SIEMBRA
REVISTA DE CIENCIAS DE LA VIDA

Original Article

Serological and molecular characterization of *Brucella* spp. in cattle farms in the Guaranda canton

Caracterización serológica y molecular de *Brucella* spp. en ganaderías bovinas del cantón Guaranda

Willian Conde Castillo [1] ✉, Franklin Román Cárdenas [1] ✉

Siembra 13 (1) (2026): e8636
DOI: 10.29166/siembra.v13i1.8636

Received: 24/08/2025
Revised: 20/10/2025 / 31/03/2026
Acepted: 09/04/2026

[1] Universidad Estatal de Bolívar. Facultad de Ciencias Agropecuarias Campus Académico "Alpachaca" Av. Ernesto Che Guevara s/n y Av. Gabriel Secaira, Guaranda, Bolívar, Ecuador.

Correspondence: froman@ueb.edu.ec

Abstract

Brucellosis is a disease caused by Gram-negative bacteria of the genus *Brucella*, primarily causing abortions and infertility, resulting in significant economic losses and posing a risk to public health. The objective of this study was to identify and determine the serological and molecular prevalence and biovars of *Brucella* in dairy cattle farms. Sampling was conducted based on the total estimated number of reproductive-age female cattle in the canton, with a margin of error of 7.3%, selecting one sample for every 146 female cattle. The iELISA test was used for serological diagnosis in 180 bovine serum samples, confirmed by PCR, and genetic sequencing was performed. The results showed a prevalence of 2.8% by iELISA and 3.8% by PCR, with an incidence of 2 new cases per farm. In the San Lorenzo parish, 1.6% of cases were identified, while the Gabriel Ignacio Veintimilla, Guanujo, Salinas, and Simiatug parishes each had a prevalence of 0.5%. PCR and sequencing were performed at Macrogen, Inc., Seoul, South Korea. The obtained sequences were cleaned using Chromas version 2.6 and assembled with BioEdit version 7.2, then entered BLAST to identify the bacteria. A phylogenetic tree of the relationships between the different *Brucella* biovars was constructed using MEGA version 11.13. This is the first study in which the bacterium has been identified through sequencing in the canton of Guaranda.

Keywords: *Brucella*, PCR, phylogenetic tree, sequencing, zoonosis

Resumen

Brucelosis es una enfermedad causada por bacterias gramnegativas del género *Brucella,* provocando principalmente abortos e infertilidad, ocasionando pérdidas económicas significativas, con riesgo para la salud pública, el objetivo es identificar y determinar la prevalencia serológica y molecular, y biovares de *Brucella* en ganaderías bovinas lecheras. Se realizó un muestreo basado en el cálculo total de hembras bovinas en edad reproductiva del cantón, con un margen de error del 7,3%, seleccionando una muestra por cada 146 hembras bovinas. Se utilizó la prueba iELISA para el diagnóstico serológico en 180 muestras de suero bovino, se confirmó mediante PCR, y se realizó secuenciamiento del material genético. Los resultados mostraron una prevalencia del 2,8% mediante iELISA y del 3,8% mediante PCR, con una incidencia de 2 nuevos casos en un mismo predio. En la parroquia San Lorenzo, con el 1,6% de los casos, mientras que las parroquias Gabriel Ignacio Veintimilla, Guanujo, Salinas y Simiatug presentaron una prevalencia del 0,5% cada una. La PCR y la secuenciación se realizaron en Macrogen, Inc., Seúl, Corea del Sur. Las secuencias obtenidas se limpiaron utilizando Chromas versión 2,6 y se ensamblaron con BioEdit versión 7,2 ingresándose posteriormente en BLAST para identificar la bacteria. Con MEGA versión 11,13 se construyó un árbol filogenético de las relaciones entre las diferentes

SIEMBRA
https://revistadigital.uce.edu.ec/index.php/SIEMBRA
ISSN-e: 2477-8850
Frecuency: half-yearly
vol. 13, issue 1, 2026
siembra.fag@uce.edu.ec

biovariedades de *Brucella*. Este es el primer estudio en el que se identifica la bacteria mediante secuenciación en el cantón Guaranda.

Palabras clave: árbol filogenético, *Brucella*, PCR, secuenciación, zoonosis

1. Introduction

Livestock farming in Guaranda not only drives the local economy but also contributes to the supply of meat and dairy products at regional and national levels. However, the presence of diseases such as brucellosis can significantly affect the productivity and sustainability of the livestock sector. Brucellosis is a highly infectious disease that causes substantial economic losses due to reduced productivity, reproductive failures, and trade restrictions, making it a major challenge for the livestock industry.

Brucellosis is caused by several bacterial species of the genus *Brucella* and affects a wide variety of animals, with cattle being the primary host species. Humans may also become infected through the consumption of raw or inadequately pasteurized milk and dairy products, or through direct contact with vaginal secretions and other reproductive fluids from infected animals during handling (World Organization for Animal Health, 2022).

Diagnostic methods for *Brucella* include serological assays for antibody detection, microbiological culture as the reference method for bacterial isolation, and molecular techniques based on the detection of specific genetic markers. Molecular methods offer rapid and sensitive identification, eliminating the need for bacterial culture and reducing the risk of laboratory exposure and contamination. In addition, these techniques enable the differentiation of *Brucella* species and biovars. Accordingly, this study aims to identify *Brucella* at the molecular level and characterize the biovars circulating in livestock farms of Guaranda Canton.

With a population of 26,327 breeding cows, the implementation of good cattle management practices in this canton is essential for maintaining food security and supporting the local economy (Agrocalidad, 2023). Brucellosis, caused by several species of the genus *Brucella*, including *Brucella abortus*, *Brucella melitensis*, and *Brucella suis*, etc., is primarily characterized by abortions and reproductive disorders in livestock, resulting in substantial economic losses for producers (World Organization for Animal Health, 2022). The information generated by this study will contribute to a better understanding of the distribution of brucellosis and provide valuable evidence for the development of effective control and prevention strategies, thereby promoting animal health, food security, and public health.

2. Materials and Methods

2.1. Sample collection

Blood samples were collected from reproductive-age female cattle located in different parishes of Guaranda Canton: Ángel Polibio Chávez (1), Gabriel Ignacio Veintimilla (13), Guanujo (64), Facundo Vela (2), Julio Moreno (1), Salinas (44), San Lorenzo (6), San Luis de Pambil (14), San Simón (9), Santa Fe (2), and Simiatug (24), resulting in a total of 180 samples.

2.2. Sample collection

To obtain the samples, the skin at the venipuncture site was disinfected, and blood was collected from the coccygeal vein using a sterile double-ended needle and a 5 mL yellow-top vacutainer tube. Each sample was labeled with a code indicating the farm of origin, parish, and sector, and subsequently stored in a cooler containing refrigerant gel until laboratory processing.

2.3. Serum collection

Blood samples were centrifuged for 5 min to separate the serum. The serum was subsequently aliquoted into sterile Eppendorf tubes and stored at −20 °C.

2.4. Serological test

Once all samples had been collected, an indirect ELISA (iELISA) test was performed using the VETLIS CHEMTEST ELISA kit to detect *anti-Brucella* antibodies. The test is based on the immobilization of antigens on a microplate, followed by incubation with bovine serum containing antibodies that specifically bind to these antigens. After a washing step, enzyme-conjugated secondary antibodies are added. Subsequently, the addition of the substrate produces a colorimetric reaction proportional to the concentration of antibodies present in the sample. Absorbance values are measured using an ELISA microplate reader and compared with the reference values provided by the manufacturer, allowing the detection of

infection and the determination of antibody levels in bovine serum. The results were interpreted according to the manufacturer's instructions.

2.5. Molecular Tests

The procedure described below was followed:

i. **Extraction of genetic material**. Molecular characterization was performed using DNA extracted from blood samples of animals that tested positive by iELISA (1M, 4M, 5M, 6M, and 7M). In addition, samples 2M and 3M from San Lorenzo Parish were included because these animals had been in direct contact with a seropositive cow from the same herd. Genomic DNA was extracted using the PureLink™ Genomic DNA Mini Kit according to the manufacturer's protocol. DNA concentration (ng uL^{-1}) and purity were determined spectrophotometrically using a NanoDrop instrument based on the A260/A280 absorbance ratio. Since nucleic acids absorb at 260 nm and proteins at 280 nm, samples with ratios between 1.8 and 2.0 were considered suitable for downstream molecular analyses.

ii. **Amplification by PCR**. The samples were sent to Macrogen, Inc. in Seoul, where the PCR analyses were performed. For this study, the universal primers B4 5′-TGGCTCGGTTGCCAATATCAA-3′ and B5 5′-CGCGCTTGCCTTTCAGGTCTG-3′ were used. These primers amplify a 223 bp fragment (Figures 1 and 2) and target an internal region of the BCSP31 gene, which is conserved among all *Brucella* species.
In addition, the universal IS711 primers were used: Forward 5′-TCGCTCGTCTGAACTCGC-3′ and Reverse 5′-AATCCGGCCGTTTGCAGG-3′. These primers amplify a 498 bp fragment and target a specific region of the IS711 insertion sequence, which is widely distributed throughout the *Brucella* genus.
The PCR test using the IS711 primers was performed according to the protocol and conditions established by Macrogen, Inc. (Standard PCR and Purification).
PCR amplification using primers B4 and B5 was performed under the following conditions at Macrogen, Inc.: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. The amplification protocol was completed with a final extension step at 72 °C for 10 min under the Custom PCR and Purification service.

iii. **Sequencing**: The PCR products were sequenced at Macrogen, Inc.

2.6. Phylogenetic analysis

After sequencing, the obtained sequences were subjected to quality control and editing. Electropherograms were visualized and edited using Chromas version 2.6, removing low-quality regions and primer sequences that were not part of the target gene. The edited sequences were then assembled into consensus sequences using BioEdit version 7.2. Subsequently, the consensus sequences were compared against the nucleotide database of the National Center for Biotechnology Information [NCBI] using the BLAST algorithm to identify homologous sequences. This analysis enabled the identification of the bacterial genus and the evaluation of sequence similarity. Fi-

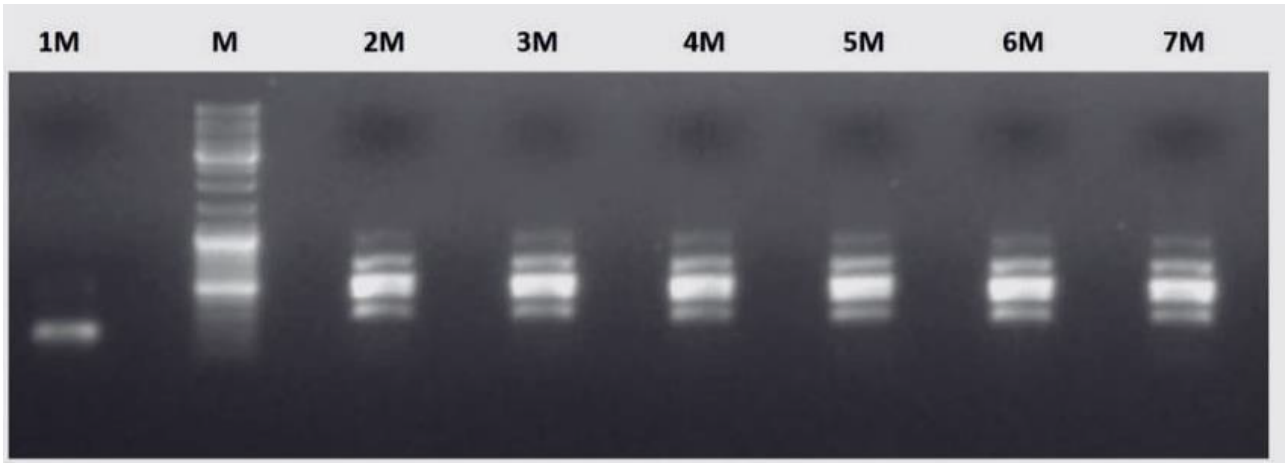

Figure 1. *Brucella* amplification by agarose gel electrophoresis: 223bp band and 498bp bands.

Note: Lane 1M utilized the universal primers B4 and B5, which amplified a specific 223-bp region of the *Brucella* BSCP31 gene, indicating a positive sample. In lanes 2M through 7M, the universal IS711 primers were used to amplify a 498-bp region, confirming the positivity of all analyzed samples. Lane M contains a molecular weight marker used as a reference.

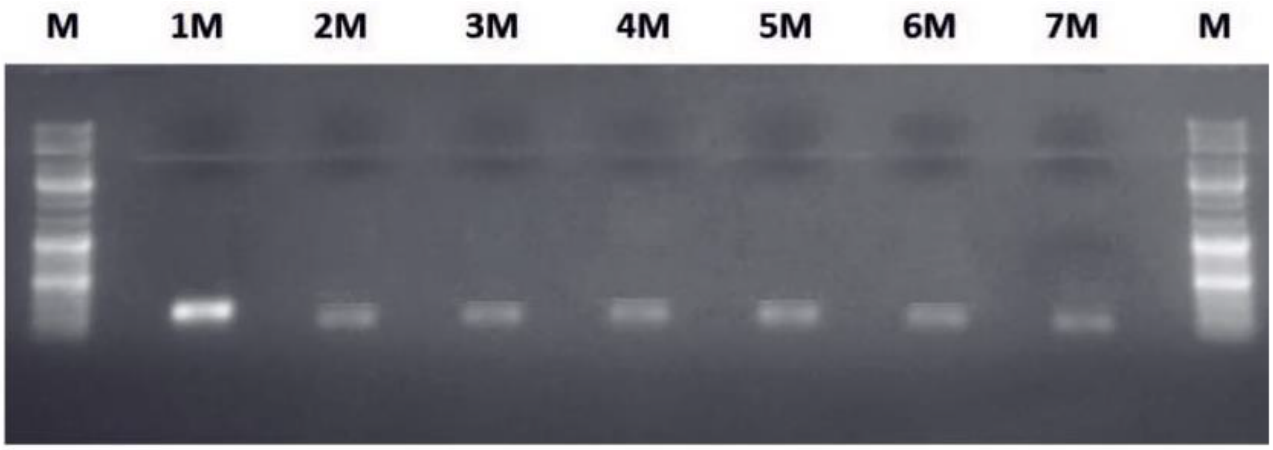

Figure 2. *Brucella* amplification by agarose gel electrophoresis, 223 bp bands.

Note: Lanes 1M through 7M utilized only the universal primers B4 and B5, which amplified a specific 223-bp region of the *Brucella* BSCP31 gene, indicating that all samples were positive. The M lanes contain a molecular weight marker used as a reference.

nally, multiple sequence alignments and phylogenetic analyses were performed using MEGA Software version 11.13 to infer the relationships among different *Brucella* biovars.

3. Results

Of the 180 serum samples collected from female cattle in Guaranda Canton and analyzed using the indirect ELISA (iELISA) test, five tested positive for *Brucella* spp., resulting in an overall prevalence of 2.8%. Positive cases were detected in the parishes of Gabriel Ignacio Veintimilla, Guanujo, Salinas, and Simiatug, each with one positive animal, corresponding to a prevalence of 0.56% per parish (Table 1).

Of the seven samples analyzed, all tested positive by PCR, indicating a prevalence of *Brucella* spp. of 3.8% across the canton. Among the parishes evaluated, San Lorenzo showed the highest prevalence, accounting for 1.6% of the positive cases (Figure 3). The parishes of Gabriel Ignacio Veintimilla, Guanujo, Salinas, and Simiatug each exhibited a prevalence of 0.5% (Table 3).

When comparing the serological and molecular tests for *Brucella* detection, all five animals that tested positive in iELISA also tested positive in PCR, achieving a sensitivity and positive predictive value of 100%. However, the two additional samples that tested positive in PCR were not compared because they were not subjected to serological testing (Table 4).

The dendrogram was generated from the alignment of *Brucella* sequences obtained from Guaranda Canton and their comparison with reference strains

Table 1. Prevalence of brucellosis through indirect ELISA.

iELISA test	Frequency	Percentage
Positive	5	2.8%
Negative	175	97.2%
Total	180	100%

Table 2. Quantity and quality in DNA extractions – NanoDrop.

Sample – Code	Volume	ng uL-1	A260/A280
1M – (7SL)	98uL	14.6	1.69
2M – (8SL)	98uL	32.6	1.79
3M – (9SL)	98uL	19.5	1.90
4M – (5Stg)	98uL	50.1	1.81
5M – (46SS)	98uL	38.5	1.76
6M – (3SIV)	98uL	31	1.76
7M – (33Gjo)	98uL	54.2	1.81

retrieved from the NCBI database (Figure 4).

Sequencing analysis of the universal primers B4 and B5, which amplify a gene encoding an outer membrane protein of *Brucella abortus*, confirmed the presence of *Brucella abortus* in the analyzed samples. This identification was supported by comparison with reference sequences and by its close phylogenetic relationship with *Brucella suis*. The observed genetic distance of 0.20 suggests that these populations share a common ancestor but have accumulated sufficient genetic mutations over time to become distinguishable from one another.

4. Discussion

The prevalence of brucellosis in a region is not merely a statistical measure; it is a key indicator of the health status, economic impact, and sanitary conditions affecting animal populations. In the present study, a prevalence of 2.8% for *Brucella* spp. was detected using the iELISA test. This value is lower than that reported by Ojeda & Román (2018), who found a prevalence of 7.4% in Zamora Chinchipe. Nevertheless, it is possible that additional infected animals

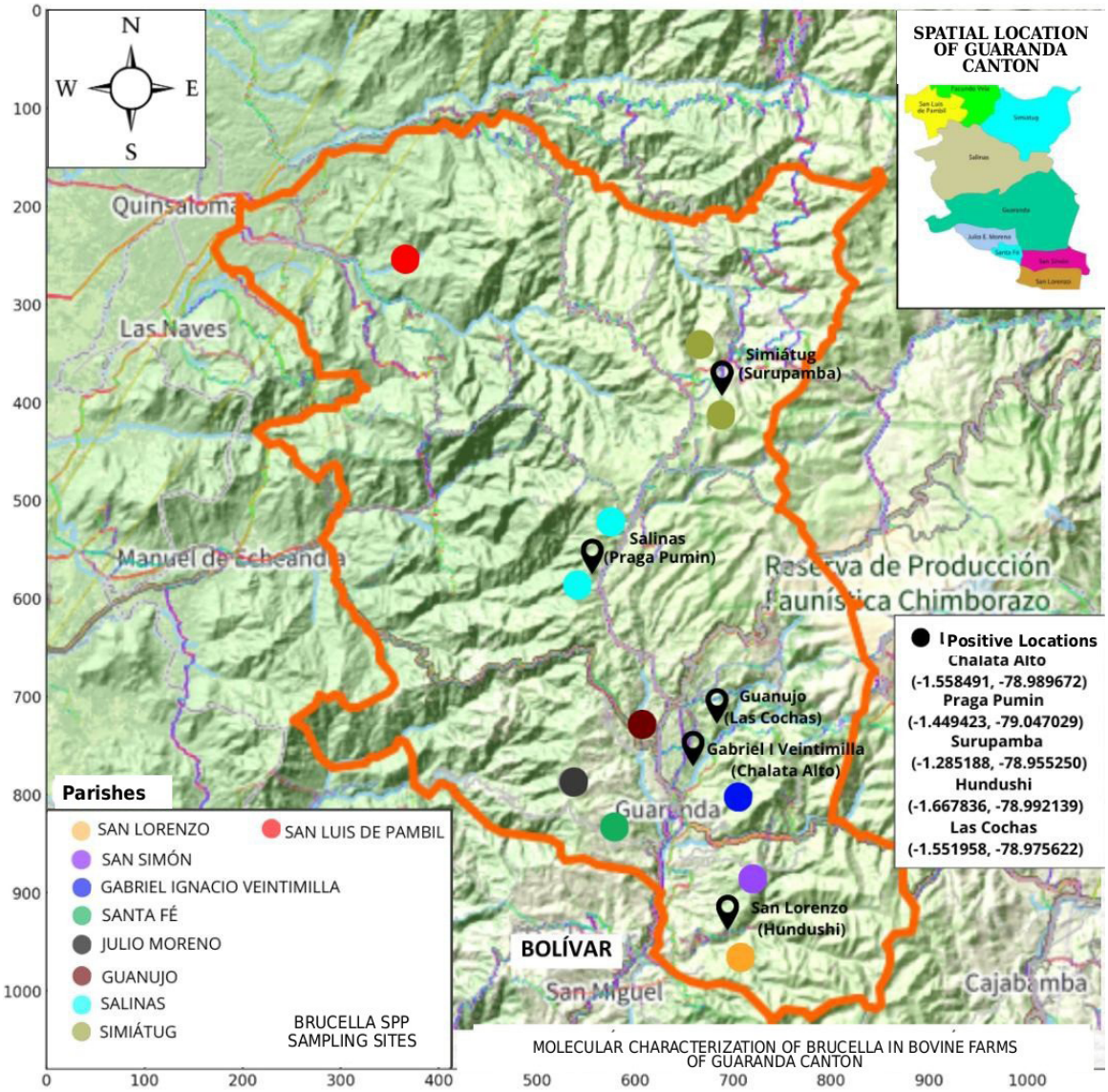

Figure 3. Location of positive cases in the Guaranda canton.

Table 3. Molecular prevalence of brucellosis through PCR testing.

PCR Test	Frequency	Percentage	Cumulative Percentage
Positive	7	3.8%	3.8%
Total	182	100%	100%

Table 4. Comparison of test positivity.

iELISA result	Positive PCR	Negative PCR	Total
Positive	5	175	180
Total	5	175	180

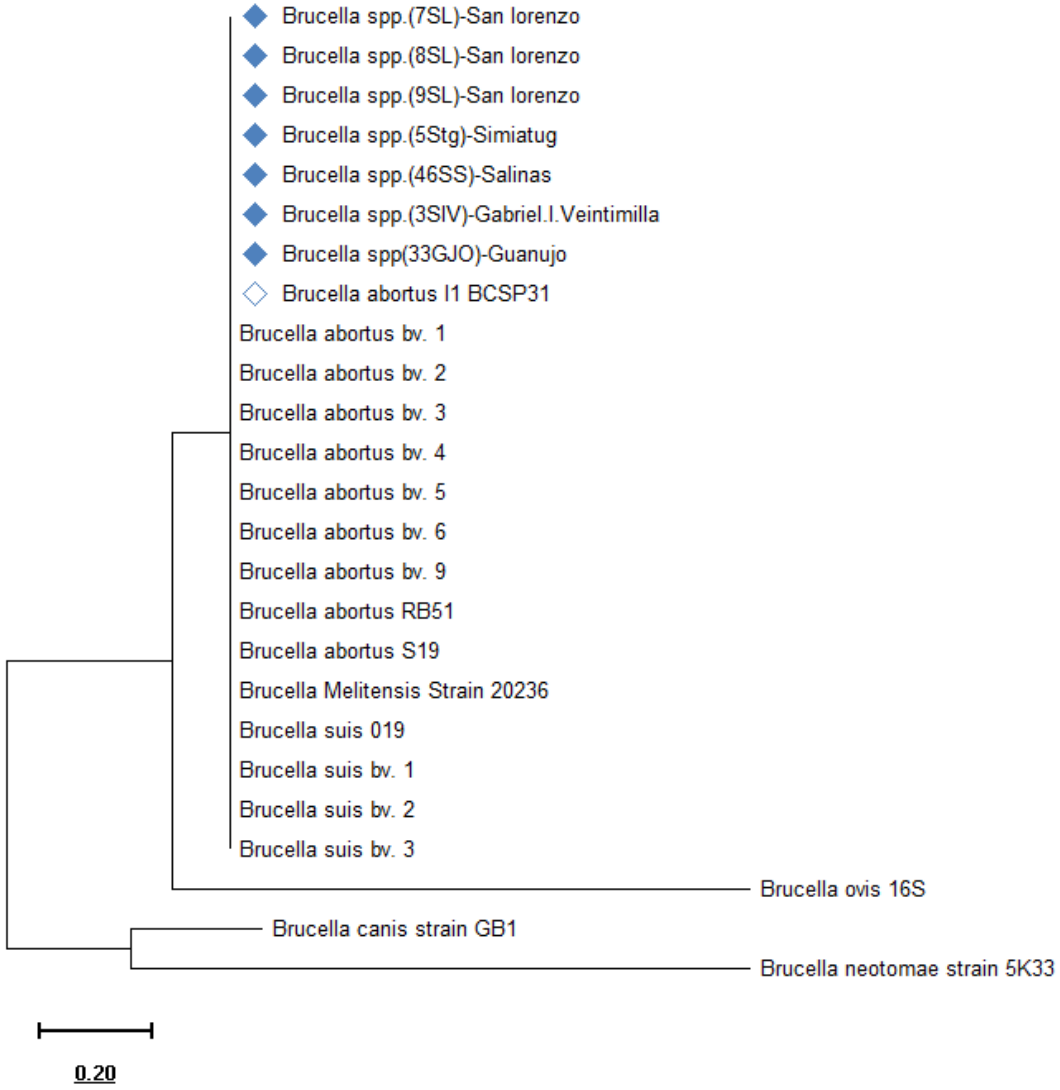

Figure 4. Phylogenetic tree design.

were present on the sampled farms but were not included in the study, which could have led to an underestimation of the true prevalence. The distribution of brucellosis varies considerably among countries and regions. Several European countries have successfully eradicated the disease or maintain prevalence rates close to 0%. In contrast, the epidemiological situation in Latin America remains heterogeneous. While countries with highly mechanized and well-controlled livestock production systems tend to report lower prevalence levels, others continue to exhibit prevalence rates ranging from 10% to 30%, reflecting differences in disease control programs, surveillance systems, and livestock management practices.

For the molecular analysis, DNA concentrations ranged from 14.6 to 54.2 ng μL^{-1}, reflecting variability in the amount of genetic material recovered from the samples. The A260/A280 ratios ranged from 1.69 to 1.90, indicating adequate DNA purity for downstream molecular applications. These results are consistent with those reported by Ojeda & Román (2018), who obtained an average A260/A280 ratio of 1.68 and DNA concentrations ranging from 18 to 60 ng μL^{-1}.

The persistence of the bacteria is influenced by multiple factors, including biological, management-related, and socioeconomic conditions. The

introduction of replacement animals without health certification and the movement of infected animals into disease-free herds are among the main factors contributing to the spread of brucellosis. Extensive livestock systems generally exhibit lower prevalence rates than intensive systems, where high animal density facilitates transmission through direct contact, aerosols, placental tissues, and fluids expelled following abortion. Likewise, natural mating with infected breeding males contributes to the maintenance of the transmission cycle. In the present study, two animals suspected of infection based on clinical signs were included in the molecular analysis. These animals had previously been exposed to a PCR-positive animal, which justified their inclusion in the diagnostic evaluation. Similar findings were reported by Gonuguntla et al. (2023), who investigated the occurrence of new cases within the same farm and identified additional animals that tested positive for the disease.

In PCR for the detection of *Brucella* spp., universal primers IS711 bind to the IS711 insertion element, which is specific to *Brucella* and present in multiple copies in its genome, offering high sensitivity and specificity (Aljanazreh et al., 2023). In contrast, primers B4 and B5 amplify a region of the bcsp31 gene, which is specific to all *Brucella* species and is usually present in fewer copies. This can result in lower sensitivity of detection, but these primers are suitable for sequencing (Zamanian et al., 2015). According to the results of this study, the molecular prevalence of *Brucella* spp. in Guaranda Canton was 3.8% using both types of primers. The difference between the serological and molecular prevalence detected is due to the fact that two additional samples were collected and subjected to molecular analysis from farms previously identified with seropositive animals, confirming that, in farms with seropositive animals, diagnostic tests should be performed on all animals.

In the research conducted by Legesse et al. (2023), it was determined that PCR is more sensitive than ELISA for the detection of *Brucella* in blood and milk samples from infected cows. In this study, all molecular tests performed on blood samples were positive, indicating the high sensitivity of PCR and confirming the results of the serological tests.

5. Conclusions

The epidemiological evidence suggests the presence of bovine brucellosis in the study area. This situation does not appear to result from a lack of diagnostic tools, as current methods provide sufficient sensitivity and specificity for reliable detection, but rather may be associated with deficiencies in the strategic management of control programs.

The diagnosis of brucellosis through serological techniques currently serves as a valuable screening tool due to its low cost. However, the standardization of molecular protocols has become indispensable for modern epidemiological surveillance because of their high analytical sensitivity and their ability to reduce diagnostic time from weeks to only a few hours.

Contributor roles

- Willian Conde Castillo**:** conceptualization, investigation, methodology, writing – original draft.
- Franklin Román Cárdenas: investigation, resources, supervision, validation, writing – review & editing.

Data Availability

Data will be made available on request.

Use of Artificial Intelligence

The authors declare that no Artificial Intelligence was used in the preparation of this manuscript.

Ethical Considerations

The authors declare that there are no ethical implications

Conflict of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

References

Agrocalidad. (2023). Buenas prácticas pecuarias en la producción de carne.

Aljanazreh, B., Shamseye, A. A., Abuawad, A., & Ashhab, Y. (2023). Genomic distribution of the insertion sequence IS711 reveal a potential role in Brucella genome plasticity and host preference. *Infection, Genetics and Evolution*, *112*, 105457. https://doi.org/10.1016/j.meegid.2023.105457

Gonuguntla, H. N., Surendra, K. S. N. L., Prasad, A., Sarangi, L. N., Rana, S. K., Manasa, G., Muthappa, P. N., Harikumar, A. v., & Sharma, G. K. (2023). Brucella melitensis: Divergence Among Indian Strains and Genetic Characterization of a Strain Isolated from Cattle. *Indian Journal of Microbiology*, *63*(3), 272–280. https://doi.org/10.1007/

s12088-023-01081-w

Legesse, A., Mekuriaw, A., Gelaye, E., Abayneh, T., Getachew, B., Weldemedhin, W., Tesgera, T., Deresse, G., & Birhanu, K. (2023). Comparative evaluation of RBPT, I-ELISA, and CFT for the diagnosis of brucellosis and PCR detection of Brucella species from Ethiopian sheep, goats, and cattle sera. *BMC Microbiology*, *23*(1), 216. https://doi.org/10.1186/s12866-023-02962-2

Ojeda, K., & Román, F. (2018). *Identificación molecular de Brucella spp. en muestras de sangre de ganado bovino de la provincia de Zamora Chinchipe.* Centro de Biotecnología UNL https://redi.cedia.edu.ec/document/270533

World Organisation for Animal Health [WOAH]. (2022). *Brucelosis*. https://www.woah.org/fileadmin/Home/esp/Health_standards/tahm/3.01.04_BRUCELL.pdf

Zamanian, M., Hashemi Tabar, G. R., Rad, M., & Haghparast, A. (2015). Evaluation of different primers for detection of Brucella in human and animal serum samples by using PCR method. *Archives of Iranian Medicine*, *18*(1), 44–50. https://www.scopus.com/pages/publications/84920949993