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Prevalence analysis of brucellosis in bovine livestock using real-time PCR technique in Salinas, Guaranda (Ecuador)

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ABSTRACT

Brucellosis, caused by the *Brucella* bacteria in cattle, has a significant impact on the health and reproduction of livestock, leading to economic and zoonotic consequences in Andean communities. Ecuador is not exempt from this reality. In this context, this study aimed to analyze the prevalence of the disease using the qPCR molecular technique. For this purpose, 50 blood samples were collected from cattle in five communities in the Salinas parish, Bolívar province. Evaluating factors such as age, sex, and gestational status revealed that in the Yacubiana community, 1 out of 14 samples tested positive for Brucella, reflecting a 7.14% prevalence in the analyzed livestock. In Mercedes de Pumín, 1 out of 11 samples tested positive, indicating a 9.09% infection rate in that community. However, Apahua, Verdepamba, and San Vicente de Plancha yielded negative results for the pathogen. In summary, 4% of the specimens analyzed showed positivity for *Brucella*, emphasizing the urgent need to implement preventive measures and monitoring in these communities to safeguard the health of the bovine population and prevent the spread of the disease in the region.

Keywords: *Brucella abortus*, brucellosis, cattle, qPCR.

INTRODUCTION

Bovine brucellosis, caused by the bacterium (*Brucella abortus*), is a zoonotic disease that has manifested globally, posing a risk to public health worldwide(1).

The Pan American Health Organization has identified zoonoses such as rabies, tuberculosis, and brucellosis as health and economic problems in Latin America and the Caribbean. These countries have implemented programs to prevent, control, and

eliminate these diseases(2). The AGROCALIDAD agency has launched the national program for the control of bovine brucellosis in Ecuador, dividing the country into five epidemiological regions for more effective management(3).

Brucellosis, an infectious and contagious bacterial disease, affects cattle, particularly pregnant females, showing symptoms such as placentitis and abortions. The highly infectious bacteria primarily target the genital tract and organs, with the mammary gland serving as a crucial source of zoonotic transmission. Besides affecting various stages of an animal's life, brucellosis can also impact other mammal species. The risk of transmission to humans is significant, underscoring the importance of control and prevention programs. Early detection and appropriate measures are essential to mitigate the impact on public health and ensure biosecurity in livestock production(4).

Species associated with this genus face exposure to common disinfectants such as sunlight and the decomposition of tissues or buried carcasses that may be contaminated. In cold climates, these species have the ability to survive for several months. However, during summer or in regions with high temperatures, the vast majority perish within 24 hours(5).

The incubation period of the bacteria varies depending on the host and the bacterial load in the early stages, persisting for several weeks. In the case of pregnancy, the period is longer initially and shorter in later stages. At the end of the pregnancy period, cows may experience premature births, eliminating bacteria located in the uterus through the expulsion of uterine discharges. Although this may temporarily disinfect the cow, it is important to note that it may continue to excrete *Brucella* throughout its productive life (6).

In older cows, the bacteria can reside in mammary glands, pregnant uteri, udders, joint capsules, and synovial sacs. In adult males, its prevalence is found in gonads, vesicular glands, and the prostate, described as accessory sex glands. The bacteria develop in the reticuloendothelial system and are transported to target organs through the blood and lymph. In sexually immature young males, the bacteria reproduce in lymph nodes but may be expelled from the body, being transient in most sexually immature calves(7).

Regarding pathogen detection, the choice of method depends on the specific situation and the study objectives. In clinical or veterinary diagnostic settings, serological tests and molecular biology techniques such as PCR and qPCR are commonly used due to their sensitivity and speed. With the aforementioned background, the aim of this study

was to analyze the prevalence of brucellosis in bovine livestock using the qPCR technique in Salinas, Guaranda, Ecuador.

MATERIALS AND METHODS

The research was conducted in five specific communities of the Salinas parish, Bolívar province, Guaranda canton: Apahua (6 samples), Mercedes de Pumín (11 samples), San Vicente de Plancha (8 samples), Yacubiana (14 samples), and Verdepamba (11 samples). In this study, fifty blood samples from reproductive stage bovines were obtained during the analysis period from February to April 2023.

Analysis methods

Regarding the analysis methods, weight was determined using a bovine-specific measuring tape expressed in kilograms, and the height at the withers was measured in each experimental unit. Age was assessed by observing the dentition of the females, with dental wear indicating the age of the animal. In terms of pregnancy status, the stage of gestation of the cow was considered, being more invasive in the early months. Conception was analyzed through artificial insemination, recognizing the risk of *Brucella abortus* transmission when the semen is infected.

The serological sample consisted of the random collection of whole blood samples from a group of animals to determine the prevalence of *Brucella* in bovine livestock.

Quantitative Polymerase Chain Reaction (qPCR)

Nucleic Acid Extraction

The genetic material extraction was performed using the KadieCheck^{TM} PCR-FLUORESCENCE DETECTION KIT. For reproducible nucleic acid isolation, it is recommended to use extraction kits validated with positive and negative controls. Both positive and negative controls should be fully involved in the nucleic acid extraction process. Ten microliters of internal control were added to the sample to be analyzed, positive control, and negative control respectively before nucleic acid extraction.

Amplification protocol for real-time PCR Bioperfectus STC-96A/96A PLUS System

1) Turn on the Bioperfectus STC-96A/96A PLUS real-time PCR system.

2) Launch version 1.0 of the Bioperfectus STC-96A/96A PLUS real-time PCR system software.
3) Click on "Experiment Wizard" and configure the appropriate parameters in "Projects" and "Settings."
4) Set up the "Plate."
5) Configure the "Sample."
6) Start the PCR: a) Insert the 96-well PCR plate or reaction tubes into the machine. b) Select the "Start Run" button.
7) After PCR, analyze the data by pressing the "Analysis" button on the left side of the menu and analyze the data using "Analyze."

Regarding the PCR description, primers B4 (5'-TGGCTCEGTTGCCAATATCAA-3') and B5 (5'-CGCGCTTGCCTTTCAGGTCTG-3') were used for the identification of *Brucella* spp. These primers amplified a 223 bp fragment that recognizes an internal region of the BCSP31 gene. This gene encodes for a 31 kDa outer membrane protein, conserved in all *Brucella* species.

The reaction conditions included an amplification time of 50-60 minutes and a temperature ranging from 60-95°C.

RESULTS AND DISCUSSION

Identification of the pathogen in the Yacubiana community

In the Yacubiana community, 14 whole blood samples were collected from different experimental units, twelve of which were females and two were males with an average age of 1 to 4 years, of Brown Swiss and Jersey breeds. Among the twelve females, none were pregnant.

Upon analyzing ribosomal DNA amplification using the qPCR technique in 13 samples, the presence of *Brucella abortus* was not detected. However, sample Y4, corresponding to a two-year-old non-pregnant Brown Swiss female, tested positive, as shown in Figure 1. In the FAM detection channel, an "S"-shaped amplification curve (S) and a Ct value < 38 indicate a positive result. This finding suggests that 7.14% of the examined cattle are infected with *Brucella* in the bloodstream.

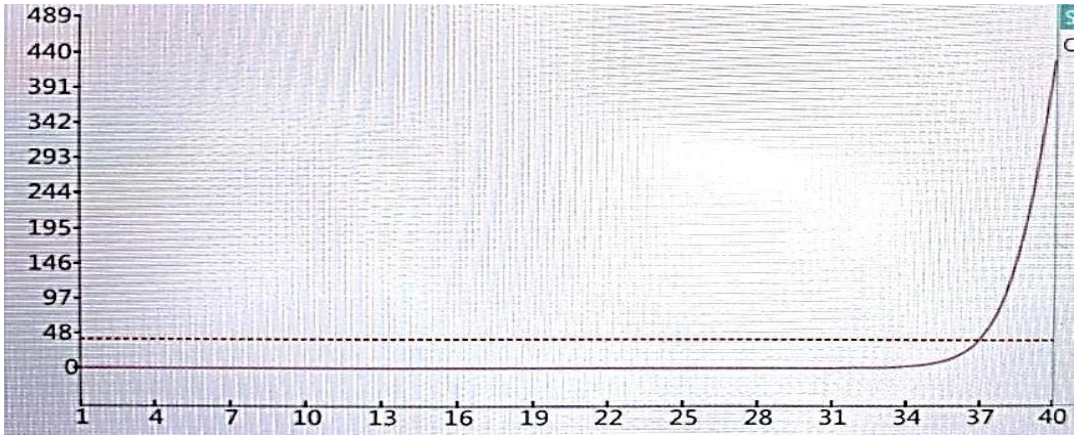

Figure 1: Real-Time qPCR Analysis of 14 Genetic Material Samples

In accordance with the obtained results, the findings of Castro & Leonyork(8) , revealed that, in their study, 5 out of the 7 analyzed samples tested positive, with an average of 71.42%. The variability in the percentages can be explained by the number of randomly selected blood samples for analysis. According to their study, a higher number of samples tends to decrease the positivity rate. This relationship suggests that the extent of sampling can significantly influence the interpretation of *Brucella* prevalence in the studied bovine population.

Identification of the pathogen in the Mercedes de Pumín community

In the Mercedes de Pumín community, 11 whole blood samples were collected from different experimental units, all belonging to approximately 2-year-old females of Holstein and Jersey breeds, with two of them being pregnant.

Upon analyzing ribosomal DNA amplification using the qPCR technique in 10 of these samples, the presence of *Brucella abortus* was not detected. However, in sample P4, belonging to the Jersey breed, weighing approximately 379 kg and aged 1 year and 6 months, tested positive, as shown in Figure 2. In the FAM detection channel, an amplification curve with a typical "S" shape (S) and a Ct value < 38 indicate a positive result. This result suggests that 9.09% of the examined cattle in this community are infected with *Brucella*, demonstrating the presence of the pathogen in the bloodstream of the evaluated bovine population.

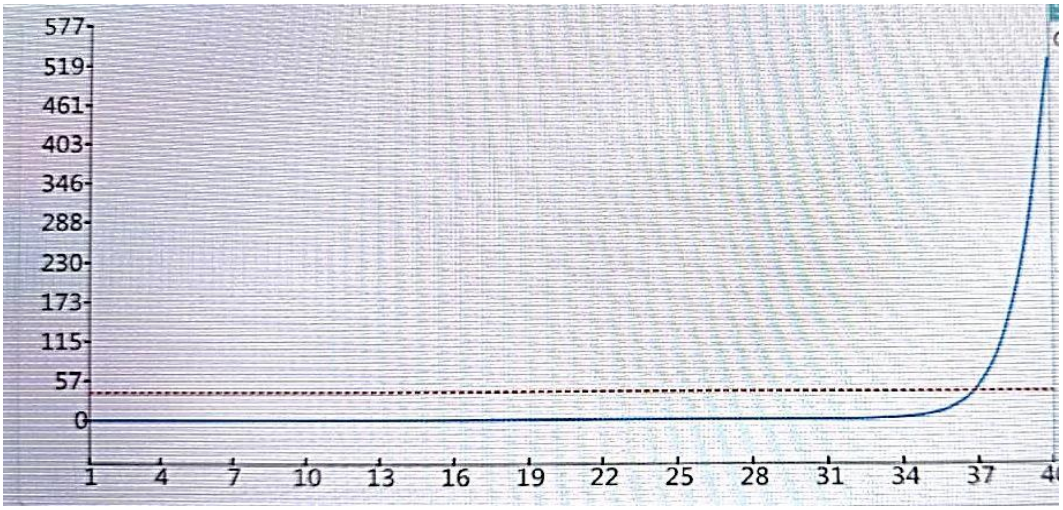

Figure 2: Real-Time qPCR Analysis of 11 Genetic Material Samples

In comparison with the results obtained by Álvarez & Chavarría(9), whose sample collection was carried out in 20 animals of Pardo Swiss breed, we observe a disparity in

the prevalence rates. According to research from the Nicaraguan Institute of Agricultural Protection and Health (IPSA), the second prevalence study per animal for Brucellosis yielded a percentage of 0.18%. However, in our study, which included the random sampling of 11 samples in the Mercedes de Pumín community, we detected a significantly higher prevalence rate, reaching 9.09%. It is important to note that this discrepancy may be attributed to differences in the examined animal populations, as well as to the specific conditions of each study environment. The identification of a single infected animal out of the 11 samples analyzed indicates a considerable presence of *Brucella* in the community, suggesting the need for more rigorous surveillance and control in this particular bovine population.

Identification of the pathogen in the Verdepamba community

In Verdepamba, 11 blood samples were collected from various experimental units, including ten females and one male, all approximately 2 and a half years old, and of Brown Swiss breed. It was identified that one female was in a pregnant state due to natural mating. After analyzing ribosomal DNA amplification using the qPCR technique, the presence of *Brucella abortus* was not detected. These findings indicate the absence of *Brucella* in the evaluated bovine population in Verdepamba, highlighting the importance of maintaining cattle health in this community.

Identification of the pathogen in the Apahua community

In Apahua, six blood samples were collected from various experimental units, including five females (weighing between 454 and 587 kg) and one male of approximately two years old, of Brown Swiss and Jersey breeds. None of the randomly selected animals showed signs of pregnancy, and according to the owner, breeding in their cattle is done through natural mating without artificial insemination. After analyzing ribosomal DNA amplification using the qPCR technique in the six samples, the presence of *Brucella abortus* was not detected. These results indicate that all the examined cattle in Apahua, who showed no signs of infection in their bloodstream, demonstrate 100% negativity for *Brucella*.

Identification of the pathogen in the San Vicente de Plancha community

In San Vicente de Plancha, eight blood samples were collected from experimental units, including seven females and one male, all approximately two years old, of Brown Swiss

and Jersey breeds. None of the randomly selected animals were in the pregnancy stage. After analyzing ribosomal DNA amplification in these samples, Brucella abortus was not detected. The absence of a characteristic "S"-shaped or linear (L) amplification curve in the FAM detection channel, and the lack of a Ct value or a value above 38, leads to classifying the result as negative. This interpretation suggests that all analyzed cattle lack evidence of *Brucella* presence in their bloodstream, indicating 100% negativity against the infection.

Given that the detection results were negative for the presence of the pathogen in the communities of Verdepamba, Apahua, and San Vicente de Plancha, this observation is supported by a study conducted by Álvarez & Chavarría (9). In this work, 173 females were examined in a serological sampling organized by age, obtaining a prevalence of 0% for Brucellosis, which aligns with the absence of infection in our three communities. This consistency of results, showing 100% negativity, supports the strength and reliability of the findings.

Consequently, it is suggested that these communities continue with the existing management protocol for cattle health, but there is an urging to maintain the prevention and control measures highlighted in our research. This will ensure not only the continuity of the absence of *Brucella* infection but also the long-term protection and welfare of the bovine population in these areas.

Conclusion

The analysis of fifty serological samples in the communities of Salinas, Guaranda, revealed a 4% positivity for *Brucella abortus* through qPCR (February-April, 2023). This zoonotic epizootic, posing a high risk to humans, could spread, causing economic losses in the livestock sector of Salinas.

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