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Research Article

Identification and Prevalence of *Brucella* Species Circulating among Cattle Slaughtered in the Douala and Buea Municipalities of Cameroon

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Brucellosis is a neglected zoonotic disease affecting the livestock sector in low-income countries. Cameroon, a lower-middle-income country in sub-Saharan Africa, has reported the prevalence of brucellosis in regions where livestock rearing is the principal economic activity. However, the presence of the disease has not been reported in southern regions receiving cattle for consumption by their population. In addition, there is no report on the *Brucella* species circulating in Cameroon. This study aimed to determine the prevalence of brucellosis in cattle slaughtered in the Buea and Douala slaughterhouses and identify the *Brucella* species circulating among these animals. A total of 576 cattle serum samples were collected from the Buea and Douala slaughterhouses and analysed by ELISA. Following the ELISA assay, all samples were subjected to polymerase chain reaction (PCR) analysis. The *bcsp31* gene primers were used for the genus-specific PCR. All *bcsp31*-positive samples were subjected to species-specific PCR. Primers targeting the *IS711* gene sequence were used to identify the abortus-melitensis-ovis-suis species. The prevalence of brucellosis in both locations was 3.1% and 5.4% using the ELISA and PCR assays, respectively. Out of the 18 ELISA-positive samples, 5 (27%) were positive with PCR, while 26 (4.7%) of the ELISA-negative samples were positive with PCR. The *Brucella* species in circulation were identified using the Sanger sequencing technique. The sequences were 99.3% to 100% identical to the *B. abortus* strain BJ1-23 and the *B. abortus* strain BJ1-1 of the 31 kDa antigen (*bcsp31*) gene from India. This is the first report on the genotypic characterisation of *Brucella* species in Cameroon and confirms brucellosis in cattle at the Buea and Douala slaughterhouses.

1. Introduction

Brucellosis, caused by a group of bacteria of the genus *Brucella*, is a widespread, endemic, and neglected zoonotic disease in most low-income countries [1, 2]. The genus *Brucella* includes twelve species, some of which are host-specific [3, 4]. The disease affects both humans and domestic animals, particularly cattle [2, 5].

Cattle brucellosis is usually caused by *B. abortus* and occasionally by *B. melitensis* and *B. suis* [6]. The disease can be spread through several routes, including ingesting contaminated feed, water, or milk. It can also be spread through the suckling or licking of an infected placenta, newborn, foetus, or the genitalia of an infected female soon after it has been aborted or after birth [7]. This disease is associated with abortion, death of young ones, stillbirth, retained placenta, the birth of weak calves, delayed calving, male infertility, and a marked reduction in milk yield [8]. Consequently, it has a negative economic impact in low-resource settings where livestock is a source of food security and income [9]. Losses

due to this disease are a direct result of abortions and the associated reduction in milk yield [10].

Brucellosis has been reported in 86 countries worldwide and is a serious threat to livestock and human health globally, with more than 500,000 documented livestock and human cases reported annually [8, 11]. Sporadic cases of cattle brucellosis are often reported in some sub-Saharan African countries, with prevalence reaching 41% in some areas [2, 5, 7]. Cattle brucellosis is endemic in Cameroon, with reported seroprevalence ranging from 2.3–30.8% [6, 10, 12–18]. A recent study investigating brucellosis in domestic animals reported an overall seroprevalence of 6.4%. Prevalence was highest in cattle (9.1%) followed by sheep (8.0%), dogs (6.1%), pigs (1.9%), and goats (1.1%) [2]. Cameroon, therefore, possesses sufficient livestock that can sustain the presence of multiple *Brucella* spp.

The management of animal brucellosis in Cameroon so far has been complex. This difficulty has been mainly due to the uncontrolled movement of diseased animals (mainly cattle) within and outside the national territory [6]. In addition, there is no surveillance system for detecting and slaughtering to eliminate infected cattle from the herds [4, 18]. A review of the literature on brucellosis research in Cameroon revealed that no study has been conducted in regions experiencing the urbanisation of cattle from the regions reported to be endemic for the disease [4, 18]. In Cameroon, only one attempt has been made at speciating *Brucella* in a study by Mitterran et al., who revealed a prevalence of 18.4% for *B. abortus* and 1.7% for *B. melitensis* in cattle [19]. Our study aimed to determine the prevalence of cattle brucellosis in the littoral and south-west regions of Cameroon, by investigating cattle slaughtered at the Buea and Douala slaughterhouses. This study also aimed to identify *Brucella* species circulating in the study areas. The species identification was done using the genus-specific PCR (targeting the 31 kDa *Brucella* antigen; *bcsp31* gene), species-specific PCR (abortus-melitensis-ovis-suis PCR; AMOS-PCR), and Sanger sequencing.

2. Materials and Methods

2.1. Study Areas. This study investigated cattle slaughtered for meat in the Douala and Buea municipalities (Figure 1). Douala is the capital of the littoral region of Cameroon. It is the largest city in Cameroon.

With a population of about 3.9 million [20], and hosts one of the three industrial slaughterhouses in the country [21], this city is located in the monomodal forest zone, characterised by humid tropical evergreen forest, with gigantic trees, and periforest savannah [22]. This vegetation disfavours cattle rearing. Hence, the population in Douala and the neighbouring towns rely on cattle transported from the Adamawa, far north, north, northwest, and west regions of Cameroon as a food source. These cattle are sold at a popular cattle market in Bojongo, Douala.

Buea is a semiurban setting with a population of about 200,000 inhabitants [23]. It has two traditional slaughterhouses. The larger slaughterhouse, serving a greater proportion of its population, was selected for this study. Its population also relies on cattle from the country's northern regions for beef. Cattle at this slaughterhouse are bought from the cattle market in Douala and transported to Buea.

2.2. Study Design and Period. This was a cross-sectional study that investigated serum samples from cattle slaughtered in the Douala and Buea municipalities in the littoral and southwest regions of Cameroon, respectively. These two regions of the country were selected because there is no documented evidence of cattle brucellosis in these regions. The lone slaughterhouse in Douala, where not less than 200 cattle are slaughtered daily, was used as a sample collection site. In Buea, samples were collected from the main slaughterhouse, where not more than five cattle are slaughtered daily, except on feast days. A total of six samples (one from Buea and five from Douala) were collected on each sample collection day. The samples from Buea were collected randomly, while the samples from Douala were collected based on a calculated sampling factor of five, every fifth cow on the chain was sampled before slaughtering. Sample collection was carried out for six months (September 2020 to February 2021).

2.3. Sample Size Estimation. The minimum sample size was calculated using the formula for a cross-sectional descriptive study assuming an unlimited population size [24]:

$$\text{Sample size } (n) = \frac{z^2 \,\mathrm{x}\, (p(1-p)}{d^2} \approx 286 \text{ cattle}, \quad (1)$$

where d is the absolute error or precision (estimated at 5%), z is the z-score of 1.96 for a 95% confidence interval, and p is the expected proportion of the population based on previous studies [24].

2.4. Ethical Considerations. Administrative authorizations to carry out this study were obtained from the Regional Delegations of Livestock, Fisheries, and Animal Husbandry of the littoral (Ref No. 28/AR/DDEPIA-WOURI of 30th June, 2020) and the south west (Ref No. MINEPIA/RD/SRAG/SW/04/1099 of 5th August, 2020) regions. In addition, verbal authorizations were obtained from the Head of each slaughterhouse.

2.5. Blood Collection and Separation of Serum. From each cow, 5 mL of blood were collected by jugular venipuncture using a sterile vacutainer. Each tube was assigned a unique identification code. The blood samples were placed in a cool box containing icepacks with the temperature maintained at +4°C, and the cold chain was maintained during sample transportation. The temperature was monitored using a temperature data logger (Testo 174T, West Chester, USA). In the laboratory, each blood sample was centrifuged at 5000 rpm for 5 min to separate the serum from blood cells. About 1 mL of serum was harvested and transferred into clean 1.5 mL Eppendorf tubes. Serum samples were stored at −20°C until they were analysed. Overall, 576 blood samples were collected.

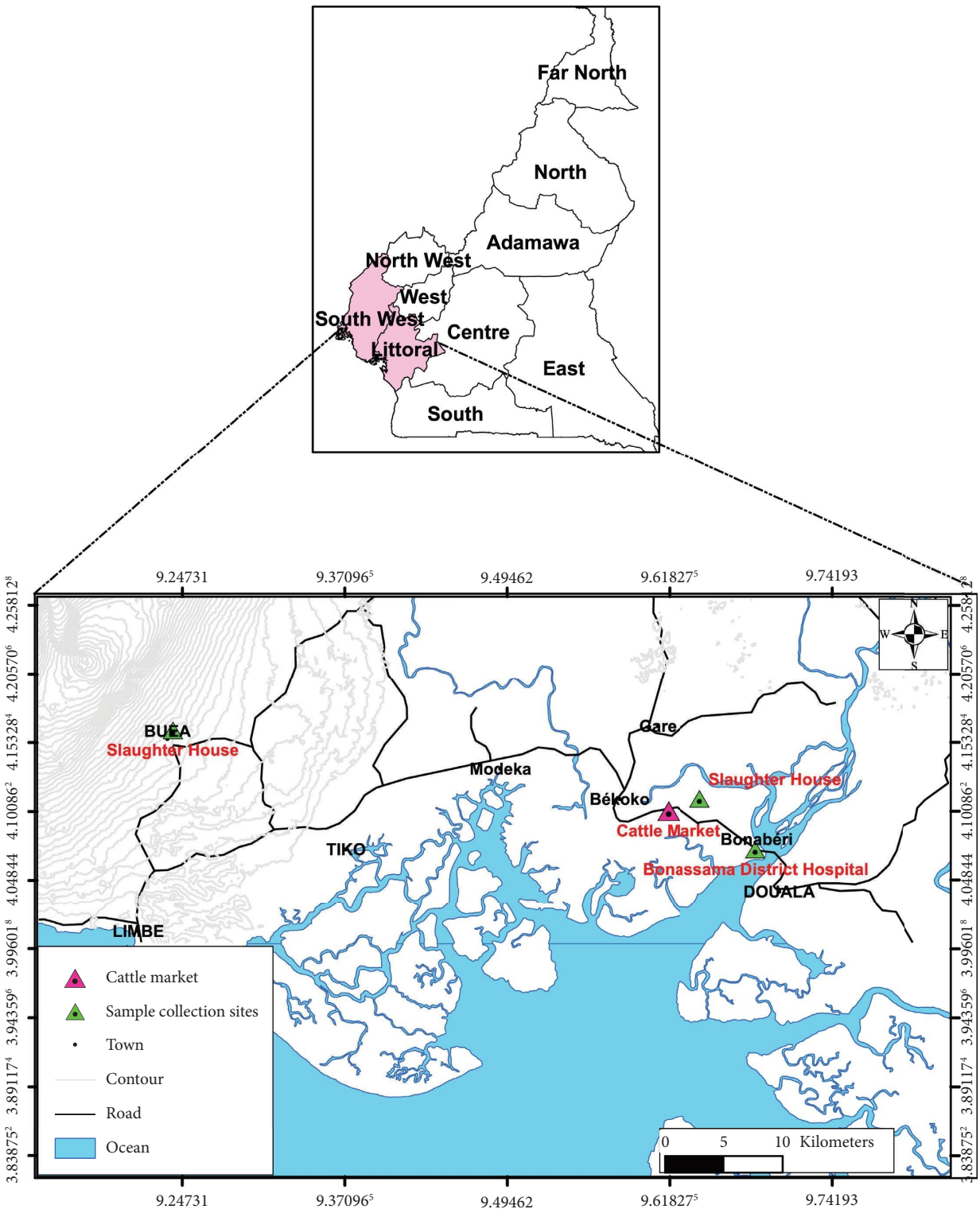

Figure 1: Map of Cameroon showing the sample collection sites in the southwest and littoral regions.

2.6. Collection of Epidemiological Data. The cattle were examined, and demographic data were obtained by trained veterinary staff at the slaughterhouses. A data collection sheet was used to record epidemiological data on cattle breeds, age, and sex. The age was estimated by dental inspection and inspection of horn rings for animals without teeth [17].

2.7. Detection of Anti-Brucella Antibodies Using an ELISA Assay. The indirect enzyme-linked immunosorbent assay (i-ELISA, DRG Instruments GmbH, Germany) was performed to detect the presence of antibodies against *Brucella* spp. according to the manufacturer's instructions without any modifications. An automatic microplate reader (ERBA

MANNHEIM Lisa Scan II) was used to measure optical density. For each sample, the ratio (S/P) was calculated as follows:

$$\frac{S}{P\%} = \frac{\left(OD_{\text{sample}} - \text{OD}_{\text{nc}}\right)}{\left(OD_{pc} - OD_{nc}\right)} \text{ x } 100, \quad (2)$$

where $\text{OD}_{\text{sample}}$, OD_{nc}, and OD_{pc} are the readings of optical densities for the sample, negative control, and positive control, respectively. The samples were considered positive if the S/P ratio was ≥0.4 and negative if <0.4. The test was considered valid if the mean value of the measured OD for the positive control diluted 1 : 100 was ≥1.0 and the mean value for the negative control diluted 1 : 250 was ≤0.35.

2.8. DNA Extraction. DNA was extracted from all serum samples individually, using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). The extraction was performed according to the manufacturer's instructions without any modifications.

2.9. Amplification and Detection of Brucella Genus-Specific DNA. The identification of bacteria of the genus *Brucella* was performed using the B4/B5 primers (Table 1). All primers (Table 1) were synthesised at Inqaba Biotec (Inqaba Biotec, South Africa). PCR reactions were carried out in a final volume of 25 μL, comprising 12.5 μL of One*Taq* 2X Master Mix (New England Biolabs, USA), 0.5 μL (from a 20 μM working solution) of each primer, 5 μL of DNA template, and nuclease-free water to make up the volume. Each PCR run included a positive control of *B. abortus* (vaccine strain) genomic DNA provided by Dr. Abel Wade, Director of the National Veterinary Laboratory (LANAVET), Yaounde, Cameroon. The PCR was performed in a MultiGene OptiMax Thermal Cycler (Labnet International, Edison, NJ, USA) under the following cycling conditions: initial denaturation at 95°C for 5 min followed by 40 cycles of amplification. Each cycle comprised denaturation at 95°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 30 s. At the end of the 40th cycle, a final extension step was performed at 72°C for 5 min.

The presence or absence of the PCR product (223 bp) was determined by agarose gel electrophoresis (1.5%) at 80 V for 45 min. The gel was stained with SYBR Safe DNA Gel Stain (Invitrogen, Thermo Fisher Scientific, USA), and the DNA bands were visualised under UV illumination using the Gel Documentation-XR (BioRAD, Hercules, CA).

2.10. Identification of Brucella Species. All positive samples for the *bcsp31* gene were subjected to the *Brucella* spp. PCR protocol targeting the *IS711* gene. A multiplex PCR assay (AMOS-PCR) using a five-primer cocktail (Table 1), as previously described, was used [26–28]. The multiplex PCR mix comprised 5 μL template DNA, 0.5 μL of each of the forward and reverse primers (to give a final concentration of 0.4 μM), 12.5 μL of One*Taq* 2X Master Mix (New England Biolabs, USA), and nuclease-free water to make up the volume. Both positive and negative controls were included in each PCR run. The PCR cycling conditions were the same as mentioned above, except for the number of cycles that was dropped to 35. Agarose gel electrophoresis was then performed to detect the presence or absence of the PCR product at 80 V for 45 min.

2.11. Differentiation between the B. abortus Wild-Type and the Vaccine Strains. A sixth primer within the *IS711* sequence (Table 1) was added to the AMOS-PCR primer cocktail to differentiate between wild-type and vaccine strains of *B. abortus*. The RB51/2308 primer amplifies a 364-bp sequence which is not present in other wild-type *Brucella* spp. [29].

2.12. Sequencing and Analysis of PCR Products. Selected amplicons of the *bcsp31* gene fragment were sequenced for further identification of bacterial spp. This selection was based on the robustness of the bands after electrophoretic separation. Sequencing was done in both directions for maximum data accuracy at Inqaba Biotech (Inqaba Biotech, South Africa).

Partial DNA sequences of reference *Brucella* spp. were retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/refseq/) for comparison with local DNA sequences of all *Brucella* spp. obtained in this study. The similarities between local sequences and reference strains were determined using the Basic Local Alignment Sequence Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Similarity matches between *Brucella* spp. reported in the study were determined in a pair-wise sequence alignment using BioEdit version 7.2.5. Multiple alignments of the nucleotide and amino acid sequences were carried out using Moleular Evolutionary Genetics Analysis (MEGA) version 11.

2.13. Data Analysis. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 25. Data were described using frequency and proportion at 95% CI. The study indicators were compared among categories of demographic information using cross-tabulation and a test of association, notably the chi-squared test of equality of proportion. This bivariate association test depicted a significant association between two variables.

3. Results

3.1. Cattle Demographic Characteristics. Blood samples were collected from 576 cattle: 465 (80.7%) from Douala and 111 (19.3%) from Buea. The majority of the cattle (77.6%) were matured, and there were more male (96.9%) than female cattle (3.1%). The most predominant breed was the White Fulani/Akou (40.8%), followed by the Red Fulani/Djafoun (27.1%) and Goudali (25.2%) (Table 2).

All cattle slaughtered at the slaughterhouses in Douala and Buea were purchased from the Douala cattle market. In 2020, this cattle market received a total of 62,203 cattle, originating from seven regions of the country. The highest number of cattle were from the north region (42%), the far north (35%), and Adamawa regions (15%) (Figure 2).

Table 1: Details of oligonucleotide primers used in this study.

Gene target	Primer sequence (5′-3′)	Amplicon size (bp)	Purpose of PCR	Reference
Bcsp31	B4-F: tggctcggttgccaatatcaa B5-R: cgcgcttgcctttcaggtctg	223	Genus specific	[25]
*IS711**	F: tgccgatcacttaagggccttcat		Species specific	[26–28]
B. abortus	R: gacgaacggaatttttccaatccc	498		
B. melitensis	R: aaatcgcgtccttgctggtctga	731		
B. ovis	R: cgggttctggcaccatcgtcg	976		
B. suis	R: gcgcggttttctgaaggttcagg	285		
RB51/2308	R: ccccggaagatatgcttcgatcc	364	Vaccine strain identification	[27]

*Same forward primer used to amplify all *Brucella* spp. [27].

Table 2: Cattle demographic characteristics.

Characteristic		Buea ($N = 111$)		Douala ($N = 465$)		Total ($N = 576$)	
		n	%	*n*	%	*n*	%
Sex							
Male	Bull	19	17.1	437	94.0	456	79.2
	Castrated	90	81.1	12	2.6	102	17.7
Female		2	1.8	16	3.4	18	3.1
Age range (years)							
<4 (young)		0	0	4	0.9	4	0.7
4 to 8 (matured)		109	98.2	446	95.9	555	96.4
>9 (old)		2	1.8	15	3.2	17	3.0
Breed							
Local	Red Fulani	43	38.7	113	24.3	156	27.1
	White Fulani	42	37.8	193	41.5	235	40.8
	Goudali	22	19.8	123	26.5	145	25.2
	Bokolo	0	0	6	1.3	6	1.0
Exotic	Crossbreed	2	1.8	29	6.2	31	5.4
	Holstein	2	1.8	1	0.2	3	0.5

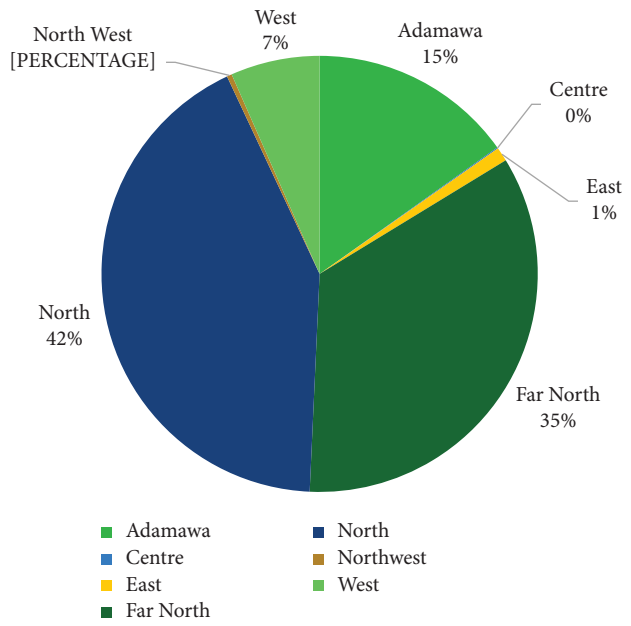

Figure 2: number of cattle that arrived at the cattle market in Douala from different regions of the country in the year 2020.

3.2. Seroprevalence of Cattle Brucellosis. The overall brucellosis seroprevalence was 3.1% (95% CI: 1.9–4.9). Of the 111 cattle from Buea, only one (0.9%, 95% CI: 0.02–4.9) had anti-*Brucella* antibodies. Of the 465 cattle from Douala, 17 (3.7%, 95% CI: 2.1–5.8) also had anti-*Brucella* antibodies (Table 3). However, the difference in prevalence between the

Table 3: Prevalence of cattle brucellosis in cattle slaughtered at the Douala and Buea slaughterhouses based on ELISA and PCR results layered by animal characteristics.

Cattle characteristics		Stats	ELISA				PCR			
			Positive	Negative	N	χ^2-test	Positive	Negative	N	χ^2-test
Sex										
Male	Bull	n	16	440	456	$\chi^2 = 7.304$ $df = 2$ $P = 0.026$	27	429	456	$\chi^2 = 1.711$ $df = 2$ $P = 0.425$
		%	3.5	96.5	100		5.9	94.1	100	
	Castrated	n	0	102	102		4	98	102	
		%	0.0	100.0	100		3.9	96.1	100	
Female		n	2	16	18		0	18	18	
		%	11.1	88.9	100		0	100	100	
Age range (years)										
<4		n	0	4	4	$\chi^2 = 0.563$ $df = 2$ $P = 0.755$	0	4	4	$\chi^2 = 5.689$ $df = 2$ $P = 0.058$
		%	0	100	100		0	100	100	
4 to 8		n	17	538	555		27	528	555	
		%	3.1	96.9	100		4.9	95.1	100	
>9		n	1	16	17		3	14	17	
		%	5.9	94.1	100		17.6	82.4	100	
Breed										
Local	Bokolo	n	1	5	6	$\chi^2 = 4.814$ $df = 5$ $P = 0.439$	0	6	6	$\chi^2 = 5.193$ $df = 5$ $P = 0.393$
		%	16.7	83.3	100		0	100	100	
	Gudali	n	3	142	145		5	140	145	
		%	2.1	97.9	100		3.4	96.6	100	
	Red Fulani	n	4	152	156		8	148	156	
		%	2.6	97.4	100		5.1	94.9	100	
	White Fulani	n	9	226	235		14	221	235	
		%	3.8	96.2	100		6.0	94	100	
Exotic	Crossbreed	n	1	30	31		4	27	31	
		%	3.2	96.8	100		12.9	87.1	100	
	Holstein	n	0	3	3		0	3	3	
		%	0	100	100		0.0	100	100	
Location										
Buea		n	1	110	111	$\chi^2 = 2.247$ $df = 1$ $P = 0.134$	3	108	111	$\chi^2 = 1.938$ $df = 1$ $P = 0.164$
		%	0.9	99.1	100		2.7	97.3	100	
Douala		n	17	448	465		28	437	465	
		%	3.7	96.1	100		6	94	100	
Total		n	**18**	**558**	**576**		**31**	**545**	**576**	
		%	**3.1**	**96.7**	**100**		**5.4**	**94.6**	**100**	

The bold values are the overall total values in each column.

two collection sites was not statistically significant (p value = 0.134). Prevalence according to sex was 11.1% for female cattle, 3.5% for males, and no infection amongst the castrated cattle. There was no discernible difference in predominance between breeds.

3.3. Prevalence of Cattle Brucellosis Using PCR. The genus-specific PCR targeting a fragment of the 31 kDa surface protein amplified DNA segments of molecular weight, 223 bp (Figure 3). The *bcsp*31 gene was detected in 5.4% (95% CI: 3.7–7.6) of the samples (Table 3). The highest infection rates were recorded amongst the bulls (5.9%), matured cattle (5.8%), and cattle from Douala (6.0%). Based on the breed, more infections were seen amongst the White Fulani (6.0%), followed by the Red Fulani (5.1%).

3.4. Correlation between the ELISA and PCR Results. The overall prevalence of brucellosis based on ELISA was 3.1%, while based on genus-specific PCR, it was 5.4% (Table 3). Out of the 18 ELISA-positive samples, 5 (27.8%) were confirmed positive by PCR, while 13 (72.2%) were PCR negative (Table 4). Of the 558 ELISA-negative samples, 26 (4.7%) were PCR positive, while 532 (95.3%) were PCR negative. The difference in results based on PCR and ELISA assays was statistically significant (p-value ≤0.001).

3.5. Identifying Brucella Species in Cattle. The AMOS-PCR was performed on all 31 samples that were positive for the *bcsp31* gene. The four primer pairs failed to amplify any of the species in the samples. The assays were validated by the amplification of the positive control (*B. abortus* vaccine strain) that produced a 498 bp band during each PCR run. Singleplex reactions were performed using each primer pair, and the same results were obtained. Additional primers were used to distinguish the wild type from the vaccine strains. In this reaction, only the positive control amplified a 364 bp DNA segment corresponding to the vaccine strain. Hence, the samples in this study contained only the wild type and not the vaccine strains.

Figure 3: A 1.5% agarose gel of amplified *bcsp31* PCR products (223 bp) from cattle blood. Lane A, 100–1000 bp molecular weight marker; lane B, negative control; lane C, positive control; lanes D, E, G, H, L, M, and N, positive samples; and lanes F, K, N, and O, negative samples.

Table 4: Table showing the cross-tabulation of ELISA and PCR *Brucella* results in cattle.

		PCR		Total
		Positive (%)	Negative (%)	
ELISA	Positive	5 (27.8)	13 (72.2)	**18**
	Negative	26 (4.7)	532 (95.3)	**558**
Total		**31 (5.4)**	**545 (94.6)**	**576**

$\chi^2 = 18.301$, d$f = 1$, and $p \leq 0.001$. The bold values are the overall total values.

3.6. Nucleotide Sequence Analyses of the Brucella bcsp31 Gene. Selected amplicons of the *bcsp31* gene segment were sequenced and analysed. At the nucleotide level, the *bcsp31* gene of the 9 DNA samples had sequence identities ranging from 99.3 to 100% to each other and the reference sequence (Table 5). The BLAST of these sequences revealed homologies of 99.38 to 100% to the *B. abortus* strain BJ1-23 reference sequence (https://www.ncbi.nlm.nih.gov/nucleotide/MK240100.1?report=genbank&log$=nucltop&blast_rank=1&RID=N37SJSN4013), and the *B. abortus* strain BJ1-1 reference sequence (https://www.ncbi.nlm.nih.gov/nucleotide/MK240101.1?report=genbank&log$=nucltop&blast_rank=10&RID=N4JH7HKH016) from India (Table 6). The sequences for eight samples showed 100% homology to each other and the reference sequences. These eight identical sequences comprised four samples from Douala (CMR_DC302, CMR_DC313, CMR_DC40, and CMR_DC182), and four samples from Buea (CMR_BC56, CMR_BC23, CMR_BC31, and CMR_BC54). The sample CMR_DC243 (from Douala) showed 99.3% homology to the local and reference sequences. This difference observed between the sequences was the absence of a nucleotide at position 47 (Figure 4).

4. Discussion

Cameroon possesses sufficient livestock, including cattle, pigs, goats, and sheep, which have the potential to sustain the presence of multiple *Brucella* spp. [18]. These animals serve as sources of food, which are an essential part of the diet of the Cameroonian population [30]. Brucellosis is more of a disease of cattle than any other animals [2]. This sentence has been rephrased to "This justifies the use of cattle for this study." Buea and Douala, the sample collection sites, are towns with minimal cattle-rearing activity. Most of the cattle slaughtered for food in these towns migrated from the country's northern regions, where the disease is reported to be endemic. Hence, the presence of brucellosis in these cattle is a risk factor for the spread of brucellosis to humans and other animals at these locations.

More samples were collected from Douala than from Buea because Douala has an industrial slaughterhouse with a slaughter capacity that is more than 10 times that of Buea. For instance, before the COVID-19 pandemic, up to about 250 cattle were slaughtered daily in Douala [21]. However, these numbers decreased greatly during our study period. The demographic data of the cattle (the majority being matured, and more bulls than cows) are in line with regulations governing the slaughter of cattle in Cameroon. This regulation is stated in Article 17 of Decree No. 76/420 of September 1976, modified by Decree No. 86/755 of 24th June, 1986. The law forbids the slaughter of female cattle under 10 years of age, and male cattle below four years, except in cases of accidents, sterility, or incurable diseases certified by a veterinary officer [31]. Although the difference in the prevalence according to sex was statistically significant ($p = 0.026$), this can be ignored because of the bias in the slaughter of female cattle. This is, therefore, not an accurate representation of the prevalence of the disease in female cattle.

The cattle population investigated in this study differed from that of Awah-Ndukum et al. (2018), who studied a cattle population in the Ngaoundere slaughterhouse. Their study analysed more cows (89.6%) than bulls (10.4%). Up to 10% of their cattle population was less than four years old. This difference could be due to the fact that Ngaoundere is a significant cattle rearing area in Cameroon and may have a population of cattle that were not thriving, such as the young and sterile females that could be sold for slaughter. On the contrary, animals sent to the cattle market in Douala travelled long distances and are expected to arrive with good body scores in order to be sold for slaughter.

Seroprevalence studies have reported higher seroprevalence values in Cameroon, including 9.2% (i-ELISA) from parts of the west and central regions [2], 3.4–5.9% (RBPT

Table 5: Percent identity among local sequences identified in this study and reference sequences retrieved from GenBank.

	MK240100.1	MK240101.1	CMR_DC243	CMR_DC302	CMR_DC313	CMR_BC56	CMR_DC40	CMR_DC182	CMR_BC23	CMR_BC31	CMR_BC54
MK240100.1	ID	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MK240101.1	1.000	ID	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CMR_DC243	0.993	0.993	ID	0.993	0.993	0.993	0.993	0.993	0.993	0.993	0.993
CMR_DC302	1.000	1.000	0.993	ID	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CMR_DC313	1.000	1.000	0.993	1.000	ID	1.000	1.000	1.000	1.000	1.000	1.000
CMR_BC56	1.000	1.000	0.993	1.000	1.000	ID	1.000	1.000	1.000	1.000	1.000
CMR_DC40	1.000	1.000	0.993	1.000	1.000	1.000	ID	1.000	1.000	1.000	1.000
CMR_DC182	1.000	1.000	0.993	1.000	1.000	1.000	1.000	ID	1.000	1.000	1.000
CMR_BC23	1.000	1.000	0.993	1.000	1.000	1.000	1.000	1.000	ID	1.000	1.000
CMR_BC31	1.000	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000	ID	1.000
CMR_BC54	1.000	1.000	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID

ID = identical.

Table 6: Percent homology between the 31 kDa antigen (*bcsp31*) gene sequences of local *Brucella* spp. identified in this study and the reference sequences from GenBank.

Sample codes	Accession number		% homology	Country	*Brucella* spp.
	Sequence from this study	Reference sequence			
CMR_DC243	ON661540	MK240100.1	97.65	India	*B. abortus* strain BJ1-23
CMR_DC302	ON661541	MK240101.1	100	India	*B. abortus* strain BJ1-1
		MK240100.1	100	India	*B. abortus* strain BJ1-23
CMR_DC313	ON661542	MK240100.1	99.42	India	*B. abortus* strain BJ1-23
CMR_BC56	ON661543	MK240100.1	100	India	*B. abortus* strain BJ1-23
CMR_DC40	ON661544	MK240100.1	99.39	India	*B. abortus* strain BJ1-23
CMR_DC182	ON661545	MK240100.1	99.38	India	*B. abortus* strain BJ1-23
CMR_BC23	ON661546	MK240100.1	100	India	*B. abortus* strain BJ1-23
CMR_BC31	ON661547	MK240100.1	99.39	India	*B. abortus* strain BJ1-23
CMR_BC54	ON661548	MK240100.1	100	India	*B. abortus* strain BJ1-23

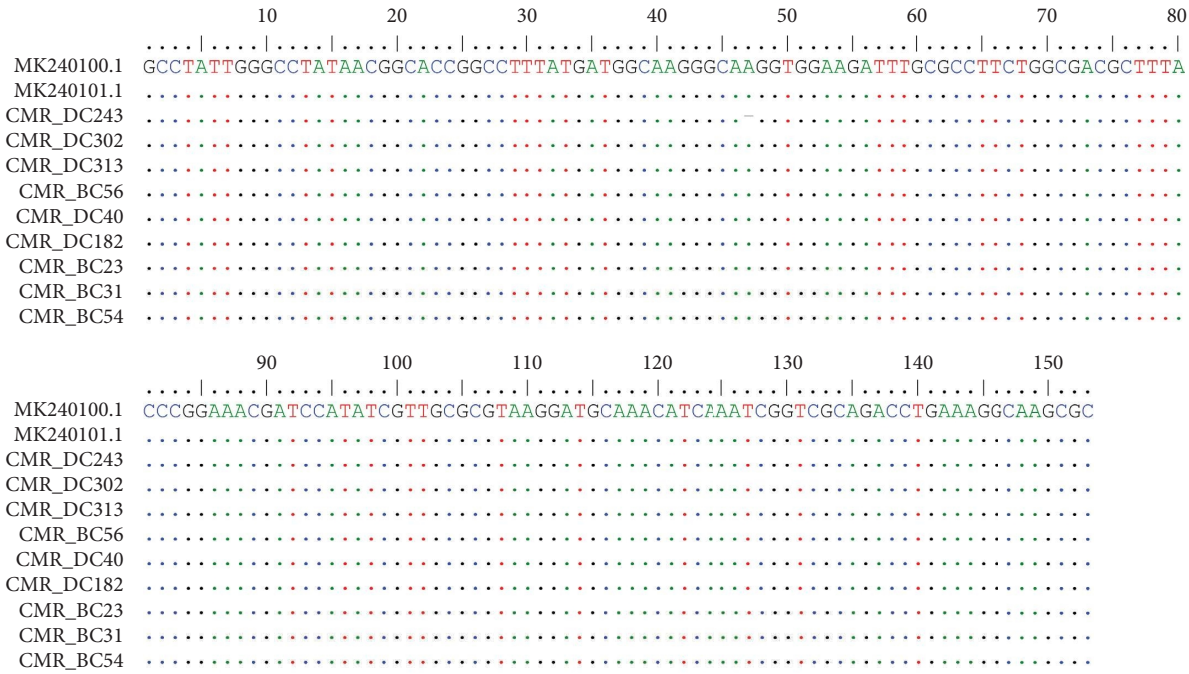

Figure 4: ClustalW alignments of the *bcsp31* DNA sequences between local sequences in this study and reference sequences. Dots represent identical nucleotides. – represents deletion of a nucleotide.

and i-ELISA, respectively) in cattle in Ngaoundere [17], 4.6% (RBPT) in the western highland savannah [16], and 5.2% (c-ELISA) in the northwest region [7]. The higher prevalence in these regions could be attributed to agricultural practices, predominated by the rearing of livestock. In these regions, practice of transhumance on most of the farms was a risk factor for infection [6]. Migratory herds get in contact with other potentially infected herds during their movement, increasing the risk of infection [7]. The differences in prevalence could also be attributed to the use of different serologic assays and the differences in the sample collection sites (some at slaughterhouses and others on farms). These factors explain the difficulty in harmonizing the results from various study sites [18].

The higher prevalence of brucellosis using PCR (5.4%) compared to ELISA (3.1%) could be explained by the ability of the PCR method to detect infection at initial stages, before antibody production [32]. It also detects DNA, which is present in both living and dead *Brucella* organisms [33]. The PCR test is therefore important in cases where animals present with brucellosis clinically and serological tests are negative, allowing the rapid confirmation of the brucellosis [33].

The AMOS-PCR, commonly used for identifying species in the genus *Brucella*, did not identify any species in our study. This may be an indication of the great diversity of *Brucella* spp. circulating in this study area. It has been reported that the AMOS-PCR does not identify all biovars of the four target species. It identifies only three *B. abortus* biovars, 1, 2, and 4; all three biovars of *B. melitensis*; and biovar 1 of *B. suis* and *B. ovis* [27, 34, 35]. In the United States of America, this method was considered sufficient for

identification of *Brucella*, since these biovars were typically being isolated from its local livestock [27]. Hence, there is a need to employ other approaches in speciating the bacteria in our study population.

Sequence analysis of the *bcsp31* gene locus revealed that the nine amplicons represented *Brucella* strains that share 99.38 to 100% identity with *B. abortus* strain BJ1-23 and *B. abortus* strain BJ1-1 reference sequences from India. This is the first report on the sequencing of *Brucella* spp. in Cameroon, and it is essential to understand the genetic diversity of the pathogen in order to control the disease.

The strains in this study were not vaccine strains because they did not amplify the 364 bp DNA segment of the vaccine strain [27]. Likewise, they were not *B. melitensis*, considering the fact that the AMOS-PCR amplifies all biovars of *B. melitensis* [27]. This could imply that the species in circulation are more diverse than those detected by the AMOS-PCR. Hence, we recommend other molecular methods, such as the real-time PCR and the multiple locus variable-number tandem repeat analysis be used for further speciation [27, 36, 37].

It was impossible to trace the origin of individual cattle to the herds as there was no cattle identification system. Identification systems for cattle are critical in tracing the source of the infection, tailoring interventions, and tracking prevalence within the area [38]. An established tracking system will foster the implementation of the test-and-slaughter approach in controlling the infection [39, 40]. The culling of animals, however, is challenging in resource-poor countries due to the mobile pastoral systems and lack of proper compensation for herders [39]. It is advised that economic sustainability should always be considered before investing resources in the prevention and control of brucellosis [18]. The species of *Brucella* implicated in natural infections must not only be detected but also identified. Because brucellosis is a zoonotic disease, the fight against it in humans and animals is centred on veterinary sanitation measures aimed at reducing or eliminating the disease in farm animals [33].

5. Conclusion

This study was conducted to determine the prevalence of brucellosis in cattle slaughtered in the Buea and Douala slaughterhouses and identify the *Brucella* species circulating among these animals. The prevalence of brucellosis in both slaughterhouses was 3.1% and 5.4% using the ELISA and PCR assays, respectively. The sequences were 99.3% to 100% identical to the *B. abortus* strain BJ1-23 and the *B. abortus* strain BJ1-1 of the 31 kDa antigen (*bcsp31*) gene from India. This report confirms *Brucella* infections in cattle slaughtered at the Buea and Douala slaughterhouses. It is the first report on the genotypic characterisation of *Brucella* species in Cameroon. Further studies are needed to identify the strain diversity in this setting.

Data Availability

All data generated or analysed during this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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