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Article

Seroprevalence and Risk Factors Associated with *Brucella* Infection in Sheep Flocks in Ecuador

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Simple Summary

Brucellosis is an infectious disease that affects livestock worldwide and causes important economic losses due to reproductive problems such as abortion, infertility, and reduced productivity. Information about brucellosis in sheep is limited in many countries, including Ecuador. This study evaluated the presence of *Brucella* spp. infection in sheep from the main production regions of Ecuador and identified factors associated with the disease. A total of 970 sheep from 385 farms were tested using a serological ELISA assay. The results showed an individual seroprevalence of 5.1% and a herd-level prevalence of 7.5%, indicating that the infection is present in sheep production systems in the country. Statistical analysis identified abortion rate and the isolation of sick animals as factors associated with infection, while access to veterinary technical advisory services was identified as a protective factor. These findings highlight the importance of improved biosecurity practices, reproductive health monitoring, and veterinary support to reduce disease transmission in sheep flocks.

Abstract

Brucellosis is an important infectious disease affecting livestock worldwide, causing reproductive losses in small ruminant production systems and representing a persistent challenge for animal health and public health under a One Health perspective. Despite its importance, epidemiological information on ovine brucellosis in Ecuador remains scarce. This cross-sectional study aimed to estimate the seroprevalence of *Brucella* spp. infection in sheep at both the individual and herd levels and to identify potential risk factors associated with infection. This study represents the largest epidemiological investigation conducted to date on ovine brucellosis in Ecuador, covering the main sheep-producing regions of the country (more than 95% of the national sheep population). Between 2024 and 2025, a total of 970 sheep from 385 farms were sampled. Serum samples were analyzed using a commercial ELISA assay, and epidemiological information was obtained through structured farm surveys. Statistical analyses included bivariate tests and a multivariable model using Generalized Estimating Equations (GEE). The overall individual seroprevalence was 5.1% (49/970), while herd-level prevalence reached 7.5% (29/385). Multivariable analysis identified abortion rate (OR = 1.045; 95% CI: 1.016–1.074) and isolation of sick animals (OR = 2.843; 95% CI: 1.150–7.027) as factors associated with seropositivity, whereas access to technical advisory services was identified as a protective factor. These findings provide essential epidemiological evidence to support surveillance programs and improve biosecurity and veterinary extension strategies in Ecuadorian sheep production systems.

Keywords: ovine brucellosis; epidemiology; seroprevalence; risk factors

1. Introduction

Brucella is a small, aerobic, non-motile, Gram-negative coccobacillus within the genus *Brucella* and is classically characterized by a rough phenotype and strong host/tissue tropism for the male genital tract of sheep. In contrast to *B. melitensis* (smooth; highly zoonotic), *B. spp.* shows a narrower host range, with rams as the preferred host, and its biological differences (including rough colony morphology and distinct growth/biochemical features) underpin important contrasts in epidemiology and diagnostic performance across *Brucella* species (Rossetti et al., 2022). At the animal level, *Brucella spp.* infection is primarily expressed as ovine epididymitis and subfertility/infertility in rams, with transmission driven by introduction of infected rams and close-contact or venereal-associated spread within mating groups. The infection may remain undetected because obvious clinical signs are often absent early on; nonetheless, infected rams can shed the organism in semen for prolonged periods, sustaining within-flock transmission and undermining reproductive efficiency (Ridler & West, 2011; Khurana et al., 2021).

Clinically, *Brucella spp.* in rams is associated with chronic epididymitis (classically pain, swelling, and induration of the epididymis), reduced semen quality, and consequent fertility losses; ewes are usually transiently infected and are considered minor in the epidemiology, although rare abortions have been described. These reproductive outcomes—often silent until lambing performance declines—make flock-level surveillance particularly relevant in extensive systems where routine breeding soundness evaluation may be inconsistent (Ridler & West, 2011; Machado et al., 2015).

The diagnosis of brucellosis relies on a combination of bacteriological, serological, and molecular methods. Direct diagnostic methods also include microbiological culture and staining techniques performed on clinical samples such as aborted fetuses, milk, semen, or lymphoid tissues. Due to these limitations, serological tests are widely used for surveillance and routine diagnosis, as they detect antibodies produced during infection. The most commonly employed assays include the Rose Bengal test, serum agglutination test (SAT), and enzyme-linked immunosorbent assay (ELISA), which are often applied in combination to improve diagnostic sensitivity and specificity (Godfroid et al., 2010; Nielsen & Yu, 2010; Ridler & West, 2011; Qureshi et al., 2023a)

From a public-health perspective, brucellosis is globally important as a zoonosis. Human disease is mainly linked to *B. melitensis*, *B. abortus*, *B. suis*, and (less commonly) *B. canis*, acquired via direct contact with infected animals/products, ingestion of contaminated dairy, or aerosols. This distinction is central when framing small-ruminant brucellosis as an occupational and foodborne hazard while correctly positioning *B. spp.* within the broader genus (Ganter, 2015; Kurmanov et al., 2022; Rossetti et al., 2022).

In humans, clinical brucellosis is typically non-specific and systemic, affecting multiple organ systems and presenting with constitutional and musculoskeletal complaints (e.g., fever, sweats, malaise, anorexia, headache, fatigue, and pain in muscles/joints/back). Contemporary reviews also highlight that the geographic footprint of human brucellosis is expanding and shaped by trade, mobility, and persistent exposure risks among livestock keepers and consumers of raw dairy products—issues that reinforce the One Health relevance of brucellosis control even when the focal livestock pathogen is not itself zoonotic (Pappas et al., 2006; Kiiza et al., 2023; Liu et al., 2024;). Brucellosis remains an important zoonotic disease in Latin America affecting humans and domestic animals. A retrospective study analyzing 1,933 *Brucella* isolates from 1968 to 2006 identified *B. melitensis*, *B. suis*, and *B. abortus* as the main species responsible for infection, with human cases reported mainly in Argentina, Mexico, and Peru. The true incidence of the disease in the region is likely underestimated due to diagnostic limitations and underreporting (Lucero et al., 2008).

Treatment and control must be framed realistically: while antimicrobial therapy can reduce shedding and improve semen parameters in some rams, it is typically reserved for high-value animals given costs and the need for follow-up testing and isolation; therefore, population-level control relies primarily on test-and-slaughter or vaccination strategies depending on feasibility. In the broader brucellosis literature (human disease), effective therapy requires intracellularly active combinations (e.g., doxycycline with rifampicin for extended courses) because *Brucella spp.* are

intracellular pathogens and monotherapy is associated with relapse (Ridler & West, 2011; Głowacka et al., 2018).

Risk-factor evidence consistently highlights management and demographic drivers that facilitate the introduction and persistence of infection. For *Brucella*, flock-level investigations indicate that advanced sheep age, larger farm area, and the absence of dedicated lambing paddocks are associated with increased infection risk—patterns consistent with longer cumulative exposure and suboptimal segregation during high-risk reproductive periods. More broadly, persistence of brucellosis in small-ruminant systems has been linked to management and husbandry conditions that facilitate introduction and spread, including limited recognition of clinical signs, insufficient vaccination where applicable, communal grazing and animal mixing, and on-farm presence of dogs, alongside consumption of raw milk and dairy products that sustains transmission at the human-animal interface. At the landscape level, deforestation, encroachment into wildlife habitats, and biodiversity loss may further intensify pathogen circulation by increasing contacts among livestock, wildlife, and people, thereby supporting endemicity in vulnerable production settings. These convergent risk domains support the rationale for Ecuador-focused studies that quantify seroprevalence and associated practices to inform feasible, context-adapted control options (Diez, 2019; Machado et al., 2015; Noguera Zayas et al., 2021).

The economic relevance of *Brucella* is driven by reduced conception rates, extended lambing periods, ram replacement, and downstream productivity losses often amplified in systems with high mating ratios or limited access to routine reproductive evaluation. More broadly, economic assessments of brucellosis control in livestock consistently emphasize the substantial costs attributable to reproductive failure, replacement, and program implementation, and show that the cost-effectiveness of interventions is sensitive to parameters such as baseline prevalence, abortion costs, and replacement prices capturing both direct and indirect burdens that can be particularly consequential for smallholder and extensive production systems (Kiiza et al., 2023a; Ridler & West, 2011).

Therefore, the aim of this cross-sectional study was to estimate the seroprevalence of *Brucella* infection in sheep in Ecuador at both the individual and herd levels and to identify potential risk factors associated with infection. The results of this study provide updated epidemiological information to support surveillance and contribute to the development of effective control strategies for brucellosis in small ruminant production systems in Ecuador.

2. Materials and Methods

2.1. Study Area

This study was conducted from February 2024 to December 2025 in the Ecuadorian Andes, where the highest sheep production in the country is concentrated, particularly in the provinces of Azuay, Bolívar, Cañar, Carchi, Chimborazo, Cotopaxi, El Oro, Imbabura, Loja, Pichincha, and Tungurahua. The remaining provinces show limited or nearly absent sheep production, especially those located in the Amazon region (Oriente). In the coastal region, only a small number of farms produce sheep for meat (Figure 1).

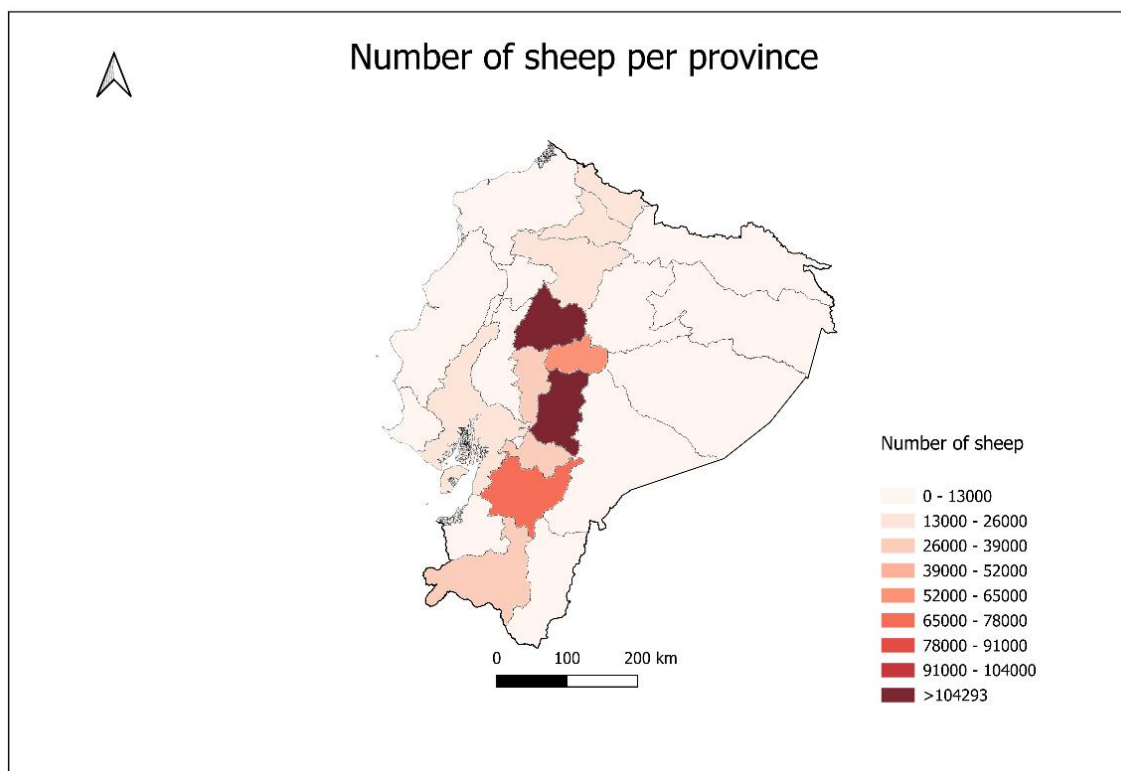


Figure 1. Distribution of sheep in Ecuador.

Ecuador is located in South America and covers 257,217.07 km², divided into four natural regions: the Andean highlands (Sierra), coastal lowlands (Costa), Amazon basin (Oriente), and the Galápagos Islands. It extends from 01°27'06" N to 05°00'56" S in latitude and from 75°11'49" E to 81°00'40" W in longitude. Owing to the presence of the Andes mountain range and the influence of both the cold Humboldt Current and the warm El Niño Current, Ecuador exhibits a wide range of climates and microclimates, with mean temperatures ranging from 4.4 °C to >32.1 °C depending on the geographic area. Mean annual precipitation varies from 96.7 to 4,360 mm, depending on the region and month. Ecuador has two main seasons: a rainy season and a dry season.

Serological samples from sheep were collected between 2024 and 2025 from farms located in Ecuador's principal sheep-producing provinces (Figure 2). Sampling was concentrated in the Andean highlands, including Loja, Azuay, Cañar, Chimborazo, Cotopaxi, Tungurahua, Bolívar, Pichincha, Imbabura, and Carchi, with additional samples obtained from highland (upland) area de El Oro, (The surveyed area represents >95% of Ecuador's total sheep population). All serological testing was performed at the Animal Health and Zoonoses Laboratory of the Universidad Técnica Particular de Loja.

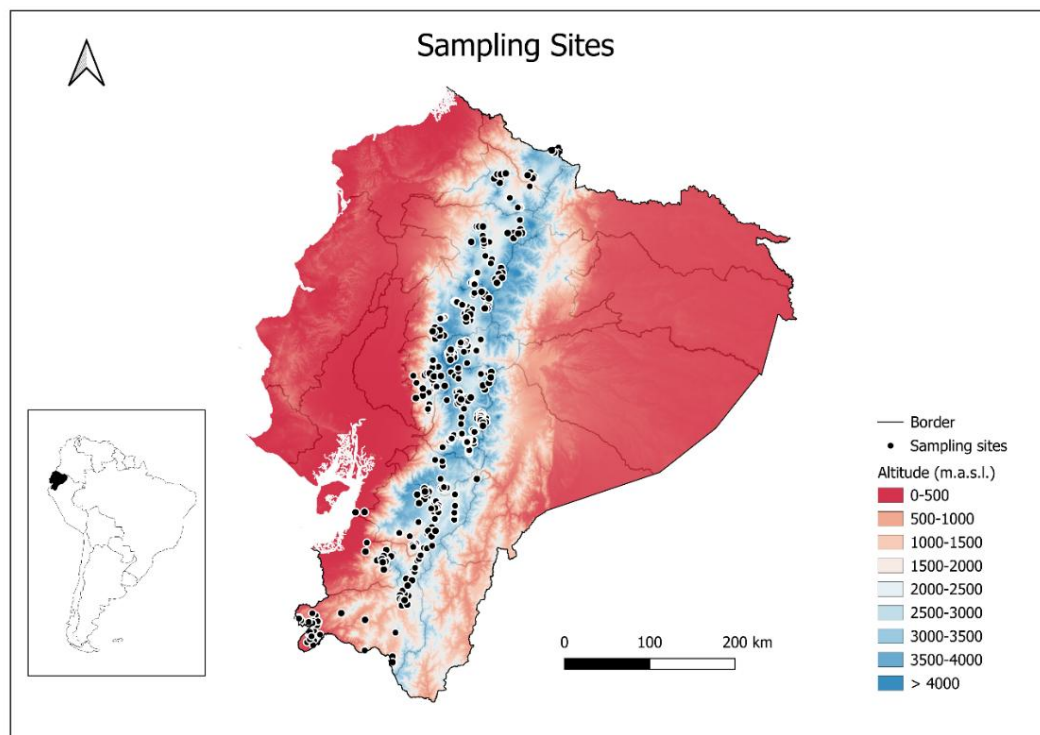


Figure 2. Location of the provinces where sampling was conducted.

2.2. Study Design

The sample size was determined using the Working in Epidemiology (Win Epi, 2023) on line statistical program, applying a 95% confidence interval (CI), 5% margin of error (ME), and an expected prevalence (EP) of 50% for a known population of 528,820 sheep (INEC, 2023), resulting in a required sample size of 385 flocks. Blood samples were collected from two/three animals older than 6 months per flock. Ultimately, 970 samples from 385 farms were analyzed. The selection criteria were based on the proportionality of samples collected per province. To conduct the sampling, the Continuous Agricultural Area and Production Survey (ESPAC, 2023) was used as a reference. A stratified sampling approach was applied, considering the provinces as units of analysis. First, the proportion of the sheep population in each province was calculated relative to the national total of 528,828 sheep (100%). Within each province, the territory was divided into 25 km² quadrants using UTM coordinates. Each cluster was numbered and randomly selected, and within each, 100% of the farms were included for analysis (Figure 2).

All animals included in the study were handled in accordance with applicable ethical standards and regulations under the Ecuadorian Sanitary Animal Health Agency (AGROCALIDAD) permit code: AGR-AGROCALIDAD/DGDA-2023-4587-E and Bioethics approval No. USFQ-2023-007 (Universidad San Francisco de Quito). In addition, access to the farms and the administration of the surveys were authorized by the farm owners.

2.3. Serological Test

Serum samples were tested using the IDEXX Ovine/Caprine Brucellosis ELISA (06-04310-12) according to the manufacturer's instructions. Microplates were read at 450 nm using a BioTek ELx800 microplate reader to obtain optical density (OD) values. Each run included the kit-provided positive (PC) and negative (NC) controls, both tested in duplicate.

A run was considered valid only if both of the following conditions were met:

$$PC\bar{x} \geq 0.350 \text{ and } \frac{PC\bar{x}}{NC\bar{x}} \geq 3.00$$

Sample calculation (M/P%) for each sample, ELISA reactivity was expressed as the sample to positive percentage (M/P%), computed by normalizing the sample OD at 450 nm to the mean negative and positive controls, as follows:

$$S/P\% = 100 \times \frac{\text{Sample } A(450) - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$$

A sample was considered positive if:

$$S/P\% \geq 120$$

2.4. Data Collection

To obtain data at both the farm and individual-animal levels, an epidemiological survey was administered to collect information on: general farm characteristics, production type (meat or wool), flock size (number of sheep), farm area, altitude, management practices, reproductive system, feeding regimen, and facilities. In addition, animal health-related variables were recorded, including: presence of respiratory diseases, umbilical cord disinfection, deworming (internal and external), implementation of quarantine, annual mortality, and abortion rate. For the individual-animal survey, the following data were recorded: animal age, breed type (local/creole or crossbred), and sex.

2.5. Statistical Analysis

Survey and serological test data were entered into SPSS Statistics v24.0 (SPSS Inc., Chicago, IL, USA) for analysis. Descriptive statistics were computed, for downstream analyses, including frequency distributions for nominal variables and measures of central tendency and dispersion for continuous variables. Continuous variables were subsequently categorized; variables with more than two categories were then recoded into dichotomous variables.

Subsequently, a bivariate analysis was performed using the Chi-square test, selecting only variables with a significance ($p < 0.2$). Identified variables were then used to construct a logistic regression model to calculate Odds Ratios (ORs) with their 95% confidence intervals (CIs), indicating both the upper and lower limits.

With respect to quantitative variables, distributional assumptions were assessed a priori using the non-parametric Kolmogorov–Smirnov test; variables showing statistically significant associations ($p < 0.02$) were considered candidates for multivariable modeling. Subsequently, a multivariable model was fitted using Generalized Estimating Equations (GEE), assuming an exchangeable correlation structure to account for potential within-group dependence. Parameter significance was evaluated using the Wald chi-square statistic, and results were reported as Exp(B) (interpreted as adjusted odds ratios) together with their 95% confidence intervals (95% CI). Model goodness-of-fit was assessed using the Quasi-likelihood under the Independence model Criterion (QIC) and its corrected version (QICC), with lower values indicating better fit. Statistical significance was set at $\alpha = 0.05$.

2.6. Map Design

The maps were designed and produced using QGIS v3.40.0. First, we generated a map displaying the sampling sites, with each sampling location linked to its corresponding altitude (m a.s.l.). A second map was created to depict the number of sheep per province. To improve visualization of the spatial patterns, we also produced two choropleth maps: one showing individual-level seroprevalence, and another showing herd-level prevalence (Figure 3 and 4).

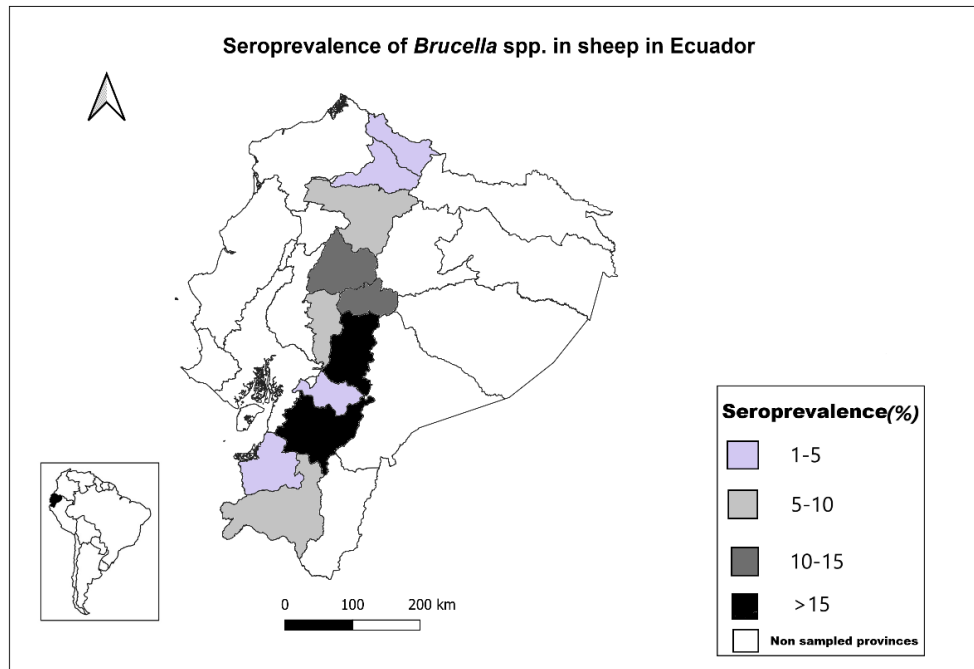


Figure 3. Seroprevalence of *Brucella* spp by individual in sheep farms by province in Ecuador.

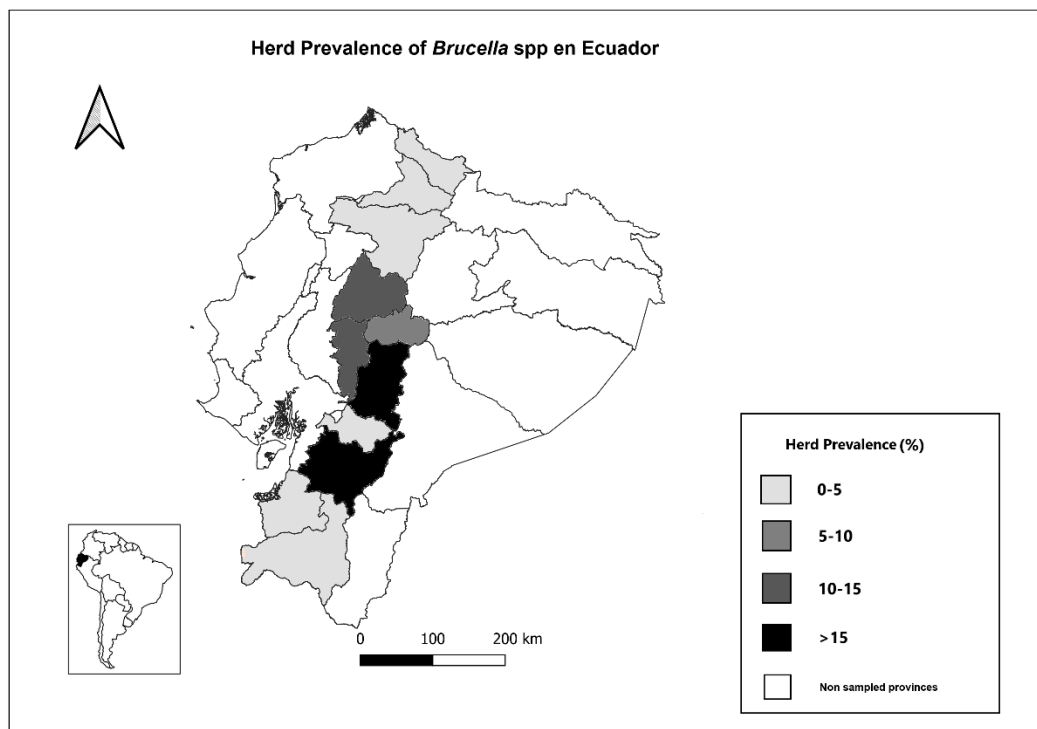


Figure 4. Dispersion of *Brucella* spp in sheep herds across the provinces of Ecuador.

3. Results

3.1. Seroprevalence of *Brucella* in Sheep in Ecuador

A total of 970 sheep serum samples were collected from 385 farms. The overall individual seroprevalence of *Brucella* was 5.1% (49/970), while the herd-level prevalence was 7.5% (29/385) Table 1. Regarding sex distribution, 9 males (0.93%) and 40 females (4.12%) tested seropositive. In relation

to breed, 27 Criollo sheep (2.8%) and 22 crossbred animals (2.27%) were seropositive. According to age groups, the highest proportion of seropositive animals was observed in sheep aged 0.5–24 months (36/49; 73%), followed by those aged 24–48 months (10/49; 20.4%) and animals older than 48 months (3/49; 6.1%).

Table 1. Seropositivity of *Brucella* spp. in sheep from Ecuador.

Seropositivity <i>Brucella</i> spp		
	Individual prevalence (%)	Herd prevalence (%)
Positive	5.1	7.5
Negative	94.9	92.5
Total	100.0	100.0

According to the data obtained, out of the 970 samples analyzed, the highest seropositivity was recorded in Cotopaxi with 38.8% (19 cases), followed by El Oro and Loja with 16.36.1%-4.1% respectively and Imbabura with 2.0% (1 case). Provinces such as Carchi, Cañar, and Pichincha did not report seropositivity. and El Oro and Imbabura presented a minimal incidence of 2.6% (1 case each) Table 2, Figure 3 and 4.

Table 2. Seroprevalence of *Brucella* in sheep in provinces in Ecuador.

	Provinces		
	%Yes	%No	Total
Loja	4.1(2)	5.8(53)	5,7(55)
Carchi	0,0(0)	1,0(9)	0,9(9)
El Oro	6.1(3)	3,1(29)	3,3(32)
Azuay	16.3(8)	16,6(153)	16,6(161)
Cañar	0,0(0)	7,2(66)	6,8(66)
Chimborazo	8.2(4)	5.1(47)	5,3(51)
Tungurahua	8.2(4)	13(120)	12,8(124)
Bolívar	16.3(8)	6,6(61)	7,1(69)
Cotopaxi	38.8(19)	33.1(305)	12,8(124)
Pichincha	0,0(0)	5,9(54)	5,6(54)
Imbabura	2.0(1)	2,6(24)	2,6(25)
Total	100,0(49)	100,0(921)	100,0(970)

Six nominal variables and twelve quantitative variables showed a significant association with *Brucella* spp. infection according to the chi-square test ($p < 0.2$) and the Kolmogorov–Smirnov Z test ($p < 0.2$). Tables 4 and 5 present the variables selected in the univariate analysis. Of the 18 variables evaluated, only two variables included in the Generalized Estimating Equation (GEE) model showed a significant association ($p < 0.2$). Isolation of sick animals, and abortion rate, while visits by technical personnel is a protective factor.

The following frequency table for categorical independent variables provides a detailed description of the distribution of each category within the analyzed variables. This analysis identifies patterns and proportions, offering valuable insights into the composition of the studied population (Table 3).

Table 3. Descriptive Analysis of independent variables.

Variable	Category	Frecuency	Porcentaje
Altitude m.a.s.l.	0-500	2	0.5
	500-1500	5	1.3
	1500-2500	50	13.0
	2500-3500	205	53.2

	>3500	123	31.9
Breed	Creole	620	63,9
	Mestizo	350	36,1
Aptitude	Meat	207	53.8
	Mixed	178	46.2
Production system	Semi-intensive	21	5.5
	Extensive	364	94.5
Irrigation	Yes	78	20.3
	No	307	79.7
Sheep pens	Yes	144	37.4
	No	241	62.6
Footbath	Yes	6	1.6
	No	379	98.4
Fences	Yes	93	24.1
	No	292	75.8
Cleaning	Cleaning is not performed	186	48.3
	Monthly	199	51.7
Bedding	Pen with bedding	165	42.9
	Soil	2	0.5
	Paddock/Pasture	218	56.6
Water	Running water	265	68,8
	Potable	5	1.3
	Well	115	29.9
Waterers	Yes	85	22.1
	No	300	77.9
Type of waterers	None	295	76.6
	Tires	1	0.3
	Buckets	5	1.3
	Rustic waterers	84	21.8
Concentrate	Yes	24	6.2
	No	361	93.8
Manufacture	On-farm/homemade	8	2.1
	Commercial	21	5.5
	None	356	92,5
Grazing	Free grazing	55	14.3
	Rotational	1	0.3
	Roping	329	85.5
Fertilization	Chemical	8	2.1
	Organic	128	33.2
	None	246	63.9
	Mixed	3	0.8
Supplementation	Yes	101	26.2
	No	284	73.8
Type of supplementation	None	280	72.7
	Salt	86	22.4
	Vitamins	3	0.8
	Concentrate (feed)	4	1.0
	Corn	9	2.3
	Multiple	3	0.8
Feeders	Yes	47	12.2
	No	338	87.8

	None	338	87.8
	Tires	26	6.8
	Buckets	11	2.9
Type of feeders	Guadua bamboo	2	0.5
	Cement	4	1.0
	Feed pans	4	1.0
	Yes	243	63.0
Abortions	No	142	37.0
	Open air	281	73.0
Disposal of aborted materials	Buried/incinerated	104	27.0
	yes	311	80.8
Respiratory diseases	No	72	19.2
	Yes	215	55.8
Ectoparasites	No	170	44.2
	Yes	204	53.0
Treatment of ectoparasites	No	181	47.0
	Annual	28	7.2
Frecuency of ectoparasites treatment	Biannual	162	42.1
	Monthly	1	0.3
	None	179	46.5
	Quarterly	15	3.9
	Yes	22	5.7
Vaccines	No	363	94.3
	Yes	72	18.7
Endoparasites treatment	No	313	81.3
	Yes	32	8.3
Antibiotics	No	353	91.7
	Yes	144	37
Diarrheas	No	241	63
	Buried/Incinerated	94	24.4
Carcass disposal	Open Air	291	75.6
	Good	226	58.7
Herd body condition	Bad	68	17.7
	Regular	91	23.6
	AI	3	0.8
Reproduction	Free mating	370	96.1
	Directed mating	12	3.1
	Pupular market	132	34.3
Origin of breeding stock	Neighbor purchase	233	60.5
	Various places	20	5.2
	Yes	140	36.3
Mixing of flocks	No	245	63.7
	Yes	288	74.8
Attends livestock fairs	No	97	25.2
	Yes	60	15.6
Animals return to the farm	No	325	84.4
	Yes	79	20.5
Quarantine	No	306	79.5
	Yes	147	38.2
Isolates sick animals	No	238	61.8
	Yes	260	67.5
Technical advisory services	No	120	31.2

Frecuency of technical visits	Monthly	1	0.3
	Annual	11	2.9
	Biannual	15	3.9
	Quarterly	12	3.1
	Never	277	71.9
	As needed	69	17.9

Table 4. Quantitative variables select in the univariate analisis for *Brucella* spp. seropositivity in sheep flocks from Ecuador ($p < 0.02$).

	Mean	Standar error	Median	Mode	Mín. ^a	Máx. ^b	K-S Z ^c	<i>p</i>
Number of sheep	17.5	1.436593	7	5	1	364	0.366	<0.001
Age (months) (meses)	26.96	0.6	24	24	4	96	0.256	<0.001
Altitude (m.a.s.l)	3074.8	21.7	3108	2780	327	4160	0.090	<0.001
Area of the APU (ha)	2.2	0.2	1	1	0	120	0.382	<0.001
Grazin area (ha)	15.5	1248.8	10	10	0	300	0.345	<0.001
Number of male	2.4	0.1	1	1	0	52	0.351	<0.001
Number of female	11.3	0.9	5	4	0	208	0.353	<0.001
Number of lambs	4.0	0.5	1	0	0	200	0.395	<0.001
Abortion rate	8.0	0.5	0	0	0	80	0.315	<0.001
Diarrhea rate	6.3	0.4	0	0	0	50	0.324	<0.001
Lambs mortality (%)	10.7	0.561542	0	0	0	90	0.305	<0.001
Adult Mortality (%)	8.2	0.446834	0	0	0	80	0.330	<0.001

a. Minimum value. b. Maximun vaue. c. Kolmogorov-Smirnov Z.

Table 5. Variables selected in the univariate analysis for seropositivity to *Brucella* spp in sheep in Ecuador ($p < 0.02$).

Variable	Categoría	Seropositividad		Chi-cuadrado	
		Positive No. (%)	Negative No. (%)	Chi	<i>p</i>
Treatment of ectoparasites	Yes	19(3.5)	525(96.5)	6.276	0.012
	No	30(7.0)	396(93.0)		
Abortions	Yes	14(2.3)	590(97.7)	24.941	0,000
	No	35(9.6)	331(90.4)		
Disposal of aborted materials	Incinerates/Buries	6(2.3)	256(97.7)	5.707	0.017
	Open Air	43(6.1)	665(93.9)		
Technical advisory services	Yes	12(1.9)	618(98,1)	37.107	0.000
	No	37(10.9)	303(89.1)		
Isolates sick animals	Yes	27(7.0)	357(93.0)	5.194	0.018
	No	22(3.8)	564(96.2)		
Footbath	Yes	0(0)	25(100)	1.365	0.020
	No	49(5.2)	896(94.8)		

The table presents both absolute values and relative percentages for each category, facilitating a clear comparison among different variables. These data are essential for interpreting the research

context and for identifying potential relationships between categorical variables and the dependent variable, serving as the basis for subsequent statistical analyses.

In the following section, the analyzed variables are presented along with their main measures of central tendency and dispersion, which are described in detail in Table 4. These values allow for the characterization and summarization of the distribution of the collected data.

3.2. Risk Factors Associated with *Brucella* Infection in Sheep

The following table presents the factors identified as relevant in association with seropositivity to *Brucella* spp. This table includes the independent variables that, in the univariate analysis, showed statistical significance with a p-value less than 0.2, a criterion used to pre-select variables for subsequent analysis (Table 5).

These variables, both categorical and continuous, reflect potential risk factors or characteristics associated with the infection in the sheep studied, such as demographic characteristics, management conditions, and epidemiological parameters. The results of this table provide a preliminary overview to identify patterns and prioritize key factors for more complex multivariate studies.

Table 6 presents the results obtained from the GEE model conducted with the variables preselected in the univariate analysis. These variables were considered due to their initial statistical significance ($p < 0.2$) and their potential association with *Brucella* seropositivity in sheep in Ecuador. The analysis allowed for the identification of the main risk factors, adjusted for the effect of other variables, highlighting those that show a significant relationship with the infection. The coefficients obtained, along with their confidence intervals and p-values, provide a detailed view of the magnitude and direction of the association, enabling a better understanding of the epidemiological determinants of ovine brucellosis in the studied context.

Table 6. Gee model^a including the risk factors significantly ($p < 0.05$) associated with *Brucella* spp. seropositivity in Ecuador.

Variable	Category	<i>p</i>	OR	CI _{95%} ^b
Abortion rate		0.002	1.045	1.016-1.074
Isolates sick animals	Yes No	0.24 -	2.843 -	1.150-7.027 -
Technical advisory services	Yes No	0.000 -	0.80 -	0.025-0.258 -

^a. Quasi-likelihood of the model under the independence model criterion (QIC): 361.09; CI_{95%} confidence interval.

4. Discussion

Epidemiological information on ovine brucellosis in Latin America remains limited, with most research focusing primarily on cattle and goats due to their greater economic importance and zoonotic impact. In Ecuador, studies addressing brucellosis in sheep are particularly scarce, highlighting a significant gap in the epidemiological understanding of the disease in small ruminants. In this context, the present study constitutes the largest epidemiological investigation conducted to date on ovine brucellosis in Ecuador, providing robust baseline data on the seroprevalence and associated risk factors of *Brucella* spp. infection in sheep populations and contributing valuable evidence to support future surveillance and control strategies in the country.

In Ecuador, available studies have largely focused on brucellosis in cattle and other livestock species, with very limited data regarding the occurrence and epidemiology of *Brucella* infection in sheep populations. Consequently, the epidemiological status of ovine brucellosis in the country remains poorly understood. Generating updated data on seroprevalence and associated risk factors

is therefore essential to improve disease surveillance and inform control strategies in small ruminant production systems (Lucero et al., 2008; Garrido-Haro et al., 2025; Qureshi et al., 2023).

The present study identified an individual seroprevalence of 5.1% and a herd-level prevalence of 7.5% of *Brucella* spp. infection in sheep. These findings indicate that although the proportion of infected animals was relatively low, the presence of positive animals in multiple flocks suggests that the pathogen is circulating within sheep production systems in Ecuador.

When compared with studies conducted outside Latin America, the individual prevalence observed in this study is within the range reported globally but higher than that reported in some developed production systems. For example, a study conducted in sheep flocks in Wyoming, USA, reported an animal seroprevalence of 0.53% and a flock seroprevalence of 22.5%, indicating a low individual infection rate but the presence of at least one positive animal in many flocks (Elderbrook et al., 2019). These differences may be associated with management practices, flock size, biosecurity measures, and the implementation of disease control programs. Similarly, a nationwide cross-sectional study in India reported a true prevalence of 3.79% in small ruminants, highlighting the endemic nature of brucellosis in several regions where sheep production is extensive (Shome et al., 2021).

Spain has significantly reduced the prevalence of ovine brucellosis through well-established control and eradication programs. Current seroprevalence in sheep ranges between 0.1% and 0.2%, a result of continuous surveillance and government-led campaigns (Khurana et al., 2021).

Other studies conducted in the Middle East have reported slightly lower individual seroprevalence values but relatively higher flock-level prevalence. For instance, research in the United Arab Emirates detected individual seroprevalence ranging from 2.31% to 4.84%, while flock seroprevalence reached up to 25.5%, suggesting that even a small number of infected animals can lead to widespread flock-level exposure (Ameni et al., 2025).

However, the situation is markedly different in developing countries, where less effective control systems result in higher prevalence rates. Factors such as limited resources, inadequate veterinary infrastructure, and traditional livestock management practices contribute to persistently high infection rates in regions like Africa and Asia. Moreover, in countries with warm and humid climates, the environmental persistence of *Brucella* increases the risk of disease transmission (Głowacka et al., 2018; World Organisation for Animal Health, 2026).

Additionally, research conducted in Portugal demonstrated that individual prevalence in small ruminants may exceed 5% in some flocks despite the implementation of vaccination campaigns, emphasizing that management practices and biosecurity measures strongly influence disease persistence (Diez, 2019).

Additional studies conducted in other regions of the world have also reported variable levels of *Brucella* seroprevalence in livestock populations. For instance, in Central Punjab, Pakistan, a cross-sectional study conducted in districts such as Lahore, Kasur, Okara and Faisalabad reported an individual seroprevalence of 3.23% among livestock (Semango & Buza, 2024). Although the animal-level prevalence was relatively low, the pathogen was widely distributed geographically, with positive animals detected in multiple villages, highlighting the endemic nature of the infection in the region (Saeed et al., 2020).

In North Africa, a cross-sectional study conducted in Algeria reported an individual seroprevalence of 3.98% and a herd prevalence of 27.95% in small ruminant flocks, which is lower at the individual level but considerably higher at the herd level than the values found in the present study. These differences may reflect variations in animal density, flock management, and the effectiveness of vaccination or surveillance programs (Ramdani et al., 2022). Similarly, a rapid review of brucellosis prevalence in East African Community countries (including Kenya, Tanzania, Uganda, Rwanda, Burundi and South Sudan) reported that animal-level prevalence in sheep ranged from 0.0% to 13.8%, indicating considerable variability depending on the country and production system studied (Djangwani et al., 2021).

In addition, broader epidemiological reviews conducted in Africa and Asia have highlighted *Brucella* spp. as one of the most common abortigenic pathogens in ruminants, often presenting seroprevalence levels exceeding 10% in several regions, particularly where surveillance systems are limited or poorly implemented (Semango & Buza, 2024).

When compared with studies conducted in Latin America, the prevalence observed in Ecuador appears to be similar or slightly higher than those reported in several countries in the region. For example, a serological study in Argentine Creole sheep reported a seroprevalence of 2.9% for *Brucella* spp., indicating a lower infection level than that detected in the present study (López et al., 2018). Likewise, in Brazil, a cross-sectional study in the state of Rio Grande do Sul found an individual prevalence of 2.89% and a flock prevalence of 2.5%, values lower than those reported here (Machado et al., 2015). In contrast, a large epidemiological survey in Costa Rica reported a very low seroprevalence of approximately 0.7% in sheep herds, suggesting that infection in small ruminants is rare in that country (Hernández-Mora et al., 2017). Similar findings have been reported in Mexico, where epidemiological modeling of infectious abortion in sheep identified *Brucella* as one of the major pathogens associated with reproductive losses, (Arteaga-Troncoso et al., 2023).

Broader regional analyses emphasize the importance of brucellosis as a zoonotic disease affecting both animal health and public health in Latin American livestock systems. For example, a systematic review conducted in Paraguay highlighted *Brucella* as one of the most important zoonotic pathogens contributing to the burden of livestock-associated diseases in the region (Noguera Zayas et al., 2021b). Similar animal-level prevalence values have been reported in Argentina. A large epidemiological survey conducted in Patagonian sheep farms revealed an average animal prevalence of 1.4-5.8% for *Brucella*, which is comparable to the individual seroprevalence observed in the present study. However, the same study reported a markedly higher farm-level prevalence, with 66.2% of farms presenting at least one infected ram, indicating widespread distribution of the pathogen within sheep production systems in the region. Santa Fe, Entre Ríos, and Santiago del Estero showed a seroprevalence of 0% (Alvarez et al., 2020; Novoa et al., 2023).

These findings suggest that even when the proportion of infected animals remains relatively low, the infection can be widely disseminated across farms due to the introduction of infected breeding rams, animal movements, and the persistence of chronically infected animals within flocks. Consequently, the relatively lower herd-level prevalence observed in the present study may reflect differences in management practices, flock structure, or animal movement patterns between Ecuadorian and Patagonian production systems. According to Godfroid (2017), effective control of the disease requires a comprehensive One Health approach, recognizing that the maintenance of infection in livestock populations is the main driver of transmission to humans and other animals. In this context, the findings of the present study provide additional evidence on key factors influencing disease dynamics within sheep flocks. Differences in prevalence among countries may be explained by factors such as livestock management practices, animal movement, implementation of vaccination programs, and the presence of national surveillance systems (Godfroid, 2017; World Organisation for Animal Health, 2026).

Overall, the seroprevalence observed in this study indicates that *Brucella* infection is present in sheep populations in Ecuador at levels comparable to those reported in several endemic regions worldwide. However, the detection of positive flocks highlights the need for strengthened surveillance, improved biosecurity practices, and the implementation of targeted control strategies to reduce the spread of the disease in small ruminant production systems.

From a policy perspective, the identification of management-related risk factors in Ecuadorian sheep farms highlights important gaps in current surveillance and control strategies for brucellosis in small ruminants. The association between abortion rate and seropositivity confirms that reproductive disorders remain key indicators of active transmission within flocks and should be integrated into routine surveillance systems as early warning signals for disease detection. Moreover, the presence of infected animals that remain within production systems, even when isolated, suggests that current farm-level control practices may be insufficient to interrupt transmission chains.

Brucellosis is widely recognized as one of the most important zoonotic diseases affecting livestock worldwide, generating substantial economic losses and posing occupational risks to farmers, veterinarians and slaughterhouse workers through contact with infected reproductive materials and contaminated environment (Ganter, 2015).

Furthermore, in many endemic regions the persistence of the disease has been linked to limited veterinary infrastructure, insufficient farmer awareness and weak implementation of coordinated control programs (Kiiza et al., 2023).

Compared to other studies conducted in the region, the prevalence reported in Ecuador is similar to that found in other Latin American countries. For instance, in Colombia, a study revealed a 2.4-6.1% seroprevalence in extensively managed sheep, while in Brazil, prevalence varies by region (4-5%) in areas with intensive production systems (Gil & Samartino, 2001; Avila-Granados et al., 2019).

In countries such as Peru, Venezuela, and Bolivia, studies on the seroprevalence of brucellosis in sheep are scarce or even nonexistent. This lack of epidemiological information limits the understanding of the real distribution and impact of the disease in small ruminant populations across the Andean region. Finally, Li et al. (2023) summarized the global seroprevalence of ovine brucellosis based on different studies included in their meta-analysis, reporting an overall average seroprevalence of 6.2% worldwide (Africa 8.5%, Asia 6.6%, and the Americas 1.9%) (Li et al., 2023).

The multivariable analysis using the Generalized Estimating Equations (GEE) model identified three variables significantly associated with *Brucella* seropositivity: abortion rate, isolation of sick animals, and access to technical advisory services. These findings highlight the importance of reproductive health indicators and management practices in the epidemiology of ovine brucellosis.

One of the most relevant findings of this study was the association between abortion rate and seropositivity. This result is consistent with the well-established pathogenesis of brucellosis, which primarily affects the reproductive system of infected animals. Infection by *Brucella* commonly leads to late-term abortions, stillbirths, and the birth of weak offspring, which are considered hallmark clinical manifestations in small ruminants (Rossetti et al., 2022; Brunetti et al., 2023; Li et al., 2023).

Abortions are a critical epidemiological event in the transmission of *Brucella*. Infected placental tissues, aborted fetuses, and reproductive secretions contain large numbers of bacteria, facilitating environmental contamination and transmission within the flock. Consequently, herds experiencing higher abortion rates are more likely to sustain transmission cycles. This mechanism is consistent with findings reported in the Ili region of China, where abortion in sheep and dairy cattle was strongly associated with infectious diseases, particularly zoonotic pathogens such as *Brucella*, which represent one of the major causes of reproductive failure in livestock. In that study, several management and environmental factors including poor biosecurity practices, inadequate hygiene in animal housing, contact with infected animals, and insufficient veterinary supervision were identified as contributors to abortion events. These findings support the results of the present study, in which the percentage of abortions was identified as a significant risk factor for *Brucella* seropositivity, highlighting the role of reproductive disorders as key indicators of infection and important drivers of pathogen transmission within sheep flocks (Ganter, 2015; Hull & Schumaker, 2018; Zhang et al., 2020).

In the present study, the isolation of sick animals was identified as a risk factor associated with *Brucella* spp. seropositivity in the multivariable GEE model. Although isolation is generally considered a biosecurity measure, this finding may reflect the presence of clinically affected animals within the flock rather than the effectiveness of the practice itself. Sheep infected with *Brucella* spp. can remain chronically infected and continue shedding bacteria in reproductive secretions, aborted fetuses, placental tissues, and other contaminated materials, thereby maintaining environmental contamination and facilitating transmission within the herd (Vection et al., 2025). This situation is particularly relevant because *Brucella* organisms are frequently present in large numbers in aborted materials and reproductive fluids, which represent the main sources of infection for susceptible animals (Ganter, 2015). Furthermore, the detection of isolation practices in positive farms may

indicate that farmers are responding to the presence of clinically ill animals rather than preventing disease introduction. If infected animals are isolated but not removed from the flock, they may continue acting as reservoirs of infection and contribute to the persistence of the pathogen. Previous studies have shown that inadequate management practices, limited recognition of brucellosis symptoms, and insufficient veterinary intervention can allow infected animals to remain within production systems, sustaining transmission cycles in small ruminant flocks (Diez, 2019). Additionally, brucellosis is characterized by chronic infections in livestock, which complicates control efforts when infected animals are not promptly identified and culled (Rossetti et al., 2022b).

Therefore, the association observed in this study suggests that the isolation of sick animals may be an indicator of underlying infection within the flock rather than a true preventive measure. Effective control of ovine brucellosis requires not only the segregation of clinically affected animals but also comprehensive surveillance, accurate diagnosis, and removal of infected individuals to interrupt transmission chains

Another key finding of this study was the identification of access to technical advisory services as a protective factor, as farms receiving veterinary support showed lower seropositivity levels. This finding suggests that veterinary guidance and farmer training play a crucial role in disease prevention by promoting improved biosecurity practices, early disease detection, and better management of reproductive health within sheep production systems. Farms receiving regular veterinary guidance are more likely to implement appropriate biosecurity measures, vaccination programs, and early disease detection strategies. Previous research has highlighted that lack of veterinary support, limited farmer education, and poor knowledge of disease symptoms are significant factors associated with higher brucellosis prevalence in sheep flocks. Furthermore, previous studies have shown that limited farmer awareness, insufficient veterinary support and poor recognition of clinical signs are major factors contributing to the persistence of brucellosis in small ruminant flocks. For example, management-related factors such as lack of veterinary guidance, limited training of farmers and inadequate disease recognition have been associated with higher prevalence of brucellosis in sheep farms (Diez, 2019; Vection et al., 2025)

In contrast, farms with greater access to veterinary extension services are more likely to implement preventive measures including surveillance, vaccination strategies where applicable, and appropriate control interventions. Brucellosis control programs worldwide emphasize the importance of coordinated veterinary surveillance and management practices, including testing, biosecurity and education of livestock producers (Kiiza et al., 2023).

Therefore, the protective effect observed in this study highlights the importance of strengthening veterinary extension programs and technical advisory services in Ecuador. Improving farmer education and facilitating access to veterinary expertise may significantly contribute to reducing the circulation of *Brucella* in sheep production systems and enhancing the effectiveness of national brucellosis surveillance and control strategies.

Mathematical models of sheep brucellosis highlight the importance of key epidemiological parameters in determining disease persistence and control in livestock populations. In particular, the basic reproduction number integrates both direct transmission between animals and indirect transmission through environmental contamination with *Brucella*. Dynamic models incorporating environmental infection pathways demonstrate that infected animals contribute significantly to environmental bacterial load through secretions and reproductive discharges, sustaining transmission within flocks. In this context, the association observed in the present study between higher abortion rates and increased seropositivity is epidemiologically consistent, as abortions represent a major route of bacterial shedding and environmental contamination. Likewise, although isolation of sick animals is intended as a biosecurity practice, infected animals may continue shedding pathogens during the infectious period, potentially maintaining environmental contamination if they are not promptly removed from the flock. Mathematical models further emphasize that effective control requires not only identification of infected animals but also appropriate testing and removal strategies, since limited diagnostic capacity or restricted culling resources may allow infected

individuals to persist in the population and sustain disease circulation (Nie et al., 2022; Yue et al., 2023).

Conversely, the protective effect of access to technical advisory services observed in this study may reflect improved implementation of biosecurity measures, disease recognition, and management practices, which are critical components for reducing transmission and lowering the effective reproduction number in endemic livestock systems. These findings emphasize the importance of strengthening extension services and veterinary outreach programs to improve disease awareness and control (Diez, 2019; Rossetti et al., 2022).

Regarding host characteristics, the present study found that most seropositive animals were females and young animals between 0.5 and 24 months of age. The higher number of positive females may be explained by the reproductive tropism of *Brucella* spp., as the bacteria preferentially infect reproductive tissues such as the placenta, uterus, and mammary glands. Infection often occurs through contact with contaminated reproductive secretions or aborted materials within the flock environment. In addition, younger animals may be more susceptible due to increased exposure during flock reproductive cycles and the absence of previous immune stimulation. However, the epidemiology of brucellosis is complex and influenced by multiple factors including management practices, herd structure, and animal movement. Breed-related differences were also observed in this study, with positive animals identified in both criollo and crossbred sheep. Although breed susceptibility to brucellosis remains poorly understood, previous research suggests that host genetic factors may influence pathogen–host interactions and disease outcomes (Rossetti et al., 2022).

From an epidemiological perspective, the detection of *Brucella* in sheep populations in Ecuador has important implications for both animal health and public health. Brucellosis remains one of the most widespread zoonotic diseases globally, causing significant economic losses in livestock production due to reproductive failure and reduced productivity (Kiiza et al., 2023).

Furthermore, the disease poses a substantial zoonotic risk, particularly for farmers, veterinarians, and slaughterhouse workers who are exposed to infected animals or contaminated biological materials. Despite progress in some developed countries, brucellosis continues to be endemic in many regions of the world, particularly in developing countries where surveillance systems and control programs are limited. Effective control strategies typically involve a combination of vaccination, surveillance, test and slaughter policies, and improved farm management practices (Ganter, 2015; Kiiza et al., 2023; Meletis et al., 2024).

In this context, the identification of management-related risk factors in Ecuadorian sheep farms provides valuable information for designing targeted control strategies.

Overall, the results of this study highlight the importance of reproductive health monitoring, farm management practices, and veterinary advisory services in reducing the risk of brucellosis transmission. Strengthening surveillance systems, improving farmer education, and promoting biosecurity practices could significantly contribute to the control of *Brucella* spp. infection in sheep production systems in Ecuador. Therefore, the consumption of aborted fetuses by dogs and other domestic carnivores may contribute to the maintenance and spread of *Brucella*, as these animals can act as potential reservoirs of the bacteria. Field observations revealed that some aborted fetuses were not properly disposed of and were subsequently consumed by domestic carnivores, particularly dogs and cats, increasing the risk of environmental contamination and pathogen transmission within and between flocks.

In various studies conducted in Latin America, abortions, the improper disposal of aborted fetuses, and the intervention of veterinary technicians have been identified as important risk factors in the transmission of ovine brucellosis. In Ecuador, spontaneous abortions in sheep are closely related to an increase in brucellosis prevalence, and the improper handling of aborted fetuses in the open air, without biosecurity protocols, facilitates the transmission of the bacteria in the herd (World Organisation for Animal Health, 2026).

The intervention of veterinarians in herd management may influence the spread of brucellosis. Although the study does not specify the lack of adequate sanitary management measures, which

could be related to veterinary intervention, it increases the prevalence of the disease in animals. Additionally, the intervention of veterinary technicians in herds does not always guarantee the proper implementation of preventive measures, as in some rural areas the lack of education and resources limits their effectiveness in disease prevention. These factors reinforce the need to establish proper sanitary management practices and continuous training for professionals in charge of animal health in rural regions of Latin America (Zhang et al., 2020; Brunetti et al., 2023).

5. Conclusions

This study provides updated epidemiological evidence on the occurrence of *Brucella* spp. infection in sheep in Ecuador, revealing an individual seroprevalence of 5.1% and a herd-level prevalence of 7.5%. These findings confirm that brucellosis is present in ovine production systems in Ecuador and highlight the importance of continued surveillance in small ruminant populations. The identification of abortion rate as a significant risk factor reinforces the well known association between *Brucella* infection and reproductive disorders in sheep. In addition, the association observed with the isolation of sick animals suggests that infected individuals may continue contributing to environmental contamination and disease persistence within flocks if not properly managed. In contrast, access to technical advisory services was identified as a protective factor, emphasizing the critical role of improved farm management practices and veterinary support in the prevention and control of brucellosis.

From an epidemiological perspective, these results underline the need to strengthen biosecurity measures, promote farmer education, and improve access to veterinary extension services in sheep production systems. Implementing targeted surveillance and control strategies could contribute to reducing the circulation of *Brucella* in Ecuadorian flocks.

The results of this study highlight the urgent need to implement effective biosecurity and health management practices in sheep production. Preventive measures should include vaccination, regular health monitoring, and isolation of infected animals.

In this context, the results of the present study underscore the need for strengthened national surveillance programs in Ecuador, improved veterinary extension services, and the integration of brucellosis control within a One Health framework that simultaneously addresses animal health, human health and livestock productivity. Strengthening diagnostic capacity, promoting farmer education and implementing targeted biosecurity measures will be essential steps to reduce the circulation of *Brucella* spp. in sheep production systems and mitigate the zoonotic risk associated with the disease.

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Informed Consent Statement: Both large and small sheep producers provided informed consent for the administration of the surveys and the collection of sheep samples, which was documented in the survey questionnaire.

Data Availability Statement: All original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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