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Research Article

Seroprevalence and Risk Factors of Brucellosis in Sedentary Livestock Farms of Hadjer-Lamis Province (Chad)

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Abstract

Brucellosis remains a major bacterial zoonosis affecting both livestock health and public health, causing severe economic losses in developing countries. This study aimed to determine the seroprevalence of brucellosis and identify the associated risk factors within sedentary livestock farms in Hadjer-Lamis Province, Chad. A cross-sectional epidemiological study was carried out between May 2024 and September 2025. A total of 299 blood samples were randomly collected from cattle, sheep, goats, and dromedaries in various sedentary farms. The collected sera were systematically screened using the Rose Bengal Test (RBT) to detect antibodies against *Brucella spp*. The overall seroprevalence of the region was 6.69% (20/299). Among the tested animals, cattle exhibited the highest prevalence rate at 8.86%. Following multivariate logistic regression analysis adjustment, only the cattle species demonstrated a significant association with brucellosis seropositivity (aOR = 3.21; 95% CI : 1.07–9.62 ; p = 0.038). This field study confirms the active circulation of *Brucella spp*. in Hadjer-Lamis Province. The findings highlight an urgent public health need to introduce the Rose Bengal Test as a mandatory sanitary prerequisite before the commercialization of fresh raw milk or its processing into dairy products in sedentary livestock farms to mitigate human contamination risks.

Keywords : Brucellosis, Sedentary livestock farming, Risk factors, Rose Bengal, Chad.

1. Introduction

Chad is among the leading livestock-producing countries in the Sahel region, where the livestock sector plays a strategic role in the national economy. According to the General Livestock Census, this sector contributes significantly to the gross domestic product, employs nearly 40% of the active population, and constitutes an essential source of income for rural households[1,2].

The national livestock herd is estimated at over 120 million head, including cattle, sheep, goats, and camels[3]. Despite this potential, the livestock sector continues to face low productivity, largely due to animal diseases. Among these, abortive diseases constitute a major obstacle, leading to abortions, stillbirths, and infertility, with significant economic consequences[4,5,6]. Some of these diseases also pose a risk to public health, notably brucellosis. This is a bacterial zoonosis caused by species of the genus *Brucella*, intracellular coccobacilli lacking a capsule and not spore-forming[7,8]. The disease primarily affects the reproductive system of animals, causing abortions, retained placentas, and decreased fertility. Transmission is facilitated by close contact between animals, sharing of pastures and watering points, and extensive livestock farming practices[9,10]. In humans, brucellosis manifests as undulant fever and constitutes a significant public health problem[11,12].

In Chad, several studies have reported the presence of brucellosis with varying prevalence levels depending on the region and animal species[6,13,14,15]. However, in the Hadjer-Lamis province, characterized by high levels of sedentary livestock farming, epidemiological data remain limited.

In this context, this study aims to estimate the seroprevalence of brucellosis and identify associated risk factors in sedentary livestock farms in this province.

2. Materials and Methods

2.1. Study Areas

The study was conducted in the Hadjer-Lamis Province, located in the Sahelian zone of Chad between 13°00' North latitude and 15°44' East longitude. It is bordered to the north by Kanem, to the south by Chari-Baguirmi, to the west by Cameroon, and to the east by Batha. It has a population of 562,957 inhabitants spread over an area of 31,426 km². The population density of the Hadjer-Lamis Province is therefore 17.9 inhabitants per km². Massaguet, Massakory, and Bokoro are the largest cities in the Hadjer-Lamis Province, which comprises four towns[1,16]. The climate is hot semi-arid, characterized by a short rainy season and a long dry season. The main economic activities of the population are livestock farming, agriculture, and trade.

The population in this area was primarily rural. Livestock farming was generally practiced by Arabs, Kourris, and Fulani. These herders were sedentary. According to the General Livestock Census, the province has approximately 1,841,028 cattle, 1,177,558 sheep, 1,543,302 goats, and 56,834 camels[2].

The study took place between May 2024 and September 2025. ArcGIS version 10.8 software was used to map the study area.

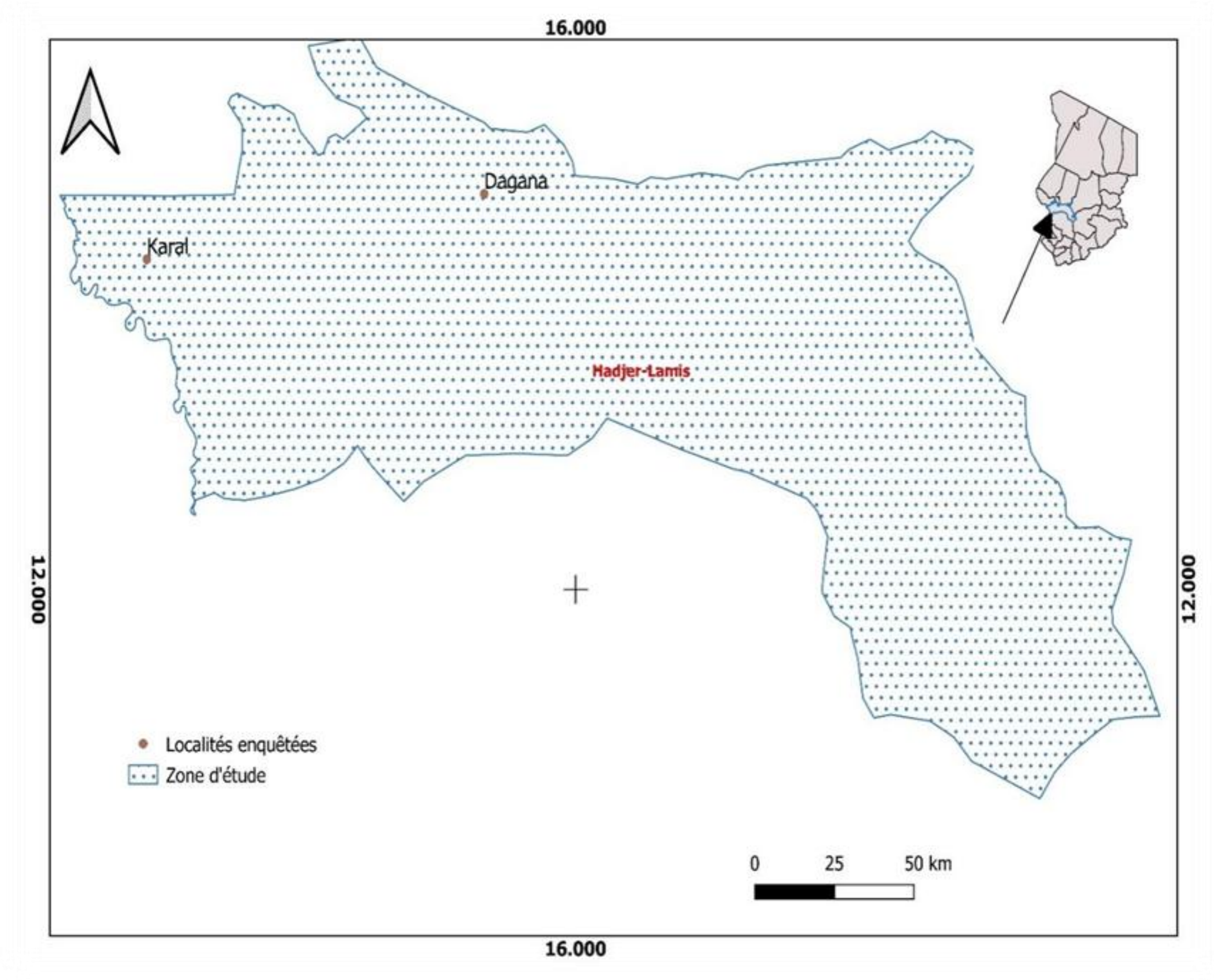

Figure 1 : Presentation of the study area

2.2. Sampling

2.2.1. Study Population

This was a descriptive and analytical cross-sectional study conducted on sedentary livestock farms.

The study population included cattle, sheep, goats, and camels from the departments of Dagana and Haraz Al Biar.

2.2.2. Sample Size :

For animals, the minimum sample size was 281. This calculation was based on the prevalence of 12% published by Abakar Fayiz et al. [13] with a 99% confidence level, given that the population of each species considered in this study in the Hadjer-Lamis region is estimated at least 1,000,000 head[2]. Sample sizes were calculated using OpenEpi version 3 software.

A total of 299 animals were randomly selected from sedentary farms in the Hadjer-Lamis province.

Inclusion criteria were based on animals present in the selected herds at the time of the survey and whose owners had given their consent.

2.3. Biological Materials

Samples were collected in the two departments of Hadjer Lamis province, namely Dagana and Haraz al-Biar. Blood samples were taken from the jugular vein. While the animals were restrained by the herders, 5 ml of blood was drawn using a sterile syringe. The blood was collected in dry tubes labeled with a code based on information about the animal (age, location, sex, breed). After blood collection, the tubes were kept for 30 minutes to one hour to allow the serum to settle. The serum was extracted manually. The samples were decanted to obtain the serum and then stored under refrigeration. The collected serum samples were transported in a cooler containing frozen ice packs to IRED.

2.4. Epidemiological Survey

In parallel with the collection of biological samples, a survey was conducted using simple questionnaires and interviews with the owners of each herd. For this survey, the list of livestock farmers was obtained from the epidemiology department of the Livestock Research Institute for Development (IRED). A random sample was then drawn, selecting approximately ten villages and farms. A total of 57 farmers agreed to participate in the study, while 2 declined. The study aimed to assess the level of knowledge about brucellosis within the communities concerned and to evaluate the risk factors associated with the disease. Information was collected individually on a survey form during household visits.

Furthermore, in each herd, symptoms related to the disease were assessed, and for each suspected animal, sociodemographic parameters such as age, sex, breed, and the farming method used by the farmer were recorded.

2.5. Rose Bengal Test Protocol

For the identification of anti-*Brucella* antibodies, serum samples were subjected to Rose Bengal tests. These tests are recommended by the World Organisation for Animal Health (WOAH [founded as OIE]) for determining the prevalence of *Brucella spp*. infection within herds and for disease surveillance[17]. The Rose Bengal test is an easy-to-implement method that is widely used in the serological screening of brucellosis, boasting a specificity of 99.95% and a sensitivity of 70–80%[18]. It serves as an excellent screening test. It is a simple, rapid reaction that remains positive for an extended period. It is a qualitative reaction in which a drop of serum is mixed with a drop of antigen on an agglutination plate to observe for an agglutination reaction[19]. The three main anti-*Brucella* immunoglobulin isotypes IgM, IgG1, and IgG2 are detected by the Rose Bengal test. The antigen consists of a suspension of killed, Rose Bengal-stained *B. abortus* cells derived from S-form (smooth) colonies[20]. Operationally, equal volumes (30 µL) of the animal serum and the standardized antigen were brought to room temperature and placed side by side on a clean white plate. The two drops were thoroughly mixed using a sterile applicator stick and rocked gently for exactly four minutes. Any visible agglutination or clump formation within this period was recorded as a positive result, confirming exposure to *Brucella spp*., whereas a completely smooth suspension was classified as negative.

2.6. Statistical Analysis :

The data were analyzed using R Studio software (version 4.5.2).

Prevalence rates were calculated with their 95% confidence intervals (Wilson's method). Associations were tested using the chi-square test. Logistic regression was used to identify risk factors. The significance level was set at $p < 0.05$.

3. RESULTS

3.1. Socio-demographic and cultural characteristics

A total of 57 herders were surveyed, representing 94 selected herds. 299 animals were randomly selected from the Hadjer-Lamis province in Chad. This study took place from May 2024 to September 2025. Most of the respondents were between 45 and 78 years old. All respondents were of Arab (48%), Fulani (34%), and Kouri (18%) ethnicity. None of the herders surveyed had any formal education (100%). The animals drank from communal wells (62.3%), thus promoting mixing and mingling among the animals, or from individual wells (34.8%). More than half of the herders (58%) shared breeding males with each other. Only 10% of the herders had heard of brucellosis; but after translation into Fulani (Bakkaleni), 90% recognized the disease. They were also able to describe some of its manifestations, such as abortion, sterility, hygroma, and juvenile mortality. However, they also knew that Chad has not yet authorized vaccination against brucellosis.

3.2. Overall Seroprevalence

A total of 299 samples were analyzed due to logistical constraints. The animals sampled were classified into three age groups: ≤ 3 years, 4–6 years, and > 6 years.

In this study, serological analyses yielded the following results:

The overall seroprevalence of brucellosis was 6.69%. It was higher in: cattle (8.86%), females (7.14%), and certain localities (Habodo Ferrick: 16.67%).

No significant differences were observed according to: sex, age, or locality (overall).

3.3. Seroprevalence by Department

Table I : Seroprevalence by Department

Modality	N=299	Positive	Prevalence (%)	IC 95% (Wilson)	p-value
Dagana	222	11	4,95	[2,79 – 8,65]	
Haraz Al Biar	77	9	11,69	[6,27 – 20,75]	
p-value (Chi²)					**0,076**

Table I shows the seroprevalence by department. It reveals that the seroprevalence in the department of Haraz Al Biar is significantly higher than that observed in the department of Dagana. Although this difference is borderline statistically significant (χ^2 = 3.13, p = 0.076).

3.4. Seroprevalence by village

Table II : Seroprevalence by village

Modality	N=299	Positive	Prevalence (%)	IC 95% (Wilson)	p-value
Amdougoula	23	3	13,04	[4,54 – 32,13]	
Daral Salam	17	0	0,00	[0,00 – 18,43]	
Djickiné	20	2	10,00	[2,79 – 30,10]	
Drigue	33	0	0,00	[0,00 – 10,43]	
Farcha Nabague	86	6	6,98	[3,24 – 14,40]	
Goz tibné	34	4	11,76	[4,67 – 26,62]	
Habodo	22	1	4,55	[0,81 – 21,80]	
Habodo Ferrick	12	2	16,67	[4,70 – 44,80]	
Kassalaye	2	0	0,00	[0,00 – 65,76]	
Koumboya	14	0	0,00	[0,00 – 21,53]	
Roumai	26	2	7,69	[2,14 – 24,14]	
Taht	10	0	0,00	[0,00 – 27,75]	
p-value (Chi²)					**0,458**

According to the results in Table II, the distribution of seroprevalence by village reveals significant spatial heterogeneity. Habodo Ferrick has the highest prevalence, followed by Amdougoula, Goz tibné, and Djickiné. Several villages show no prevalence, notably Drigue, Daral Salam, Taht, and Kassalaye. This inter-village variability does not reach the threshold of significance (χ^2 = 10.77, p = 0.458).

3.5. Seroprevalence by animal species

Table III : Seroprevalence by animal species

Modality	N=299	Positive	Prevalence (%)	IC 95% (Wilson)	p-value
Cattle	158	14	8,86	[5,35 – 14,32]	
Goat	31	1	3,23	[0,57 – 16,19]	
Dromedary	80	3	3,75	[1,28 – 10,45]	
Sheep	30	2	6,67	[1,85 – 21,32]	
p-value (Chi²)					**0,407**

Table III shows that seroprevalence varies according to animal species : it is highest in cattle, followed by sheep, dromedaries, and goats. This inter-species difference is not statistically significant (χ^2 = 2.91, p = 0.407).

3.6. Seroprevalence by sex

Table IV : Seroprevalence by sex

Modality	N=299	Positive	Prevalence (%)	IC 95% (Wilson)	p-value
Female	210	15	7,14	[4,38 – 11,45]	
Male	89	5	5,62	[2,42 – 12,49]	
p-value (Chi^2)					**0,818**

Table IV shows seroprevalence by sex. This table indicates that seroprevalence is slightly higher in females than in males. The difference between the two sexes is not statistically significant (χ^2 = 0.055, p = 0.818).

3.7. Seroprevalence by age group

Table V : Seroprevalence by age group

Modality	N=299	Positive	Prevalence (%)	IC 95% (Wilson)	p-value
≤ 3 years	75	6	8,00	[3,72 – 16,37]	
4-6 years old	141	9	6,38	[3,39 – 11,69]	
> 6 years	83	5	6,02	[2,60 – 13,34]	
p-value (Chi^2)					**0,867**

According to Table V, the animals were divided into three age groups : ≤ 3 years (n=75), 4–6 years (n=141), and > 6 years (n=83). The prevalences were 8.00%, 6.38%, and 6.02%, respectively. No significant difference was observed between the groups (χ^2 = 0.282, p = 0.867).

3.8. Risk Factors for Brucellosis

3.8.1. Multivariate Logistic Regression

Table VI : Risk factors for brucellosis

Variable (category vs ref.)	β	SE	aOR	IC 95% Down	IC 95% High	p-value	Sig.
Species							
Cattle vs. Other Species	1,166	0,559	**3,21**	1,07	9,62	**0,0378**	*
Sex							
Female vs Male	0,247	0,580	1,28	0,41	4,01	0,6666	ns
Department							
Haraz Al Biar vs Dagana	0,381	0,550	1,46	0,50	4,27	0,4928	ns
Age range (ref: 3-5 years)							
≤3 years (Ref.)	-0,274	0,908	0,76	0,12	4,62	0,7650	ns
4-6 years old	-0,013	0,592	0,99	0,31	3,14	0,9843	ns
>6 years	-1,268	1,149	0,28	0,03	2,68	0,2717	ns

*β: logistic regression coefficient. SE: standard error. aOR: adjusted odds ratio. 95% CI calculated by exponential transformation (β ± 1.96 × SE). Significance: * p < 0.05; ns: not significant. Method: BFGS. Species reference = other species; sex reference = male; department reference = Dagana; age reference = 3–5 years.*

According to Table VI, based on multivariate logistic regression, the bovine species is considered a factor influencing the prevalence of brucellosis (aOR = 3.21) [95% CI : 1.07–9.62] p = 0.038*. Cattle are 3.2 times more likely to be seropositive compared to other species, all other things being equal. The other variables studied did not have a significant influence on infection.

Based on the p-value (5%), the adjusted odds ratio (aOR) for the bovine species is 3.21. It is therefore greater than 1, which mathematically confirms that it is a risk factor.

3.8.2. Bivariate Analyses

Table VII : Risk Factors — Animal Species (Ref : Bovine)

Species	N	Pos.	Prevalence (%)	OR raw	IC 95%	IC 95% High	p-value
Cattle [Ref]	158	14	8.9%	—	—	—	—
Dromedary	80	3	3.8%	0.40	0.11	1.44	0.118
Sheep	30	2	6.7%	0.73	0.16	3.41	1.000
Goat	31	1	3.2%	0.34	0.04	2.71	0.472

OR : Crude Odds Ratio. 95% CI calculated using Woolf's method. p-value: two-sided Chi^2 test. [Ref] : reference category.

According to the results in Table VII, cattle exhibit the highest seroprevalence, making them the reference category (OR=1.00). Dromedaries (OR=0.42), sheep (OR=0.34), and goats (OR=0.20) all have odds ratios below 1, and no difference was statistically significant at the α=0.05 level in bivariate analysis.

4. Discussion

The present study aimed to estimate the seroprevalence and risk factors for brucellosis in sedentary livestock herds in the Hadjer-Lamis province (Chad). The main finding of this study is the identification of cattle as a major risk factor, with a risk increased 3.2-fold after adjustment for other variables. This study revealed that brucellosis is endemic in Chad—specifically in the Hadjer-Lamis province—and underscores the need to implement the Rose Bengal test.

4.1. Methodology

While the sample size is normal, it has limitations. It is particularly marked by reductions in the sample size, notably among goats and in certain villages. This reduction led to wider confidence intervals and may have reduced the statistical power to detect other potentially real differences. Furthermore, the cross-sectional nature of the study highlights associations but does not allow for the establishment of a formal causal link. However, the great strength of this study lies in the use of multivariate logistic regression. This analytical method made it possible to control for potential confounding factors and to robustly identify cattle as an independent risk factor, thus providing valuable epidemiological data.

4.2. Socio-demographic and Cultural Characteristics

Brucellosis remains a neglected zoonotic infection in Chad. According to the survey results, all the livestock farmers interviewed belonged to the Arab (48%), Peulh (34%), and Kouri (18%) ethnic groups. Ethnic affiliation appears to be linked to specific livestock management practices that facilitate the spread of brucellosis—an observation that corroborates findings from West Africa, where herds belonging to Fulani herders showed significantly higher infection rates[21].

The complete lack of formal education among the surveyed livestock farmers (100%) likely contributes to the persistence of practices associated with a high risk of transmission : watering at communal wells (62.3%), lending of breeding males (58%), failure to isolate females during calving (98%), and a lack of disinfection (96%) or protective measures (91%). Paradoxically, while few farmers were familiar with the medical term "brucellosis," 90% recognized the disease by its name in the Peulh language ("Bakkaléni") and were able to describe its clinical manifestations, such as abortion, sterility, hygroma, and juvenile mortality[8].

Furthermore, the absence of vaccination in Chad is primarily attributable to a lack of financial and logistical resources, but also to the failure to enforce existing regulations. Although animal brucellosis is officially designated as a disease under surveillance (Law No. 04-009/PR/2004), the responsible ministry has not organized any vaccination campaigns since 2014[15].

4.3. Prevalence of Brucellosis

The overall seroprevalence obtained in this study (6.69%) falls within the range observed across the African continent, varying from 0.034% in Botswana to 30% in Niger[22]. However, it proves to be lower than the rates reported by other West African studies—such as in Togo, where Bawa et al. [8] and Koutinhouin [23] found respective rates of 13.46% across different prefectures ; or in Mali, with 22.08%[24]; and in Burkina Faso, with 13.2%[25]. In countries neighboring Chad, prevalence rates vary : 12.2% in cattle in the Central African Republic[22], 14.8% in goats in Libya[26], 13.3% in Nigeria[27], and 11.09% in Niger[28]. In Cameroon, Eko et al. [11] report a prevalence ranging from 1.1% to 9.12%, while in Sudan, Musa and Shigidi [29] obtained a rate of 10.3%.

These variations can be attributed to differences in production systems (extensive vs. intensive) and to the practice of transhumance in the Sahelian zone. The extensive movement of animals in search of water and pasture facilitates their infection and the dissemination of the disease across these territories.

Finally, this average prevalence rate is also explained by the diagnostic tool utilized. The Rose Bengal test is highly sensitive for detecting recent or active cases of the disease, as it identifies both IgM (recent infection) and IgG (past infection). While simple and rapid for herd screening, this test does not, however, allow for the confirmation of chronic or subclinical infections, which are often asymptomatic[30]. Furthermore, Rose Bengal can agglutinate other Pathogens such as *Yersinia*, *Salmonella*, *Escherichia coli*, or the causative agent of tuberculosis which may lead to false positives[24].

5. Conclusion :

This study clearly demonstrates the presence of seroprevalence and risk factors for brucellosis in sedentary livestock farms in the Hadjer-Lamis province (Chad). It confirms the circulation of *Brucella spp*. bacteria in the Hadjer-Lamis province and identifies cattle as a priority target population. The 3.2-fold increased risk identified in cattle justifies the implementation of control measures. These control measures will be implemented in farms throughout Chad and underscore the need to introduce the Rose Bengal test prior to the marketing of fresh milk or its processing into other products in sedentary livestock farms.

Conflict of Interest : The authors declare that there are no conflicts of interest.

Authors' Contributions :

D.M.: Study design, data collection, writing.

N.R.B.N.: Scientific supervision, critical review.

A.M.S.: Contribution to writing.

L.G.R. : Statistical support and data analysis.

B.Z.: Critical review.

M.M. : Critical review.

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