	0.09	16c	0.09276
Education level	No schooling	88a	0.08	77a	0.10561	80a	0.10	93.5a	0.06	80a	0.10	100a	0.00	100a	0.00	100a	0
	Primary education	12b	0.08	23b	0.10475	20b	0.10	6.5b	0.06	20b	0.10	0b	0.00	0b	0.00	0b	0
Religion	Animism	0c	0.00	4.8b	0.05279	5b	0.05	2b	0.03	10.9b	0.08	0c	0.00	11c	0.08	11c	0.07726
	Christianity	3b	0.04	1c	0.02438	5b	0.05	0c	0.00	1.6c	0.03	12b	0.08	26b	0.11	26b	0.10747
	Islam	97a	0.04	94.2a	0.05682	90a	0.07	98a	0.03	87.5a	0.08	88a	0.08	63a	0.12	63a	0.11737
Ethnicity	Bariba	0c	0.00	13b	0.08114	23b	0.10	0c	0.00	35b	0.12	0c	0.00	0b	0.00	0b	0
	Dendi	9b	0.07	5c	0.05219	11c	0.07	2b	0.03	10c	0.07	25b	0.10	0b	0.00	0b	0
	Peulh	85a	0.08	82a	0.09132	66a	0.11	96a	0.05	55a	0.12	75a	0.10	100a	0.00	100a	0
	Somba	6b	0.06	0d	0	0d	0.00	2b	0.03	0	0.00	0c	0.00	0b	0.00	0b	0

Intra-class percentages that are followed by different letters are significantly different at the 5% threshold. AEZ : agro-ecological zone N : total sample size P : percentage CI : confidence interval

3.2 Seroprevalence of bovine brucellosis

Two complementary indicators of disease burden were computed (Tables 2). Herd-level seroprevalence the proportion of herds with at least one seropositive animal varied significantly across zones (χ^2, $p < 0.001$). The highest rates were recorded in AEZ 4 (92.31%), AEZ 1 (89.23%), and AEZ 5 (86.15%), all of which were significantly higher than the remaining zones (Table 2). The lowest herd-level seroprevalence was observed in AEZ 7 (38.46%) and AEZ 3 (41.62%). Apparent within-herd prevalence, the proportion of individual animals testing seropositive, ranged from 2.7% (AEZ 7) to 13.8% (AEZ 4). Although inter-zonal differences did not reach statistical significance at the individual level, a north-south gradient was observed, with AEZ 4 (13.8%), AEZ 1 (12.48%), and AEZ 5 (12.48%) exhibiting the highest individual seroprevalence (Table 2). The spatial distribution of bovine brucellosis seroprevalence is presented in Figure 1.

Table 2: Seroprevalence of bovine brucellosis according to agro-ecological zones and herds

AEZ	Individual-level prevalence				Herd-level prevalence			
	Herd size (total cows/herd	Observed herd size	Prevalence (%)	CI (%)	Total herds	positive	Prevalences	CI (%)
1	28	3.49	12.48a	0.12	65	58	89.23a	0.08
2	71	7.89	11.11a	0.07	65	40	61.54b	0.12
3	30	1.38	4.6a	0.07	65	27	41.62d	0.11
4	13	1.79	13.8a	0.19	65	60	92.31a	0.06
5	13	1.62	12.48a	0.18	65	56	86.15a	0.08
6	13	1.01	7.8a	0.15	65	34	52.31bc	0.12
7	14	0.38	2.7a	0.08	65	25	38.46d	0.11
8	10	0.49	4.9a	0.13	65	28	43.08cd	0.12
Legend: AEZ: Agro-Ecological Zone					Significance threshold: ***			

Significance threshold: $p < 0.001$ (χ^2). Values followed by different superscript letters differ significantly at the 5% level (pairwise Z-tests).

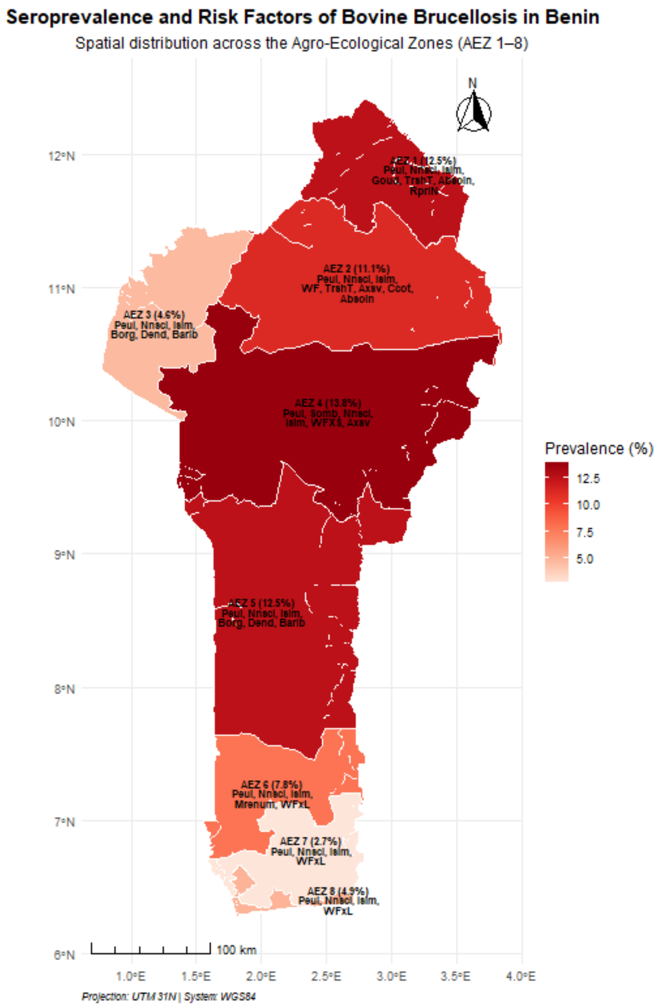


Figure 1. Spatial distribution of bovine brucellosis herd-level seroprevalence across the eight agro-ecological zones of Benin. Colour gradient indicates herd-level prevalence (%). Abbreviations within each zone refer to associated risk factors identified by Correspondence Analysis (see Section 3.4 and Table 4 for details). Projection: UTM 31N; Datum: WGS84.

3.3 Spatial autocorrelation and cluster identification

Global spatial autocorrelation analysis yielded a significantly positive Moran's I index (I = 0.38; Z = 1.80; p = 0.036). The observed index exceeded its theoretical expectation under the null hypothesis of spatial randomness (E[I] = −0.14) by more than 1.5 standard deviations, confirming that geographically proximate AEZs tend to exhibit similar seroprevalence profiles. This spatial clustering is consistent with the epidemiology of bovine brucellosis in West African pastoral systems, where transhumance circuits, seasonal cattle congregation at shared water points, and cross-border trade create spatially correlated exposure conditions.

Local spatial analysis (LISA) classified the AEZs into four quadrants (Figure 2). AEZs 1, 2, 4, and 5 were consistently assigned to the **High-High quadrant**, indicating a coherent high-prevalence cluster in the northern and central regions. AEZs 6, 7, and 8 formed a **Low-Low** southern coldspot. AEZ 3, despite being geographically surrounded by high-prevalence zones, was classified in the **Low-High** quadrant, reflecting its relatively low seroprevalence.

The Kulldorff spatial scan statistic identified a single most likely cluster comprising **AEZ 2 and AEZ 4** in the north-central region (Figure 3).

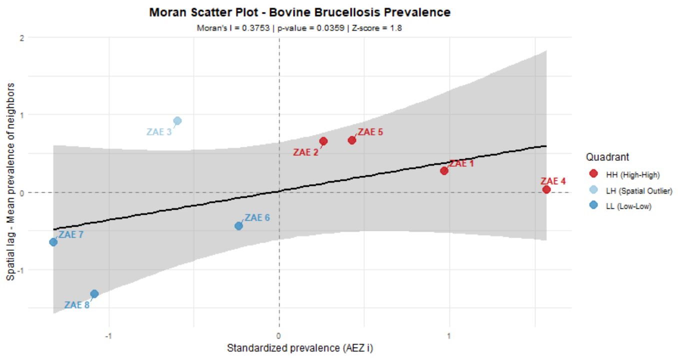


Figure 2. Moran scatter plot of AEZ-level bovine brucellosis seroprevalence. The x-axis represents standardized prevalence; the y-axis represents the spatially lagged prevalence (weighted mean of neighbors). The regression line slope corresponds to Moran's I = 0.38 (p = 0.036). Quadrant colours: red = High-High; blue = Low-Low; orange = High-Low; light blue = Low-High.

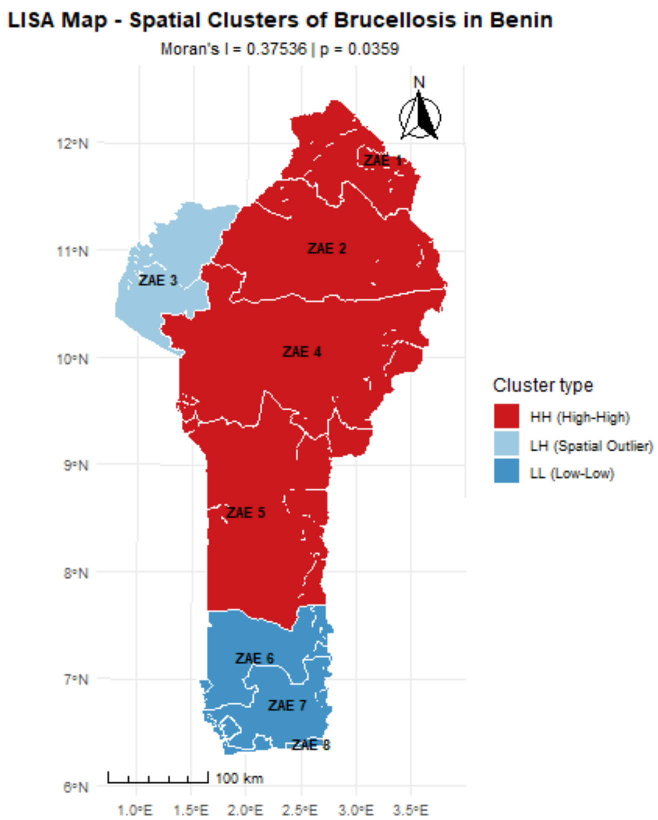


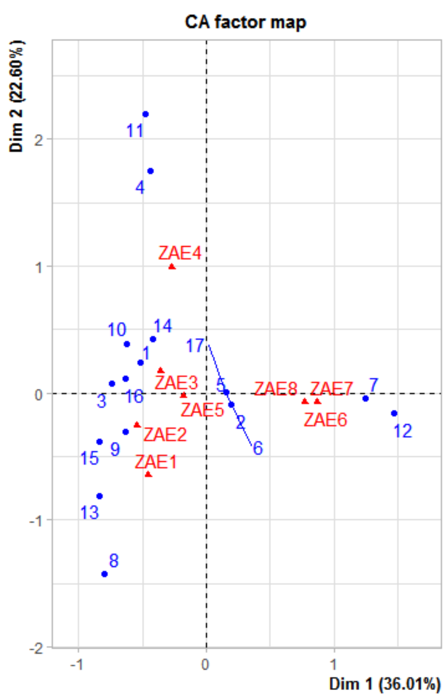
Figure 3. LISA cluster map showing the spatial classification of agro-ecological zones based on local Moran's I values. The primary Kulldorff cluster (AEZ 2 and AEZ 4) is delineated.

3.4 Risk factor profiles identified by Correspondence Analysis

Correspondence Analysis (CA) was performed to explore the multivariate structure of risk factor distribution across the eight AEZs. The first two factorial dimensions accounted for 58,61% of the total inertia. Four distinct zone–risk factor clusters were identified (Figure 4):

- **Group 1 (AEZ 1 and 2):** characterized by White Fulani and Goudali zebu breeds, cotton cultivation, cross-border transhumance, limited access to veterinary care, and unmanaged reproduction.
- **Group 2 (AEZ 3 and 5):** distinguished by a multi-ethnic herder population (Dendi, Bariba, Fulani), Borgou cattle, contact with wildlife, and absence of veterinary care.
- **Group 3 (AEZ 4):** defined by the Somba ethnic group, White Fulani × Somba crossbred cattle, proximity to wildlife, and uncontrolled breeding.
- **Group 4 (AEZ 6, 7 and 8):** marked by the use of hired (paid) labor and White Fulani × Lagunaire crossbred cattle.

Fulani ethnicity, lack of formal education, and Islamic religion were shared characteristics across nearly all zones and did not contribute to inter-zonal differentiation.



Legend: ZAE= Agro-ZoneEcological;
1. Barib=Bariba;
2.Peul=Peulh;
3 Dend=Dendi;
4.Somb=Somba;
5.Nnscl=No formal education;
6. Islm=Muslim;
7. Mrenum= hired labour;
8. Goud= Goudali;
9. WF=White Fulani;
10. Borg=Borgou;
11. WFXS=White Fulani x Somba;
12.WFxL=White Fulani x Lagunaire;
13. TrshT= Cross-border transhumance;
14.Axsv=Wild animals;
15.Ccot=Cotton cultivation;
16.Absoin= Absence of healthcare;
17.RprIN=Unmanaged reproduction

Figure 4. Correspondence Analysis (CA) biplot of risk factor profiles across agro-ecological zones of Benin. Points represent AEZs (triangles) and risk factor modalities (circles). Proximity on the plot indicates statistical association.

3.5 Quantification of risk factor associations

To complement the exploratory CA and quantify the strength of association between risk profiles and herd seropositivity, ecological odds ratios (OR) were computed by contrasting herds from exposed AEZs (where each factor was identified as characteristic by the CA) against herds from all remaining AEZs (Table 3).

Five of the seven ecological risk factors reached statistical significance. The most striking finding was the nearly sixfold increase in the odds of herd seropositivity for the northern high-risk cluster (AEZ 1, 2, 4, 5) compared with the southern zones (OR = 5.96; 95% CI: 3.99–8.90), corroborating the spatial clustering identified by the LISA and Kulldorff analyses.

Among individual risk factors, the predominance of susceptible zebu breeds (Goudali and White Fulani) was the strongest predictor (OR = 3.89; 95% CI: 2.56–5.92). Contact with wildlife near the W-Arly-Pendjari (WAP) biosphere reserve (OR = 2.37; 95% CI: 1.50–3.73) and cross-border transhumance in the far north (OR = 2.13; 95% CI: 1.36–3.33) both significantly increased herd-level infection risk. Cross-border transhumance was further corroborated by a significant Spearman correlation with AEZ-level mean seroprevalence (ρ = 0.732; p = 0.039).

Conversely, the use of hired labor emerged as a significant protective factor (OR = 0.37; 95% CI: 0.25–0.56), suggesting that professional herd management improves disease surveillance and reduces transmission risk.

Neither large herd size (OR = 1.07; 95% CI: 0.74–1.55) nor cotton cultivation (OR = 0.93; 95% CI: 0.54–1.58) reached significance at the ecological level, likely because their effects are confounded by other zone-specific management factors.

Table 3. Ecological odds ratios (OR) and 95% confidence intervals (CI) for risk factors associated with bovine brucellosis herd seropositivity (N = 520 herds).

Risk factor	Exposed AEZs	Exposed (+/–)	Non-exposed (+/–)	OR	95% CI	p
Northern high-risk cluster	1, 2, 4, 5	214 / 46	114 / 146	5.96	3.99 – 8.90	< 0.001
Goudali / White Fulani breeds	1, 2, 4	158 / 37	170 / 155	3.89	2.56 – 5.92	< 0.001
Wildlife contact (protected areas)	2, 4	100 / 30	228 / 162	2.37	1.50 – 3.73	< 0.001
Cross-border transhumance	1, 2	98 / 32	230 / 160	2.13	1.36 – 3.33	< 0.01
Hired labor (protective)	6, 7	59 / 71	269 / 121	0.37	0.25 – 0.56	< 0.001
Large herd size (mean > 20 head)	1, 2, 3	125 / 70	203 / 122	1.07	0.74 – 1.55	ns
Cotton cultivation	2	40 / 25	288 / 167	0.93	0.54 – 1.58	ns

(+) seropositive herds; (–) seronegative herds; ns: not significant. OR > 1 = risk factor; OR < 1 = protective factor.

4 Discussion

This study constitutes the first spatially comprehensive assessment of bovine brucellosis across all eight agro-ecological zones of Benin. The results reveal a marked spatial heterogeneity in herd-level seroprevalence, ranging from 38.46% (AEZ 7) to 92.31% (AEZ 4), with a clear north-south gradient confirmed by spatial autocorrelation (Moran's I = 0.38; p = 0.036). The ecological odds ratio analysis identified susceptible zebu breeds, wildlife contact, cross-border transhumance, and the absence of professional herd management as significant determinants of herd seropositivity. These findings are discussed below in light of regional and international evidence.

4.1 Spatial heterogeneity and the north-south gradient

The LISA analysis classified AEZs 1, 2, 4, and 5 as a coherent High-High spatial cluster, with a nearly sixfold increase in herd seropositivity compared with the southern zones (OR = 5.96;

95% CI: 3.99-8.90). This north-south gradient is consistent with patterns reported across West Africa. In Niger, Boukary et al. (2013) recorded herd-level seroprevalence of 46.8% in the Niamey peri-urban area and up to 62.5% in transhumant systems, both significantly higher than in sedentary southern herds. Similarly, Akinseye et al. (2016) documented in a systematic review of bovine brucellosis in Nigeria that prevalence was consistently higher in the northern pastoral states (13–20% individual-level) than in the southern humid zones (3-8%). In Ethiopia, Megersa et al. (2011) observed that pastoral areas in the lowlands exhibited significantly higher seroprevalence than mixed crop-livestock highland systems, a gradient attributed to herd mobility and intermingling.

Our findings extend this well-documented West African pattern to Benin, confirming that the northern pastoral corridor, where livestock mobility, porous borders, and limited veterinary infrastructure converge constitutes a persistent hotspot for *Brucella* transmission. The Kulldorff scan statistic specifically identified the AEZ 2- AEZ 4 dyad as the most likely cluster, an area that straddles the W-Arly-Pendjari biosphere reserve and multiple international borders.

4.2 Breed susceptibility as the primary individual risk factor

The predominance of Goudali and White Fulani zebu breeds was the strongest individual-level predictor of herd seropositivity (OR = 3.89; 95% CI: 2.56-5.92). This finding aligns with observations by Kassa (2017) and Assani et al. (2015), who reported greater susceptibility of these zebu types to brucellosis and pasteurellosis in northern Benin compared with local taurine breeds. The Borgou breed (AEZ 3), a stabilized taurine-zebu cross, has been described as inheriting complementary resistance traits from its taurine ancestry (Gbodjo et al., 2014; Koudandé et al., 2009). This may partly explain the significantly lower seroprevalence in AEZ 3 despite its geographic proximity to the high-prevalence cluster, a finding reflected in its Low-High LISA classification.

From a broader perspective, the enhanced susceptibility of *Bos indicus* (zebu) types to *Brucella abortus* relative to *Bos taurus* (taurine) breeds has been documented in several African contexts. In Nigeria, Cadmus et al. (2006) reported significantly higher seroprevalence in trade zebu cattle compared with indigenous taurine breeds. Ducrotoy et al. (2017) emphasized that breed susceptibility, combined with management practices, is a key driver of heterogeneous brucellosis distribution across sub-Saharan Africa. This finding has direct implications for the ProSeR initiative in Benin: any programme introducing improved zebu genetics into southern zones must incorporate systematic pre-movement screening and post-introduction surveillance to prevent the southward expansion of brucellosis.

4.3 Cross-border transhumance and wildlife interfaces

Cross-border transhumance, characteristic of AEZs 1 and 2 along the Benin–Niger border, doubled the odds of herd infection (OR = 2.13; 95% CI: 1.36–3.33) and was significantly correlated with AEZ-level seroprevalence (Spearman ρ = 0.732; p = 0.039). Transhumant herds are exposed to multiple risk pathways: mixing with animals of unknown sanitary status at shared water points and pastures, contact with herds from neighbouring countries where brucellosis is endemic, and prolonged absence from veterinary services (Assani et al., 2015; Lesse et al., 2015). Comparable findings have been reported in Niger by Boukary et al. (2013), who identified transhumance as the primary risk factor for Brucella seropositivity, and in Mali by Sanogo et al. (2012), where nomadic herds showed two to threefold higher seroprevalence than sedentary ones.

Contact with wildlife, assessed through proximity to the WAP biosphere reserve (AEZs 2 and 4), was also significantly associated with herd seropositivity (OR = 2.37; 95% CI: 1.50–3.73). Wildlife–livestock interfaces are increasingly recognized as maintenance reservoirs for *Brucella spp.* in sub-Saharan Africa. Godfroid et al. (2011) demonstrated that African buffalo (*Syncerus caffer*) and other wild ungulates can harbour *Brucella abortus* and serve as spillback sources to domestic cattle. In East Africa, Muendo et al. (2012) reported significantly higher brucellosis seroprevalence in cattle herds adjacent to wildlife conservancies in Kenya. The WAP complex, one of the largest protected area networks in West Africa, supports substantial populations of buffalo, hippopotamus, and wild bovids, all of which represent potential reservoirs. Despite legal restrictions, transhumant herders continue to access peripheral zones of the reserve (Lesse et al., 2015), creating persistent wildlife–livestock contact.

4.4 Protective role of professional herd management

The use of hired (paid) labor for herd management, predominantly observed in the southern AEZs (6 and 7), emerged as a significant protective factor (OR = 0.37; 95% CI: 0.25–0.56). This result suggests that the professionalization of daily herd supervision, through dedicated workers who ensure regular monitoring, prompt removal of aborted material, and timely veterinary consultations, effectively reduces transmission risk. Kassa et al. (2016) noted that hired herders in the Borgou region were more consistently present during calving periods and more likely to implement basic sanitary measures than family members juggling multiple activities. Similarly, Houndékon and Ahossin (2024) observed that the division of labor in Beninese agricultural enterprises, including livestock operations, was positively associated with productivity and health outcomes.

This finding contrasts with the situation in AEZs 1 and 2, where cotton cultivation competes for farmers' attention and labor allocation. Katè et al. (2015) documented that in the Banikoara area (AEZ 2), herders who prioritize cotton farming frequently neglect livestock monitoring during the growing season, a period that coincides with peak calving and maximum brucellosis transmission risk. The non-significance of cotton cultivation as an independent ecological risk factor (OR = 0.93; 95% CI: 0.54–1.58) in our analysis suggests that its effect is mediated through reduced herd supervision rather than acting as a direct pathway.

4.5 Implications for brucellosis control under a One Health framework

From a One Health perspective, the spatial clustering of bovine brucellosis in northern Benin has direct consequences for public health. Brucellosis is a priority zoonosis whose control depends on the active participation and behavioural change of livestock keepers (Zinsstag et al., 2012). Our data indicate that the populations most at risk, Fulani herders managing large zebu herds through cross-border transhumance, are also those with the least access to formal education and veterinary extension services. Dean et al. (2012), in a systematic review of human and animal brucellosis in sub-Saharan Africa, emphasized that effective control requires coordinated surveillance at the human-animal-environment interface, particularly in pastoral settings where exposure pathways are continuous and informal.

The identification of a primary Kulldorff cluster in AEZs 2 and 4 provides a precise geographic target for priority intervention. Ring vaccination strategies, systematic screening of transhumant herds at border checkpoints, and community-based education programmes, delivered through culturally appropriate channels, including Qur'anic schools and local pastoral organizations, represent actionable first steps. Additionally, the protective effect of hired labor (OR = 0.37) suggests that investments in professional herder training programmes may yield measurable reductions in seroprevalence.

5 Conclusion

This study provides the first comprehensive national mapping of bovine brucellosis seroprevalence across all eight agro-ecological zones of Benin. Herd-level seroprevalence ranged from 38% to 92%, with a statistically significant spatial cluster in the northern and central zones (OR = 5.96; 95% CI: 3.99–8.90). The predominance of susceptible zebu breeds (OR = 3.89), wildlife contact near the WAP reserve (OR = 2.37), and cross-border transhumance (OR = 2.13) were identified as significant risk factors, while the use of hired labor acted as a strong protective factor (OR = 0.37). These findings provide a solid evidence base for prioritizing geographically targeted surveillance and control measures in AEZs 1, 2, 4, and 5, and for integrating brucellosis risk assessment into national livestock improvement programmes such as ProSeR.

6 Study limitations

This study has limitations that should be considered when interpreting the results. First, the purposive selection of pastoral camps, chosen based on histories of reproductive disorders, means the study population represents an at-risk sub-population rather than a random sample of all Beninese cattle herds. Consequently, the reported seroprevalence may overestimate the national baseline. Second, the indirect ELISA, while sensitive, may yield false-positive results due to cross-reactivity with antibodies against other Gram-negative bacteria, particularly *Yersinia enterocolitica* O:9 (OIE, 2021). Confirmatory testing using the Rose Bengal test or complement fixation was not performed, which may have led to an overestimation of prevalence. Third, the ecological design of the risk factor analysis, whereby exposure was defined at the zone level rather than at the individual herd level, limits causal inference and may mask within-zone heterogeneity. Finally, unmeasured variables such as herd density, vaccination history, and the timing of transhumance relative to sampling may have residually confounded the associations.

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