Original Article





Spatiotemporal Variation of Bovine Brucellosis (*Brucella abortus*) in Ecuador: A Retrospective Analysis From 2016 to 2023

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How to Cite This Article Barragán-Taco, E., Ordóñez- Andrade, G. M., & Núñez, P. (2026). Spatiotemporal Variation of Bovine Brucellosis (*Brucella abortus*) in Ecuador: A Retrospective Analysis From 2016 to 2023. *Iranian Journal of Veterinary Medicine*, 20(1), 23-34. http://dx.doi.org/10.32598/ijym.20.1.1005793



ABSTRACT

Background: Bovine brucellosis is a zoonotic disease of global significance due to its impact on public health and the economy, particularly in developing countries across Latin America, Africa, and Asia, where control and eradication programs are often ineffective.

Objectives: This study analysed the prevalence of bovine brucellosis (*Brucella abortus*) in Ecuador between 2016 and 2023.

Methods: A total of 1,279 reported cases of bovine brucellosis in cattle, obtained from the records of the Agency for Phytosanitary and Zoosanitary Regulation and Control (AGROCALIDAD), were examined to assess the spatiotemporal variation in prevalence across the country's geographic regions.

Results: The overall prevalence for the 7-year study was 13.7% (95% CI, 10.9%, 16.5%), with the Andean and Amazon regions exhibiting the highest average prevalence rates, at 15.7% and 15.3% respectively, while the Coastal region reported a lower average of 9.5%. Within the Andean region, prevalence varied significantly by province, ranging from 7.8% in Carchi to 29.6% in Loja. In the Amazon region, seroprevalence ranged from 6.8% in Morona Santiago to 22.9% in Napo. Temporally, bovine brucellosis in Ecuador showed considerable fluctuations. During the study period, prevalence ranged between approximately 2% and 52%, with an anomalous peak occurring in late 2022.

Conclusion: Bovine brucellosis in Ecuador shows significant regional variation, with the highest prevalence in the Andean and Amazon regions, especially Loja and Napo, and the lowest rates in the Coastal region. Effective control requires targeted surveillance, mandatory vaccination, and movement restrictions in high-risk areas, supported by training and improved epidemiological data integration to move toward eradication.

Keywords: Animal health, Brucella abortus, Retrospective Study, Zoonotic Disease

Article info:

Received: 01 May 2025 Accepted: 30 Jun 2025 Publish: 01 Jan 2026

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Introduction

in cattle (Constable et al., 2017).

ovine brucellosis is a zoonotic disease with a wide global distribution. It has been successfully controlled and, in some cases, eradicated in several developed countries (More et al., 2017). Brucellosis is primarily caused by *Brucella abortus*, a gram-negative, facultative intracellular coccobacillus. However, in some cases, *Brucella melitensis* and, less frequently, *Brucella suis* have also been identified as causative agents associated with the disease

Cows infected with B. abortus may exhibit infertility problems, characterized by endometritis, retention of fetal membranes, which can lead to the birth of weak calves, decreased milk production, reduced fertility, and abortion (Khairullah et al., 2024). In males, orchitis and epididymitis may occur, beginning with an acute inflammatory phase in the seminal vesicles followed by a chronic phase marked by fibrinoid hardening, necrotic areas encapsulated by fibrous tissue, and in some cases, the formation of soft lesions containing purulent exudate (Khurana et al., 2021).

Globally, numerous studies report varying prevalence rates of bovine brucellosis. For instance, Warioba et al. reported an animal-level seroprevalence of 25.9% in commercial cattle farms in the eastern coastal region of Tanzania (Warioba et al., 2023). In contrast, studies conducted in Ethiopia between 2019 and 2023 reported lower prevalence values, ranging from 1.2% to 2.8%. These differences were associated with risk factors, such as herd size, introduction of new animals, geographic location of the farm, history of abortions, and retention of foetal membranes, both at the individual and herd levels (Demissie et al., 2024; Getahun et al., 2023). This variability in prevalence levels may be related to differences in farmers' knowledge, livestock management practices, and national policies for disease control, as noted by Djibril et al. (Djibril et al., 2024).

According to Bonilla-Aldana et al., the overall prevalence of brucellosis in Latin America and the Caribbean is approximately 4.0%, although significant regional and national differences are observed (Bnilla-Aldana et al., 2023). The prevalence is higher in Central America and the Caribbean (8%) compared to South America (4.0%).

In Ecuador, Garrido-Haro et al. reported a herd-level prevalence of bovine brucellosis at 21.3% and an animal-level prevalence of 6.2% (Garrido -Haro et al.,

2023). They identified herd area (>70 ha) and the number of parturitions per animal (two or more) as significant risk factors. Additionally, geographic location appeared to influence disease occurrence, with the Amazon region showing the lowest prevalence and an absence of reported clinical signs.

Although significant progress has been made in the control and eradication of brucellosis in countries such as Australia, New Zealand, Canada, Japan, and 16 European Union member states, the disease remains a major concern in Turkey and other regions. In these areas, not only does brucellosis affect animal health, but it also poses a serious threat to human health, with reported seroprevalence rates ranging from 34% to 74.4% (Constable et al., 2017; Kazak et al., 2016). The high morbidity rates of human brucellosis underscore its importance as a public health issue, necessitating the implementation of preventive measures and public education initiatives (Varikkodan et al., 2024).

The lack of public awareness, along with the high potential for brucellosis to spread into new areas and its transmission through wild and domestic animals, poses a significant epidemiological challenge for many countries (Khurana et al., 2021). These facts highlight the need for studies that assess the historical prevalence of brucellosis in Ecuador. Therefore, the present study conducted a retrospective analysis of brucellosis across different provinces during the 2016–2023 period, aiming to identify spatio-temporal differences in the prevalence of bovine brucellosis in Ecuador.

Materials and Methods

The research was based on a retrospective study of the prevalence of bovine brucellosis across the different provinces of Ecuador, using data provided by the Agency for Phytosanitary and Zoosanitary Regulation and Control (AGROCALIDAD) from 2016 to 2023. A total of 1,279 cases of bovine brucellosis were analysed, as reported in AGROCALIDAD's official records. To determine the seroprevalence of B. abortus in cattle, a two-tiered serological testing approach was employed in accordance with internationally recognised protocols established by the World Organization for Animal Health (WOAH, 2023). The initial screening was conducted using the Rose Bengal test (RBT) (Barkay et al., 2024) new approaches that can be useful in clinical practice are required, and we aimed to evaluate this situation in our study. Methods: 171 of 213 patients followed in our center between January 2021 and April 2024 were included in the study. A total of 150 patients were included in the

study as a control group. Rose Bengal test (RBT. All RBT-positive samples were subsequently analysed using a competitive enzyme-linked immunosorbent assay (c-ELISA) to increase specificity and reduce false positives due to cross-reactivity with Yersinia enterocolitica O:9 and other Gram-negative organisms. The c-ELISA was performed following the manufacturer's protocol, targeting antibodies against the Brucella smooth lipopolysaccharide antigen (WOAH, 2023).

This dual testing strategy is recognised as a robust approach for large-scale seroepidemiological studies, ensuring both high sensitivity and specificity for the detection of B. abortus infection (Xie et al., 2024).

The analysis assessed variations in the prevalence percentages of *B. abortus* over the years included in the study, as well as fluctuations by month, province, and geographic region within Ecuador. For analytical purposes, data from the three main geographic regions were considered, following the official national territorial classification: the Coastal region, the Andean (Sierra) region, and the Amazon region.

The prevalence of bovine brucellosis in Ecuador was evaluated using descriptive statistical analysis, performed with SPSS software, version 22.0 (IBM Corp.). A time series analysis was conducted to model and fore-

cast the monthly prevalence of bovine brucellosis in Ecuador from January 2016 to December 2023, with projections extending through December 2028.

Results

Prevalence and fluctuation of bovine brucellosis in Ecuador

The overall prevalence for the 7-year study was 13.7% (95% CI, 10.9%, 16.5%). The average prevalence of brucellosis showed slight variations among the three geographic regions of Ecuador during the study period, with higher values recorded in the Andean region and the Amazon region compared to the Coastal region. However, these differences were not statistically significant (P≥0.05; F=0.119; df=2) (Figure 1). The average prevalence in the Andean and Amazon regions was 15.7% (95% CI, 10.9%, 20.4%) and 15.3% (95% CI, 15.4%, 17.%), respectively, while the Coastal region reported a lower average of 9.5% (95% CI, 5.5%, 13.4%).

At the provincial level, prevalence in the Coastal region ranged from 6.0% in Santa Elena, with a single case reported in July 2016, to 15.2% in Guayas, which registered 19 cases between 2016 and 2023 (P< 0.05; F=2.385; df=21) (Table 1). In the Andean region, a wider variation in prevalence levels was observed, ranging

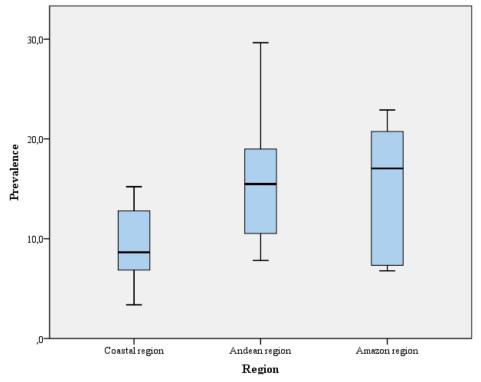


Figure 1. Box-and-whisker plot showing variations in the percentage of bovine brucellosis prevalence among the Coastal, Andean, and Amazon regions during the 2016–2023 period

from 7.8% in Carchi to 29.6% in Loja. In the Amazon region, seroprevalence ranged from 6.8% in Morona Santiago to 22.9% in Napo (Table 1).

Significant differences were identified among provinces (P<0.05; F=2.385; df=21). The highest prevalence rates were reported in Loja (Andean region), Napo, Pastaza, and Sucumbíos (Amazon region), as well as Bolívar, Cañar, Azuay, and Chimborazo (Andean region), with values ranging from 29.64% to 18.39%. These were fol-

lowed by Guayas, Orellana, and Santo Domingo de los Tsáchilas with prevalence rates of 15.21%, 14.4%, and 13.64%, respectively.

Three additional groups with lower prevalence rates were distinguished. The first group included the Andean provinces of Tungurahua, Cotopaxi, and Pichincha, with average values of 12.56%, 12.32%, and 10.52%, respectively, and the Coastal province of Los Ríos, with an average of 11.93%. The next group included Esmeraldas

Table 1. Variation in the prevalence of bovine brucellosis across the provinces of Ecuador from 2016 to 2023

Province	No. of Susceptible Cases	No. of Reported Cases	Mean Prevalence Rate
Loja	27	8	29.64±0.629ª
Napo	87	20	22.9±0.369 ^a
Pastaza	43	9	20.74±0.562ª
Sucumbíos	40	8	19.94±0.029 ^a
Bolívar	83	16	19.24±0.060°
Cañar	58	11	18.98±0.0523ª
Azuay	102	19	18.64±0.074°
Chimborazo	392	72	18.39±0.031°
Guayas	158	24	15.21±0.124 ^b
Orellana	191	27	14.14±0.015 ^b
Santo Domingo de los Tsáchilas	572	78	13.64±0.011 ^b
Tungurahua	645	81	12.56±0.009°
Cotopaxi	1080	133	12.32±0.036°
Los Ríos	159	19	11.93±0.515°
Pichincha	3165	333	10.52±0.018 ^c
Esmeraldas	568	49	8.63±0.002 ^d
Imbabura	711	61	8.58±0.009 ^d
Carchi	2506	196	7.82±0.013 ^d
Manabí	1102	85	7.71±0.739 ^d
Zamora Chinchipe	54	4	7.34±0.205 ^d
Morona Santiago	147	10	6.78±0.006 ^d
Santa Elena	17	1	6±0.004 ^d
El Oro	415	14	3.37±0.000°
	12324	1278	13.7

Note: Means in a column followed by the same letter did not show significant differences (P<0.05).

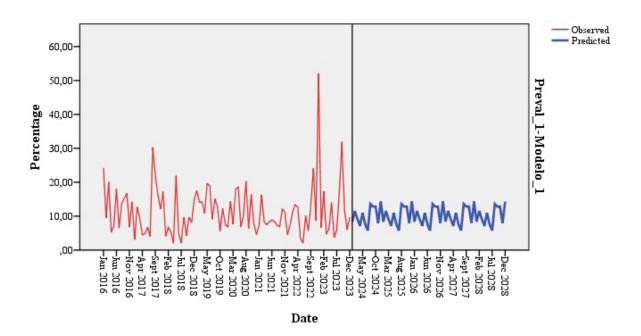


Figure 2. Time series and prediction of incident brucellosis trend for the coming years in Ecuador, 2016-2023

(8.63%), Imbabura (8.58%), Carchi (7.82%), Manabí (7.71%), Zamora Chinchipe (7.34%), Morona Santiago (6.78%), and Santa Elena (6.00%). Finally, El Oro province (Coastal region) reported the lowest prevalence at just 3.37%, which was below the national average.

Temporal variation in the prevalence of bovine brucellosis in Ecuador from 2016 to 2023

The observed data (Figure 2, red line) exhibited marked intra-annual variability, consistent with a seasonal pattern characterised by biannual peaks. During the study

period, prevalence ranged between approximately 2% and 52%, with an anomalous peak occurring in late 2022, suggesting a potential outbreak or intensified surveillance activity. The modelled forecasts (Figure 2, blue line) were generated using an ARIMA approach, optimised to capture both seasonal and non-seasonal components. The fitted model predicted a relatively stable trajectory in prevalence for the period 2024-2028, oscillating around a central mean of $\sim 10\%$. Seasonal fluctuations were retained in the projection, although the model did not anticipate the occurrence of extreme values as seen in past observations.

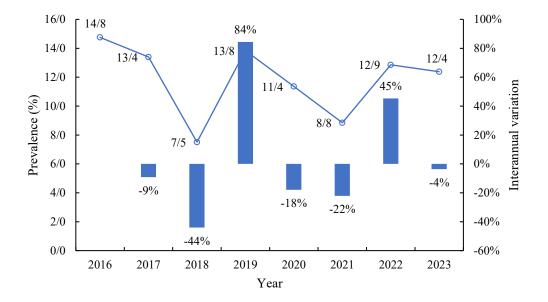


Figure 3. Dynamics of the evolution of bovine brucellosis in Ecuador during the period 2016–2023

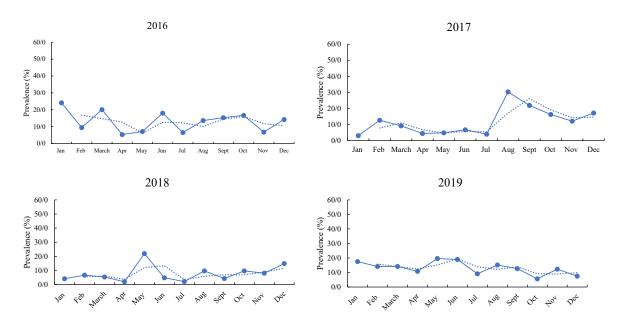


Figure 4. Monthly fluctuations in bovine brucellosis prevalence from January to December during the years 2016 to 2019

Notably, the forecasted series revealed neither a statistically significant upward nor downward trend, indicating the persistence of endemic transmission without evidence of progressive intensification or sustained control. The model performance suggests a good fit for capturing regular seasonality, though it appears to smooth irregular peaks, an expected limitation of ARIMA in the absence of exogenous explanatory variables.

The prevalence of bovine brucellosis in Ecuador exhibited temporal fluctuations from 2016 to 2023, although no statistically significant differences were detected (P≥0.05; F=1.602; df=7) (Figure 3). Between 2016 and 2018, the national prevalence percentage declined from 14.75% to 7.51%, representing a 44% decrease. This was followed by a notable increase in 2019, reaching 13.84%, which marked an 84% year-over-year rise compared to 2018. Subsequently, prevalence declined to 11.36% in 2020 and further to 8.84% in 2021, corresponding to annual variations of -18% and -22%, respectively. In 2022, a resurgence was observed, with a 45% increase over the previous year, while in 2023, a slight decrease of 4% was recorded.

A statistically significant effect on the seasonal pattern was found as a result of the interaction between month and year (P<0.05; $F_{7, 11}$ =1.570), with the highest prevalence values recorded in December 2022, while the lowest values were observed in 2021 (Figures 4 and 5). Most years displayed two distinct peaks in prevalence: the first generally occurring in the first half of the year (April to June), and the second in the latter half (August to December).

In 2016, prevalence was high in January, declined through May, and then increased steadily until the end of the year. In 2017, prevalence remained around 10% from January to July, followed by an increase in August. Although it began to decrease after September, prevalence remained higher in the second half of the year than in the first. A similar pattern was observed in 2018, with low prevalence between January and April (4.0–6.0%). A sharp increase occurred in May, reaching 21.9%, followed by a decrease in June and July. Prevalence then showed a slight but sustained increase through December. In contrast, 2019 exhibited a generally higher prevalence throughout the year, with a decreasing trend during the second half (Figure 4).

In 2020, two distinct peaks were again observed: the first between April and May, with average values of 18.0% to 18.6%, and the second between August and October, with values of 20.3% and 16.4%, respectively. The pattern in 2021 was similar to 2019, with relatively low and stable prevalence throughout the year, except for slight upticks in February and again in October and November. Finally, in 2022 and 2023, the trend was more atypical, with relatively high peaks during the second half of the year. The highest prevalence reached 52.0% in December 2022 and 31.9% in September 2023 (Figure 5).

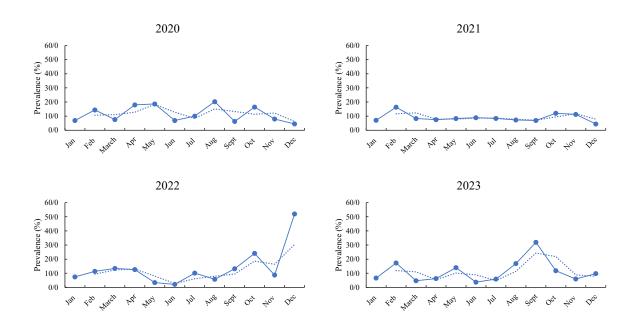


Figure 5. Monthly fluctuations in bovine brucellosis prevalence from January to December during the years 2020 to 2023

Discussion

The decomposition of the original time series confirmed the presence of a strong seasonal component superimposed on a slowly varying trend. This cyclical behaviour aligns with the known ecology of *B. abortus*, and may reflect seasonal calving patterns, variations in herd management, and climatic influences on pathogen transmission dynamics.

Overall, the results support the endemic and seasonally recurrent nature of bovine brucellosis in Ecuador. The predictive component of this analysis offers valuable insight for national surveillance and control strategies, enabling resource allocation to be optimised in anticipation of high-risk periods. These results demonstrated that brucellosis remains a significant animal health concern affecting a large proportion of cattle herds in Ecuador, particularly in the Andean and Amazon regions. According to Román-Cárdenas & Luna-Herrera (2017), between 2006 and 2015, a total of 6,806 cases of B. abortus infection were reported in Ecuador, with the majority occurring in the provinces of Pichincha (2,207 cases) and Carchi (993 cases), accounting for 32.4% and 14.6% of total national cases, respectively. These findings align with the results of the present study, which also identified a higher prevalence in Andean provinces. Conversely, Grrido-Haro et al. (2023) found the highest prevalence of brucellosis in both the Andean and Coastal regions, with average prevalence rates of 23.7% and 22.3%, respectively, while the Amazon region exhibited a lower prevalence of 8.8%. This discrepancy may be attributed to differences in study periods, as Grrido-Haro et al. (2023) focused exclusively on data from 2017, whereas the present study analysed data spanning a broader time frame (2016 to 2023). On the other hand, geographical heterogeneity within the Amazon region may also explain these findings. Garrido-Haro et al. may have sampled in localities or provinces where disease circulation was minimal at the time of study (Grrido-Haro et al., 2023). In contrast, our data derived from national surveillance records include provinces, such as Napo and Pastaza, which reported consistently high prevalence over several years. This suggests localised endemic foci of infection within the Amazon basin. Additionally, increased detection and reporting in recent years may have contributed to the observed rise in Amazonian prevalence. Enhanced laboratory capacity, improved farmer awareness, or intensified control campaigns may have led to better case identification, especially in remote regions that historically faced barriers to diagnosis and reporting.

Climatic conditions are known to play a pivotal role in the distribution of both vector organisms and pathogens. Studies have established strong associations between climate variability, climate change, and the emergence or re-emergence of infectious diseases, including brucellosis, which continues to pose a public health threat globally from highly endemic to less affected regions (Rodriguez-Morales, 2013).

Regarding temporal variations in bovine brucellosis prevalence, similar trends were observed in northern China from 2005 to 2018, where epidemiological curves showed seasonal peaks in incidence between April and July, with the highest rates in May. These peaks were correlated with daylight hours, evaporation, and most notably, temperature fluctuations. Temperature, in particular, has been shown to have a more substantial impact on brucellosis outbreaks than other climatic factors (Liu et al., 2020). Prior studies have also demonstrated that climate variability can affect the epidemiological parameters of infectious diseases, making it a valuable criterion for forecasting outbreaks weeks or even months in advance (Morin et al., 2018; Xiang et al., 2018). Transmission and persistence of *Brucella* spp. have been shown to increase with rising temperatures, reaching a cumulative effect at 15.2 °C over four months (Liu et al., 2020). Elevated temperatures accelerate the development and replication rates of Brucella spp. within the host, while also indirectly increasing the exposure frequency of susceptible animals and humans (Li et al., 2013).

Additionally, Faramarzi et al. found that brucellosis incidence was positively correlated with altitude (Faramarzi et al., 2019). In northern Iran, cities located at higher elevations and exposed to lower temperatures favoured the survival and reproduction of the bacterium. This may help explain why Ecuador's highland provinces consistently exhibit higher brucellosis prevalence than other regions. Similarly, Chen et al. reported higher brucellosis incidence during spring and summer, attributing this to the strong association between Brucella and the immune status of cattle, which may be compromised due to reduced feeding rates during these seasons, thereby increasing infection risk (Chen et al., 2023).

The seasonal peaks in brucellosis prevalence observed in this study, typically during the first (April-June) and second (August-December) halves of the year, are consistent with global evidence linking climatic variability to the epidemiology of infectious diseases, being the temperature a crucial factor determining Brucella transmission and persistence since elevated ambient temperatures may enhance bacterial replication, extend environmental survival, and increase host exposure frequency (Li et al., 2013; K. Liu et al., 2020). These climatic dynamics may explain the recurrent mid-year and end-of-year surges documented in the present study. Additionally, higher altitudes, as found in Ecuador's Andean provinces, have been correlated with increased brucellosis incidence due to environmental conditions favourable to pathogen persistence (Faramarzi et al., 2019). Thus, these findings support the role of climate and seasonality as key determinants of brucellosis dynamics in Ecuador and underscore the need for temporally targeted control strategies.

Given its impact on both animal and human health, the World Health Organization (WHO) and the WOAH (formerly OIE) have established brucellosis control and prevention strategies aimed at reducing zoonotic transmission and mitigating its public health burden (Rubacha et al., 2013). Vaccination has been widely recognized as an effective tool in brucellosis prevention and control; however, recent studies have emphasized that vaccination alone is insufficient. Liu et al. highlighted the need for complementary strategies, including disinfection, selective culling of infected animals, and health education, as essential components of an effective control program (Liu et al., 2025). Therefore, a multifaceted, integrated approach is vital to reduce disease transmission and its impact on both animal and human populations.

Nonetheless, the success of such strategies has been limited, with effective control achieved only in certain high-income countries (Zhang et al., 2018). In contrast, brucellosis continues to be a serious public health concern in most Latin American countries, largely due to the lack of attention and prioritization given to its control (Khoshnood et al., 2022; Zhang et al., 2018). This poses a major challenge for low-income countries, where implementing effective control measures requires substantial investments in economic resources and time and labour (Zhang et al., 2018). The situation is further complicated by the limited availability of resources and the absence of sustainable surveillance and control programs in many affected regions.

Another obstacle in the fight against brucellosis is the lack of epidemiological data in several Latin American countries, stemming from the scarcity of up-to-date publications and research. This information gap hinders accurate estimation of global annual prevalence (Khoshnood et al., 2022), impeding decision-making and the formulation of effective policies.

In response to this challenge, AGROCALIDAD launched the national bovine brucellosis control program in 2008. This program included vaccination with strain 19 in calves aged 3 to 6 months, epidemiological surveillance, removal of positive animals, improved diagnostic laboratory capacity, and awareness campaigns targeting livestock producers (SESA, 2008). However, the program had limited reach, partly due to poor uptake among small and medium-scale farmers, who were responsible for covering implementation and diagnostic costs. Moreover, the program was not mandatory and did not offer compensation for culled animals or financial incentives for smallholders (Aguayo & Ruano, 2018; Vinueza et al., 2023). Sample collection and transportation

also posed logistical barriers, as producers were required to deliver refrigerated samples (2-8 °C) to AGROCALI-DAD laboratories near the capital or to regional offices.

More recently, in June 2024, Ecuador organized a series of webinars in collaboration with the Pan American Health Organization (PAHO), supported by the Pan American Centre for Foot-and-Mouth Disease and Veterinary Public Health (PANAFTOSA/VPH), along with the Ministry of Public Health and AGROCALIDAD. These sessions aimed to provide up-to-date scientific and technical information and promote cooperative approaches for brucellosis surveillance, prevention, control, and treatment under the "one health" framework. The initiative also sought to reinforce the coordinated efforts led by the Ministry of Health and AGROCALIDAD, underscoring the importance of a multisectoral response to this disease (PAHO, 2024).

These initiatives illustrate that effective brucellosis prevention and control, both in animals and humans, requires a multidimensional strategy combining vaccination, sanitary control, education, and international cooperation. Only through sustained commitment, grounded in scientific evidence and integrated efforts across human and animal health sectors, can the burden of brucellosis in Latin America be reduced and meaningful progress made toward its eradication.

Conclusion

Bovine brucellosis in Ecuador exhibits marked regional and provincial disparities, with the highest prevalence observed in the Andean and Amazon regions, particularly in Loja and Napo provinces, while the Coastal region showed the lowest rates, with El Oro significantly below the national average.

Between 2016 and 2023, the disease's prevalence fluctuated notably, declining until 2018, spiking in 2019, and stabilizing thereafter, indicating potential links to climate variability and livestock management practices.

These findings underscore the need for region-specific studies on risk factors, enabling the development of targeted and effective brucellosis control strategies tailored to the diverse realities of Ecuadorian cattle farming. To mitigate the burden of bovine brucellosis, it is critical to strengthen regional risk-based surveillance, implement mandatory vaccination campaigns, and enforce movement control of livestock, particularly in high-prevalence zones. Additionally, capacity-building programs for local farmers and veterinary personnel, alongside

improved epidemiological data integration, are essential for transitioning from control to eradication strategies.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the Master's Programme in Animal Health at the Technical University of Ambato, Ambato, Ecuador for its support in the realisation of this research.

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