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Prevalence and risk factors associated with bovine brucellosis in dairy farms in the province of Azuay-Ecuador

Prevalencia y factores de riesgo asociados a brucelosis bovina en ganaderías lecheras de la provincia del Azuay-Ecuador

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Abstract

The health of herds that are not within the official Brucellosis control program in the province of Azuay is unknown, and there may be areas with a higher frequency of seropositive herds. This paper aims to determine the prevalence and risk factors associated with bovine brucellosis in dairy farms. An epidemiological study was carried out in 436 farms, for which milk samples were taken from producers in collection centers, collecting trucks and herds. A georeferenced survey was used to collect information on the management of the herds. The milk was analyzed by indirect-ELISA, and thirty-seven farms were seropositive, obtaining a prevalence of 8,5%. The percentages of seropositivity were: Cuenca (14.84%), Girón (23.07%), Nabón (8.21%), Oña (11.53%), San Fernando (33.33%), Sevilla de Oro (7.14%), Sigsig (4.16%). The Rose Bengal and competitive ELISA tests were performed on bovines that contributed to the milk pool in 34 herds, establishing a 100% concordance of indirect ELISA to detect seronegative farms. In the logistic regression analysis, a significant association ($P < 0,05$) was determined between seropositivity and factors such as: geographic location, extension of the farm, exploitation system, presence of other domestic species, elimination of placental remains, reproduction system, having a higher probability of seropositivity in herds that presented abortions ($OR = 2,71$), estrus problems ($OR = 2,09$), birth of weak calves ($OR = 3,24$) and extensive management ($OR = 3,67$). These findings constitute serological evidence that *Brucella spp.* circulates in farms in the area.

Keywords: Prevalence, Brucellosis, Enzyme Linked Immunoadsorbent Assay, Risk factors.

Resumen

Se desconoce el estatus sanitario de ganaderías que no están dentro del programa oficial de control de Brucelosis en la provincia del Azuay, pudiendo existir zonas con mayor frecuencia de rebaños seropositivos. Este trabajo pretende determinar la prevalencia y factores de riesgo asociados a brucelosis bovina en predios lecheros. Se llevó a cabo un estudio epidemiológico en 436 fincas, para lo cual se tomaron muestras de leche de productores en centros de acopio, camiones recolectores y hatos. Se usó una encuesta georeferenciada a fin de recopilar información del manejo de las ganaderías. La leche se analizó mediante ELISA-indirecto, 37 fincas resultaron seropositivas, obteniendo una prevalencia de 8,5%. Los porcentajes de seropositividad fueron: Cuenca (14,84%), Girón (23,07%), Nabón (8,21%), Oña (11,53%), San Fernando (33,33%), Sevilla de Oro (7,14%), Sigsig (4,16%). Se realizaron las pruebas Rosa de Bengala y ELISA-competitivo a bovinos que aportaron al pool de leche en 34 ganaderías, estableciéndose una concordancia del 100% de ELISA-indirecto para detectar fincas seronegativas. En el análisis de regresión logística se determinó una asociación significativa ($P < 0,05$) entre la seropositividad y factores como: ubicación geográfica, extensión de la finca, sistema de explotación, presencia de otras especies domésticas, eliminación de restos placentarios, sistema de reproducción, teniendo una mayor probabilidad de seropositividad las ganaderías que presentaron abortos ($OR = 2,71$), problemas de celo ($OR = 2,09$), nacimiento de terneros débiles (OR=3,24) y manejo extensivo ($OR = 3,67$). Estos hallazgos constituyen evidencia serológica que *Brucella spp.* circula en ganaderías de la zona.

Palabras clave: Prevalencia, Brucelosis, Ensayo Inmunoadsorvente Ligado a Enzimas, Factores de riesgo.

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1 Introduction

Brucellosis is a zoonotic bacterial disease caused by several species of the genus *Brucella spp.* that infects domestic and wildlife animals (Ledwaba et al., 2019), affecting the reproductive system and causing abortions, weak offspring and producing economic losses due to the slaughter of infected animals and the limitation to trade (Assenga et al., 2015). Symptoms of brucellosis in humans are fever, fatigue, arthralgia, muscle pain and sweating, sometimes producing physical disability (Zheng et al., 2018).

Twelve species are known of which *B. abortus* affects cattle, *B. mellitensis* causes abortions in goats, *B. suis* infects pigs, *B. canis* is specific in canines, *B. ovis* infects sheep, *B. neotomae* has been reported in rats (Suárez-Esquivel et al., 2017); two species *B. pinnipedialis* and *B. ceti* were isolated in marine mammals (Kroese et al., 2018); *B. microti* has been identified in a variety of animals such as voles, wild boars; *B. papionis* has been described as host to baboons, *B. vulpis* in red foxes; *B. inopatia* has been isolated in humans although the animal reservoir has not been identified (Leclercq et al., 2020). Zoonotic capacity is more expressed in *B. mellitensis*, but *B.abortus* is also responsible for brucellosis in humans (Awah-Ndukum et al., 2018).

Transmission to humans occurs by the consumption of milk, infected dairy products, inhalation of aerosolized particles, and direct contact with tissues of diseased animals (Dal et al., 2019). Sources of infection for animals include aborted materials, vaginal secretions, milk, semen, water consumption, contaminated feed, and infection in calves can occur through the uterus and by colostrum (Ogugua et al., 2018). A close relationship between wildlife and cattle would provide potential opportunities for the transmission and persistence of brucellosis (Godfroid et al., 2013). Likewise, some studies suggest that the bacterium may circulate among several susceptible wildlife species, thus remaining permanently in ecosystems (Aruho et al., 2021).

Infections declared by the World Organization for Animal Health (OIE) as zoonotic diseases require prevention, diagnosis and control measures. For this reason, it is essential to identify risk factors associated with the pathogenesis of *Brucella spp.* infection in the different livestock management systems responsible for the spread of the disease, thus allowing effective management for its management and control (OIE, 2018).

Brucellosis is one of the most important zoonoses with more prevalence in Latin America. Argentina reports a prevalence of 19.7% in herd (Aznar et al., 2015); Uruguay 0.02% (Baruch et al., 2020); Colombia 22% (Cárdenas et al., 2018). It is difficult to establish official prevalence data in Ecuador because it has been under-reported to the OIE. However, studies on the presence of antibodies against *Brucella spp.* have been reported, varying between regions, even within regions. A nationwide study in 1979 reported a seroprevalence in the north Highland of 1.97 to 10.62%, in the Cost of 4.2 to 10.62% and in the south Highland of 1.3 to 2.6%. Another study reports a prevalence of 6% (Salguero, 2011; Román-Cárdenas and Luna-Herrera, 2017). In recent years, some research allow updating the seroprevalence level of this disease, with a significant variability ranging from 1.80-12% throughout the country (Zambrano et al., 2016).

It is necessary to understand the epidemiology of brucellosis in other regions of the country where serological surveillance is not performed as a requirement, prior to implementing control programs and determining the areas with the highest prevalence of the disease. There are several tests for diagnosing it in blood or milk. Currently, the tests prescribed for international cattle trade are Rose Bengal (RBT), Buffered Plate Agglutination (BPAT), ELISA-I (ELISA-indirect), ELISA-C (ELISA-competitive), Complement Fixation (CFT), and Polarized Fluorescence (PF) (Vhoko et al., 2018).

An initial step to set appropriate brucellosis control programs at the local level would be the georeferencing of the infection of some dairy areas that would allow measuring the disease at the farm level and generating epidemiological evidence of the endemicity of the bacterium.
Hence, the aim of the paper was to estimate the prevalence of bovine brucellosis in cattle farms in the province of Azuay, using the ELISA-I technique in milk samples. Likewise, the associated factors that could cause the appearance of the disease will be evaluated, such as presence of abortions, increased calving intervals, birth of weak calves, veterinary

assistance, absence of vaccination, herd size, among others, related to the pathogenesis and signs of brucellosis (Akinseye et al., 2016; Mugizi et al., 2015).

2 Materials and methods

2.1 Study area

This research was conducted in Cuenca, Santa Isabel, Gualaceo, Paute, Sigsig, Sevilla de Oro, Girón, San Fernando, Pucará, Oña, Nabón, El Pan and Chordeleg, belonging to the province of Azuay, located in the southern region of Ecuador, with an approximate area of 8.639 km^2. There are two distinctive zones: the east, with eastern Andes, and the west, with the coastal region. The climate varies from warm to cold due to the altitude, the presence of the Andes massif and subtropical vegetation. To the west, the province is climatologically divided in different sectors. In addition, due to its location, each climatic zone has only two defined seasons: wet and dry. In the West, the temperature ranges between 20°C and 33°C, while in the Andean zone, it is usually between 10°C and 28°C (Cárdenas and Murillo, 2018).

2.2 Study population

It consisted of agricultural production units (PU) dedicated to milk production regardless of size, which included lactating cows during the research period. The Holstein Friesen breed predominated (Instituto Nacional de Estadísticas y Censos, 2019). Herd ranged from 5 to 120 animals; the management system covered a wide range, from extensive technified herds larger than 50 ha, medium-sized herds between 5 to 50 ha, and small farms with traditional extensive management with little technology, smaller than 5 ha. For this research, dairy production zones were defined by the highest concentration of farms that supply this raw material (Ortega et al., 2017).

2.3 Study design

A descriptive cross-sectional study was conducted between 2019 and 2020. The unit of analysis consisted of milk samples obtained from collection centers, collection vehicles and directly from farms. The Win Epi epidemiological program (De Blas et al., 2006) was used to calculate the number of farms to be sampled. The total population was taken as the 15,784 production units (PU) included in the program for the control and eradication of Foot and Mouth Disease in Azuay (Agrocalidad, 2019). Since there were no previous studies in this area on the prevalence of brucellosis, we assumed an expected prevalence of 50%, an estimated error of 5% and a confidence level of 95%. The program yielded a figure of 376 farms to be sampled; however, a total of 436 cattle farms were assessed.

Proportional sampling was used to determine the number of PUs to be studied in each parish. The farms were selected randomly, according to accessibility to the area, distance, time to reach the farms, availability of resources, willingness of producers, collection centers and transporters with the greatest feasibility to participate in this research. A georeferenced survey was conducted with each owner using Survey 123 ArcGis software installed on mobile devices. None of the farms reported having a vaccination program against brucellosis.

2.4 Georeferenced survey

A geo-referenced survey was conducted with questions designed to obtain information on animal health status, and farm management based on existing literature (Cárdenas et al., 2019) with the objective of determining the possible risk factors for suffering brucellosis considering: reproductive management, animal replacement, origin of drinking water, presence of susceptible domestic animals, farming system, knowledge of the disease, reproductive problems, presence of abortions, management of waste after parturition or abortions (Cárdenas et al., 2019). Informed consent for questionnaire administration and sample collection was obtained verbally from owners prior to sampling and interview.

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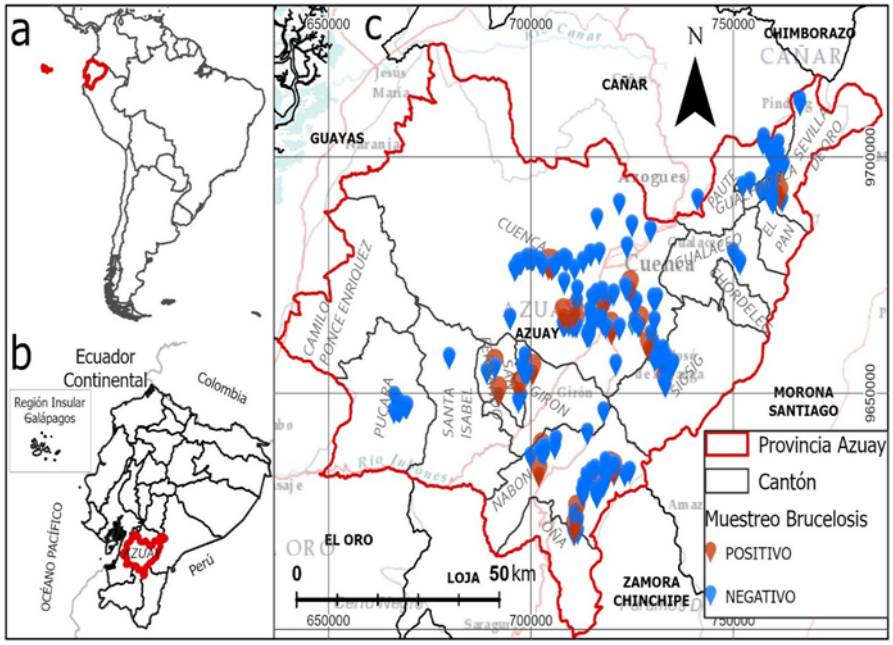

Figure 1. a) Location of Ecuador in South America b) Location of the project at the national level c) Distribution of parishes with seropositive cattle in the province of Azuay.

2.5 Analysis of milk samples by indirect ELISA

The samples were collected in sterile containers in a quantity of 100 ml. The containers were transported refrigerated to the Microbiology Laboratory of the Faculty of Agricultural Sciences of the University of Cuenca, where they were stored at $-20°C$. The ELISA-I kit (Innovate Diagnostic, France) was used to identify the presence of antibodies to *Brucella spp.*, for which the milk samples were previously centrifuged at 8 000 rpm for 10 minutes to separate the lacto-serum from the fat. A 96-well plate impregnated with *Brucella abortus* LPS was used; 100 μl of negative control and positive control were distributed in duplicate and then 100 μl of the samples were added to the remaining wells. The plate was sealed and incubated at $21°C$ for 45 min, then each well was rinsed with 300 μl of wash solution three times. 100 μl of conjugate (peroxidase-labeled ruminant IgG) was added, incubated at $21°C$ for 30 minutes, the washing process was repeated and then 100 μl of developer solution (tetramethylbenzidine) was added to all wells. The plate was incubated again for 15 minutes at $21°C$ and finally 100 μl of stop solution was added to stop the reaction.

Optical density (OD) values of samples (m) and controls were read at 450 nm (wavelength), using an ELISA plate reader (Biotek 800TS, USA). Positive controls (cp) and negative controls (cn) were used to validate the assay. Percent inhibition (PI) was calculated using Equation 1. A sample was considered positive when its PI was higher than 50 %.

$$PI = \frac{OD_m - OD_{cn}}{OD_{cp} - OD_{cn}} \times 100 \quad (1)$$

2.6 Serology for identifying seropositive animals

We had access to 34 cattle farms to perform RBT and ELISA-C tests on all the cows that contributed to the milk pool to individually confirm the presence of seropositive animals. For this, 9 ml of blood were taken from the coccygeal region in vacuum tubes without anticoagulant, which were transported to the laboratory at a temperature of $8°C$. Centrifugation was performed at 8 000 rpm (Dynac, Clay Adams, USA), for 10 minutes, to extract the blood serum to be stored in eppendorf tubes and frozen at $-20°C$.

2.7 Rose Bengal

Sera extracted from peripheral blood obtained without anticoagulant were subjected to the RBT test (Innovate Diagnostics, France), according to the manual of the World Organization for Animal Health (OIE). A gridded glass plate was used; 40 ul of the reagent were mixed with the same amount of serum to be analyzed, and the plate was lightly shaken for 4 minutes. The agglutination appearance within one minute was scored as 4+ (++++), between 1 and 4 min was scored 1+ to 3+ (+, + + + and + + + + + +) according to the different agglutination degrees. The absence of agglutination within 4 minutes was considered negative.

2.8 ELISA-C as confirmatory test

The ELISA-C kit (Svanova, Sweden) was used to confirm the presence of animals that were seropositive to *Brucella spp.* The assay was performed by adding 45 μl of dilution solution in all wells and then adding 5 μl of positive, weak and negative controls in duplicate, as well as 5 μl of dilution solution as a conjugate control; 5 ul of the samples were added afterwards. Next, 50 μl of the pre-diluted mouse monoclonal antibodies (mAb) solution, specific for a common epitope of the smooth O-polysaccharide of LPS molecule, were added to both control and sample wells. The plate was sealed and shaken for 5 minutes, then incubated for 30 minutes at $20°C$. After incubation, the plate was rinsed 4 times with PBS-Tween Buffer solution, 100μl of conjugate solution (goat anti-mouse IgG antibody bound to horseradish peroxidase, HRP) was immediately added to each well and incubated at $20°C$ for 30 minutes.

The washing process was repeated and then 100 μl of substrate (hydrogen peroxidase and ABTS chromogen) was added. It was incubated at $20°C$ for 10 minutes and the reaction was stopped by adding 50 μl of the stop solution (H2SO4) (Viveros, 2019). The microplate was read at 450 nm with a spectrophotometer (Biotek 800TS, USA), calculating the percentage inhibition (PI) for each sample using Equation 2.

$$PI = \frac{OD_m - OD_{cn}}{OD_{cp} - OD_{cn}} \times 10 \quad (2)$$

Where, OD_m, OD_{cp}, OD_{cn} are the optical density readings for the samples, positive control and negative control, respectively. Samples were classified as positive if the antibody titers recorded a $PI \geq 30\%$, defined by the supplier. In addition, the fact that $OD_{cp} > 0,350$ and $OD_{cp}/OD_{cn} > 3$, confirmed that the test worked correctly.

2.9 Statistical analysis

The analyses were performed using Infostat software version 2020 (Di Rienzo et al., 2020). The absolute and relative frequency of antibody seropositive farms in milk samples against *Brucella spp*. infection was calculated. The Chi-square test was used to analyze if there was a relation between each of the risk factors and seropositivity. The influence of the factors was investigated using the logistic regression model. Double-entry tables were made to perform Odds Ratio calculations to estimate the relative risk of an event. The confidence interval was 95% for the logarithm of the odds ratio as 1.96 standard errors on both sides of the estimate, in addition to the *P* value in each case, stablishing statistical significance when $P \leq 0,05$

3 Results

3.1 Seroprevalence of bovine brucellosis at the farm level

Antibodies to *Brucella spp.* were found in 37 milk samples from a total of 436 farms analyzed (Figure 1), with a prevalence of 8.5%. The lowest seropositivity percentage was found in Sigsig parish, with 4.16%, and the highest in San Fernando with 33.33%. No seropositivity was found in the samples from the other six parishes, so the confidence intervals (–) are recorded without values (Table 1).

3.2 Confirmation of seropositive animals on ELISA-I positive and negative farms in milk

Serological tests with RBT and ELISA-C were performed in 34 farms on the cows that contributed to the milk pool to check the presence of seropositive animals, with a 100% negative diagnosis with ELISA-I in milk samples from 20 farms, as no seropositive animals were detected, and in 14 farms whose milk samples were positive, only 12 of them had cows with antibodies (Table 2).

4 Risk factors for the presence of infection

Logistic regression revealed that abortions, geographic location, farm extension, farming system, presence of other domestic species on the farm, estrus problems, elimination of placental remains, birth of weak calves and the reproduction system were significantly related with brucellosis seropositivity ($P < 0,05$), being the herds that presented abortions the ones that showed higher risks of contracting the disease ($OR = 2,71$). Likewise, herds whose animals presented problems of repeated estrus were more likely to be infected ($OR = 2,09$). The birth of weak calves was also a factor associated with more predisposition ($OR = 3,24$).

The association with factors such as veterinary assistance, calving area, water sources, replacement animals, breed, and the presence of retained placenta did not show significant differences ($P < 0,05$) (Table 3).

Table 1. Percentage of brucellosis seropositive herds according to parishes.

Parish	Analyzed farms	Seropositive farms	% Positivity	95 % IC Inferior limit	95 % IC Superior limit
Cuenca	128	19.00	14.84	8.65	20.95
El Pan	49	0.00	0.00	–	–
Girón	13	3.00	23.07	0.19	46.00
Guachapala	15	0.00	0.00	–	–
Gualaceo	4.00	0.00	0.00	–	–
Nabón	73	6.00	8.21	1.90	14.50
Oña	26	3.00	11.53	0.00	23.00
Paute	19	0.00	0.00	–	–
Pucará	26	0.00	0.00	–	–
San Fernando	6.00	2.00	33.33	0.00	71.00
Santa Isabel	1.00	0.00	0.00	–	–
Sevilla de Oro	28	2.00	7.14	0.00	16.00
Sigsig	48	2.00	4.16	0.00	9.00
TOTAL	**436**	**37.00**	**8.50**	**5.00**	**10.00**

5 Discussion

The presence of antibodies to *Brucella spp.* in milk samples by ELISA-I and confirmed with the existence of seropositive animals in RBT and ELISA-C suggests a high exposure to the bacterium of cattle herds in Azuay, which has been previously described by the identification of Brucella abortus strains in cattle, as well as in humans, in several regions of Ecuador (Ron-Román et al., 2014; Rodríguez-Hidalgo et al., 2015).

The correlation between ELISA-I in milk, RBT and ELISA-C in blood serum, indicate a high sensitivity of ELISA-I to diagnose brucellosis-positive farms with 100 % specificity. Only in two farms positive in milk with ELISA-I, no animals were found positive to RBT or ELISA-C, possibly due to the movement of these animals to the drying pen or to reproductive problems at the time of individual sampling or to the refusal of some owners to take samples in pregnant females. An antigenic cross-reaction with other bacterial infections (*Yersinia spp, Salmonella spp, Streptococcos spp, E. coli*) could lead to false positive results in serological diagnosis (Bonfini et al., 2018), although, according to Nielsen et al. (2004), this is unlikely, due to the high specificity of serological tests for brucellosis in milk.

Table 2. Results with ELISA-I, RBT, and ELISA-C in 34 cattle farms according to geographical location.

Parish	Farms positive in milk	Farms with seropositive animals	Seropositive animals			Farms negative in milk	Farms with seropistive animals	Seropositive animals		
			Sampled animals	RBT	ELISA-C			Sampled animals	RBT	ELISA-C
Cuenca	6	6	236	29	28	10	0	239	0	0
Santa Isabel	–	–	–	–	–	1	0	24	0	0
Girón	2	2	145	31	31	2	0	41	0	0
Sevilla de Oro	–	–	–	–	–	2	0	36	0	0
Oña	1	1	31	1	1	3	0	64	0	0
San Fernando	2	2	50	3	3	–	–	–	–	–
Nabón	1	–	18	0	0	1	0	14	0	0
Sigsig	2	1	16	1	1	–	–	–	–	–
TOTAL	14	12	496	65	64	20	0	418	0	0

The prevalence of brucellosis may vary depending on the study zones, influenced by different management practices, the origin of replacement animals, the farming system, and the greater permanence of the bacteria due to variations in climate, among other factors. *Brucella spp.* is very susceptible to sunlight and heat, surviving a few hours in hot and dry months, while in summer it can survive in humid soil for approximately 7 days (Matope et al., 2010), prevailing in endemic areas, due to the wide range of susceptible hosts, capable of transmitting the disease (Ducrotoy et al., 2017; Musallam et al., 2019).

In parishes with higher prevalence of affected farms, such as San Fernando, Girón, Cuenca, brucellosis could be due to the management system, mainly due to the mixing of animals from different herds within the same geographical area (Craighead et al., 2018), because these are places with a higher number of dairy herds and have an important cattle trade. In the epidemiological surveys, most producers stated that they were unaware of the symptoms of the disease, there was an absence of serological monitoring by laboratory analysis and no discarding of infected animals that are commonly traded, thus spreading the disease. In areas detected with low prevalence, such as the parishes of Paute, El Pan, Guachapala, Gualaceo, Pucará and Santa Isabel, there are low transmission rates, possibly due to agro-ecological factors that limit contact between herds.

The prevalence values obtained in this study (8.5%) are lower than those found by Mainato and Vallecillo (2017), in the neighboring province of Cañar (13.63%) where they report a higher presence of seropositive farms in the parishes of Biblián and Cañar. An epidemiological study of Brucellosis at national level (Carbonero et al., 2018) includes the province of Azuay with a herd level prevalence of less than 10%; Pichincha 37.5%; Santo Domingo 26.8%; Tungurahua 25.3% and Zamora with 4.8%. On the other hand, Poulsen et al. (2014), in a study to determine the prevalence in two provinces of Northern Ecuador, refer a value of 7.2%. These variations at the country level could be due to sampling techniques, test interpretation, reagents used, and number of animals sampled.

Among the risk factors, farm size was a significant factor associated with a higher brucellosis seroprevalence, probably due to hygiene problems resulting from high animal density in extensive production systems. Berhe et al. (2007) reported a seropositivity risk of 8.5 and 4.3 times higher in large and medium herds, respectively, compared to small herds, since the risk of contact with animals from other herds decreases in these herds. Similarly, McDermott and Arimi (2002), state that the size of herds in extensive systems, common crossbreeding with other animals and meeting at common grazing and watering points increase the risk of contagion of the disease.

A history of abortions or stillbirths was associated with brucellosis seropositivity. Aborted fetuses and uterine secretions provide a constant supply of the bacterium, maintaining the transmission of new infections (Sanchez et al., 2020). A relationship with estrus problems in animals was also observed, which is in line with other research (Asgedom et al., 2016), that also identified an increase in the number of services per calving when cattle presented reproductive problems due to brucellosis, which affects the genital tract, leading to uterine infection and poor conception rate.

Table 3. Risk factors associated with bovine brucellosis.

Factor	Variable	N° positive farms	Seropositivity ELISA-I%	Odd ratio	95% IC	P value
Abortions	Yes	58(10)	17.24	2.71	1.25 - 5.86	0.001
	No	378(27)	7.14	0.37	0.17 - 0.80	
Parish	Cuenca	127(19)	14.96	–	–	0.001
	El Pan	49(0)	0			
	Girón	13(3)	23.07			
	Guachapala	15(0)	0			
	Gualaceo	4(0)	0			
	Nabón	73(6)	8.21			
	Oña	26(3)	11.53			
	Paute	19(0)	0			
	Pucará	26(0)	0			
	San Fernando	6(2)	33.33			
	Santa Isabel	1(0)	0			
	Sevilla de Oro	28(2)	7.14			
	Sigsig	46(2)	4.34			
Farm extension	Big	208(25)	12.01	–	–	0.0043
	Big 2	44(6)	13.64			
	Medium	78(0)	0			
	Small	106(6)	5.66			
Exploitation system	Extensive	196(27)	13.78	3.67	1.76 -7.69	0.0003
	Rope attained	240(10)	4.17	0.27	0.13 - 0.57	
Presence of domestic species	Can, OVI, EQUI	256(17)	6.64	–	–	0.01
	POR, EQUI, OVI	19(2)	11.11			
	CAN, POR, EQUI, OVI	48(3)	6.25			
	Others	49(7)	14.29			
	Do not have other species	64(7)	10.9			
Estrus problems	Yes	95(13)	13.68	2.09	1.03 – 4.25	0.03
	No	341(24)	7.04	0.48	0.24 – 0.97	
Elimination of placenta remains	Buries, trash, burns	103(15)	14.56	–	–	0.03
	Eaten by the animal / others animals	164(12)	7.32			
	Leave in the area	169(10)	5.92			
Birth of weak calves	Yes	45(9)	20	3.24	1.44 – 7.28	0.0034
	No	391(28)	7.16	0.31	0.14 – 0.69	
Reproduction system	MN (own bull/borrowed)	333(20)	6	–	–	0.0001
	AI	78(16)	20.51			
	MN / AI	25(1)	4			
Veterinary assistance	Yes	140(10)	7.14	1.3	0.62 – 2.74	0.48
	No	296(27)	9.12	0.77	0.37 – 1.61	
Calving areas	Paddocks	13(0)	7.69	–	–	0.26
	Pens	423(37)	8.74			
Water Sources	Ditch, river, well	398(37)	9.3	–	–	0.14
	Drinking water	27(0)	0			
	AP, river, ditches, well	11(0)	0			
Replacement animals	Of the farm	352(25)	7.1	–	–	0.18
	PR and nearby farms	60(9)	15			
	Out of State	6(1)	16.67			
	PR and outside the state	18(2)	11.11			
Breed	Holstein	321(24)	7.48	–	–	0.5
	Half-Breed	69(7)	10.14			
	Brown Swiss	20(2)	10			
	Jersey	26(4)	15.38			
Retained placenta	Yes	74(8)	10.81	1.39	0.62 – 3.12	0.43
	No	362(29)	8.01	0.72	0.32 – 1.61	

CAN= canines; OVI= sheep; POR= porcine; EQUI= equine; MN= natural mating; AI= artificial insemination; AP= drinking water; PR= of the farm.

In relation to the farming system, there are similar results to those reported in this work, where traditional management would facilitate the spread of the disease due to poor animal movement control (Fero et al., 2020). However, Kumar et al. (2016) mention that horizontal transmission of the disease in organized farms would be related to overcrowding, high animal density and poor hygienic practices, such as inadequate disposal of aborted fetuses, fetal membranes and vaginal secretions, which help to spread the infection.

Logistic regression associated inadequate disposal of placenta and fetuses as a predisposing factor for the transmission of infection, because millions of Brucellae are excreted during normal parturition or abortions of infected cows, which can maintain infectivity for several months, given a suitable environment of temperature, sunlight and pH (Sussex, 2016). Similarly, John et al. (2010) indicate that herd owners who improperly disposed biological waste after calving, abortions or placental retentions were more likely to have at least one seropositive animal when compared to those who properly disposed these materials.

Although in this work, the association with the introduction of animals of unknown health status into the herd did not have a significant effect, the percentage of positive animals increases when animals from other farms are introduced. Kanouté et al. (2017) determine a higher probability of observing Brucella positive herds when untested cattle enter endemic areas. They emphasize the need to monitor cattle before entering the farm, and also to promote replacement with animals from brucellosis-free farms.

According to the epidemiological survey, most of the evaluated areas were unaware of the existence of preventive immunization programs against brucellosis, so we can infer that the presence of seropositive animals was due to contact with Brucella in the field and not to post-vaccination reactions. Dorneles et al. (2015) point out that vaccination is a determining strategy for brucellosis control and eradication programs. Likewise, Pascual et al. (2018) state that eradication programs should include diagnostic tests, discarding infected animals and the incorporation of vaccination, which has been shown to reduce infections and abortions in animals.

Olsen and Stoffregen (2005) have proven that the percentage of reactors in infected herds is lower in vaccinated animals compared to non-vaccinated animals. According to their data when using full doses of strain 19 in calves and evaluating protection in cattle up to 9 years old, they estimated that approximately 65-75% of all vaccinated animals were completely protected during their productive life. Undoubtedly, the high prevalence of brucellosis detected in the area is also related to the absence of vaccination.

The breeding system can also influence *Brucella spp.* infection especially through sexual contact with neighboring herds or through the exchange of bulls coming from infected farms (Nardi et al., 2017). Breed and water sources were not shown in this study to be predisposing factors for infection. Although other management factors not considered could influence, our findings are in line with the general epidemiology of bovine brucellosis observed in other parts of the world (Franc et al., 2018; Hull and Schumaker, 2018), understanding that the high prevalence of this disease, represents an important public and animal health problem in Ecuador.

6 Conclusions

This study provides serological evidence of the presence of brucellosis in dairy herds with different seropositivity levels in the province of Azuay, with a high prevalence (8.5%), associated with risk factors involved in the pathogenesis of the disease and responsible for its spread. The ELISA-I test in milk is a useful diagnostic tool to identify brucellosis-positive farms with very high specificity, reducing sampling time, cost, and effectively analyze a larger number of farms at the same time. It is necessary to carry out sero-surveillance in cattle farms to understand the spatial distribution of the disease in the country, prior to implementing control programs and raising public awareness of the zoonotic transmission of brucellosis.

Ethical approval

All procedures were carried out in accordance with experimental practice and international standards

for animal welfare.

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References

Agrocalidad (2019). Programa de erradicación de fiebre aftosa, I Fase. techreport, Jefatura de Sanidad Agropecuaria Azuay.

Akinseye, V., Adesokan, H., Ogugua, A., Adedoyin, F., Otu, P., Kolawole, N., Awosanya, E., Cadmus, E., Cadmus, S., and Kwaghe, A. (2016). Seroepidemiological survey and risk factors associated with bovine brucellosis among slaughtered cattle in nigeria. *Onderstepoort Journal of Veterinary Research*, 83(1):1–7. Online:https://bit.ly/3LQAZG2.

Aruho, R., MacLeod, E., Manirakiza, L., and Rwego, I. (2021). A serological survey of brucellosis in wildlife in four major national parks of uganda. *BMC Veterinary Research*, 17(1):1–10. Online:https://bit.ly/40bOWTr.

Asgedom, H., Damena, D., and Duguma, R. (2016). Seroprevalence of bovine brucellosis and associated risk factors in and around alage district, ethiopia. *Springerplus*, 5:1–8. Online:https://bit.ly/3FOELvP.

Assenga, J., Matemba, L., Muller, S., Malakalinga, J., and Kazwala, R. (2015). Epidemiology of brucella infection in the human, livestock and wildlife interface in the katavi-rukwa ecosystem, tanzania. *BMC veterinary research*, 11:1–11. Online:https://bit.ly/3nlZRLu.

Awah-Ndukum, J., Mouiche, M., Bayang, H., Ngu Ngwa, V., Assana, E., Feussom, K., Manchang, T., and Zoli, P. (2018). Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the adamawa and north regions of cameroon. *Veterinary Medicine International*.

Aznar, M., Linares, F., Cosentino, B., Sago, A., La Sala, L., Leon, E., Duffy, S., and Perez, A. (2015). Prevalence and spatial distribution of bovine brucellosis in san luis and la pampa, argentina. *BMC Veterinary Research*, 11(1):1–7. Online:https://bit.ly/40bRa5f.

Baruch, J., Suanes, A., Piaggio, J., and Gil, A. (2020). Analytic sensitivity of an elisa test on pooled sera samples for detection of bovine brucellosis in eradication stages in uruguay. *Frontiers in Veterinary Science*, 7:178. Online:https://bit.ly/3FIQpIM.

Berhe, G., Belihu, K., and Asfaw, Y. (2007). Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of tigray region of ethiopia. *International Journal of Applied Research in Veterinary Medicine*, 5(2):65. Online:https://bit.ly/40cJyzq.

Bonfini, B., Chiarenza, G., Paci, V., Sacchini, F., Salini, R., Vesco, G., Villari, S., Zilli, K., and Tittarelli, M. (2018). Cross-reactivity in serological tests for brucellosis: a comparison of immune response of escherichia coli o157: H7 and yersinia enterocolitica o: 9 vs brucella spp. *Vet Ital*, 54(2):107–114. Online:https://bit.ly/3G5RKcK.

Carbonero, A., Guzmán, L., García-Bocanegra, I., Borge, C., Adaszek, L., Arenas, A., and Saa, L. (2018). Seroprevalence and risk factors associated with brucella seropositivity in dairy and mixed cattle herds from ecuador. *Tropical animal health and production*, 50:197–203. Online:https://bit.ly/40vkZ0C.

Cárdenas, C. and Murillo, M. (2018). Calidad bacteriológica de la leche cruda en ganaderías de la provincia del azuay. Master's thesis, Universidad de Cuenca.

Cárdenas, L., Melo, O., and Casal, J. (2018). Evolution of bovine brucellosis in colombia over a 7-year period (2006-2012). *Tropical animal health and production*, 50:19–27. Online:https://bit.ly/3G42KXT.

Cárdenas, L., Peña, M., Melo, O., and Casal, J. (2019). Risk factors for new bovine brucellosis infections in colombian herds. *BMC Veterinary Research*, 15:1–8. Online:https://bit.ly/3Ky3QOd.

Craighead, L., Meyer, A., Chengat, B., Musallam, I., Akakpo, J., Kone, P., Guitian, J., and Häsler, B. (2018). Brucellosis in west and central africa: A review of the current situation in a changing landscape of dairy cattle systems. *Acta tropica*, 179:96–108. Online:https://bit.ly/40F6RC1.

Dal, T., Kara, S., Cikman, A., Balkan, C., Acıkgoz, Z., Zeybek, H., Uslu, H., and Durmaz, R. (2019). Comparison of multiplex real-time polymerase chain reaction with serological tests and culture for diagnosing human brucellosis. *Journal of Infection and Public Health*, 12(3):337–342. Online:https://bit.ly/3UcbvFt.

De Blas, I., Ruiz-Zarzuela, I., and Vallejo, A. (2006). Winepi: Working in epidemiology. an online epidemiological tool. In *ISVEE 11: Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics*.

Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., and Robledo, C. (2020). Infostat versión 2020. techreport, Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Online: http://www.infostat.com.ar.

Dorneles, E., Sriranganathan, N., and Lage, A. (2015). Recent advances in brucella abortus vaccines. *Veterinary research*, 46(1):1–10. Online:https://bit.ly/3U6W6Gl.

Ducrotoy, M., Bertu, W., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A., Welburn, S., Ocholi, R., Blasco, J., and Moriyón, I. (2017). Brucellosis in sub-saharan africa: Current challenges for management, diagnosis and control. *Acta tropica*, 165:179–193. Online:https://bit.ly/3MhYWGx.

Fero, E., Juma, A., Koni, A., Boci, J., Kirandjiski, T., Connor, R., Wareth, G., and Koleci, X. (2020). The seroprevalence of brucellosis and molecular characterization of brucella species circulating in the beef cattle herds in albania. *PloS one*, 15(3):e0229741. Online:https://bit.ly/40IYntH.

Franc, K.and Krecek, R., Häsler, B., and Arenas-Gamboa, A. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC public health*, 18(1):1–9. Online:https://bit.ly/40NZ19y.

Godfroid, J., Garin-Bastuji, B., Saegerman, C., and Blasco, J. (2013). Brucellosis in terrestrial wildlife. *Revue Scientifique et Technique. Office International des Epizooties*, 32(1):27–42. Online:https://bit.ly/3UbHCoC.

Hull, N. and Schumaker, B. (2018). Comparisons of brucellosis between human and veterinary medicine. *Infection ecology & epidemiology*, 8(1):1500846. Online:https://bit.ly/3ZIbytu.

Instituto Nacional de Estadísticas y Censos (2019). Encuesta de superficie y producción agropecuaria continua espac-2019. INEC. Online:https://bit.ly/3K3sg0A.

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G., MacMillan, A., and Cleaveland, S. (2010). Quantifying risk factors for human brucellosis in rural northern tanzania. *PloS one*, 5(4):e9968. Online:https://bit.ly/3UdPwxU.

Kanouté, Y., Gragnon, B., Schindler, C., Bonfoh, B., and Schelling, E. (2017). Epidemiology of brucellosis, q fever and rift valley fever at the human and livestock interface in northern côte d'ivoire. *Acta tropica*, 165:66–75. Online:https://bit.ly/435rxVy.

Kroese, M., Beckers, L., Bisselink, Y., Brasseur, S., van Tulden, P., Koene, M., Roest, H., Ruuls, R., and Backer, J.and IJzer, J. (2018). Brucella pinnipedialis in grey seals (halichoerus grypus) and harbor seals (phoca vitulina) in the netherlands. *Journal of wildlife diseases*, 54(3):439–449. Online:https://bit.ly/411lDTr.

Kumar, A., Gupta, V., Singhi, A., and Reddy, N. (2016). Seroprevalence and risk factors associated with bovine brucellosis in western uttar pradesh, india. *Indian journal of animal sciences*, 86(2):131–135. Online:https://bit.ly/3zxcMx9.

Leclercq, S., Cloeckaert, A., and Zygmunt, M. (2020). Taxonomic organization of the family brucellaceae based on a phylogenomic approach. *Frontiers in Microbiology*, 10:3083. Online:https://bit.ly/3GiJaXY.

Ledwaba, M., Gomo, C., Lekota, K., Le Flèche, P., Hassim, A., Vergnaud, G., and Van Heerden, H. (2019). Molecular characterization of brucella species from zimbabwe. *PLOS Neglected Tropical Diseases*, 13(5):e0007311. Online:https://bit.ly/3MsDoXE.

Mainato, S. and Vallecillo, A. (2017). Seroprevalencia de la brucelosis bovina en la provincia del cañar, ecuador. *Maskana*, 8:25–28. Online:https://bit.ly/3KdWuye.

Matope, G., Bhebhe, E., Muma, J., Lund, A., and Skjerve, E. (2010). Herd-level factors for brucella seropositivity in cattle reared in smallholder

dairy farms of zimbabwe. *Preventive Veterinary Medicine*, 94(3-4):213–221. Online:https://bit.ly/3KyFrIy.

McDermott, J. and Arimi, S. (2002). Brucellosis in sub-saharan africa: epidemiology, control and impact. *Veterinary microbiology*, 90(1-4):111–134. Online:https://bit.ly/3zFy63q.

Mugizi, D.and Boqvist, S., Nasinyama, G., Waiswa, C., Ikwap, K., Rock, K., Lindahl, E., Magnusson, U., and Erume, J. (2015). Prevalence of and factors associated with brucella sero-positivity in cattle in urban and peri-urban gulu and soroti towns of uganda. *Journal of Veterinary Medical Science*, 77(5):557–564. Online:https://bit.ly/40LTbVU.

Musallam, I., Ndour, A., Yempabou, D., Ngong, C., Dzousse, M., Mouiche-Mouliom, M., Feussom, J., Ntirandekura, J., Ntakirutimana, D., and Fane, A. (2019). Brucellosis in dairy herds: A public health concern in the milk supply chains of west and central africa. *Acta tropica*, 197:105042. Online:https://bit.ly/3KCjfNN.

Nardi, G., Megid, J., Mathias, L., Paulin, L., Vicente, A., Cortez, A., Listoni, F., Lara, G., Motta, R., and Chacur, M. (2017). Performance of microbiological, serological, molecular, and modified seminal plasma methods in the diagnosis of brucella abortus in semen and serum of bovine bulls. *Biologicals*, 48:6–9. Online:https://bit.ly/40IGM5i.

Nielsen, K., Smith, P., Widdison, J., Gall, D., Kelly, L., Kelly, W., and Nicoletti, P. (2004). Serological relationship between cattle exposed to brucella abortus, yersinia enterocolitica o: 9 and escherichia coli o157: H7. *Veterinary microbiology*, 100(1-2):25–30. Online:https://bit.ly/418YWwR.

Ogugua, A., Akinseye, V., Cadmus, E., Jolaoluwa Awosanya, E., Alabi, P., Idowu, O., Akinade, S., Dale, E.and Perrett, L., and Taylor, A. (2018). Prevalence and risk factors associated with bovine brucellosis in herds under extensive production system in southwestern nigeria. *Tropical animal health and production*, 50:1573–1582. Online:https://bit.ly/416zU1a.

OIE (2018). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. World Organization for Animal Health.

Olsen, S. and Stoffregen, W. (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert review of vaccines*, 4(6):915–928. Online:https://bit.ly/3MjqATs.

Ortega, V., Dután, J., Ayala, L., Rodas, R., Nieto, P., Vázquez, J., Pesántez, J., Andrade, O., Pesántez, M., and Guevara, R. (2017). Caracterización productiva de las ganaderías en los cantones occidentales de la provincia del azuay. *Maskana*, 8:145–147. Online:https://bit.ly/4182Gic.

Pascual, D., Yang, X., Wang, H., Goodwin, Z., Hoffman, C., and Clapp, B. (2018). Alternative strategies for vaccination to brucellosis. *Microbes and infection*, 20(9-10):599–605. Online:https://bit.ly/3Ke4XS1.

Poulsen, K., Hutchins, F., McNulty, C., Tremblay, M., Zabala, C., Barragan, V., Lopez, L., Trueba, G., and Bethel, J. (2014). Brucellosis in dairy cattle and goats in northern ecuador. *The American Journal of Tropical Medicine and Hygiene*, 90(4):712. Online:https://bit.ly/414gha4.

Rodríguez-Hidalgo, R., Contreras-Zamora, J., Benitez Ortiz, W., Guerrero-Viracocha, K., Salcan-Guaman, H., Minda, E., and Ron Garrido, L. (2015). Circulating strains of brucella abortus in cattle in santo domingo de los tsáchilas province–ecuador. *Frontiers in Public Health*, 3:45. Online:https://bit.ly/3m8noQb.

Román-Cárdenas, F. and Luna-Herrera, J. (2017). Revisión actualizada de la epidemiología de brucelosis (brucella abortus, brucella mellitensis, brucella suis, brucella canis) en el ecuador y el mundo. *Centro de Biotecnología*, 6(1):82–93. Online:https://bit.ly/3ZGYWTy.

Ron-Román, J., Ron-Garrido, L., Abatih, E., Celi-Erazo, M.and Vizcaíno-Ordóñez, L., Calva-Pacheco, J., González-Andrade, P., Berkvens, D., Benitez-Ortiz, W., and Brandt, J. (2014). Human brucellosis in northwest ecuador: typifying brucella spp., seroprevalence, and associated risk factors. *Vector-Borne and Zoonotic Diseases*, 14(2):124–133. Online:https://bit.ly/3KjgKyf.

Salguero, A. (2011). Determinación de la prevalencia serológica de brucelosis en bovinos de las provincias de carchi , esmeraldas e imbabura y análisis de factores de riesgo. Master's thesis, Universidad Central del Ecuador.

Sanchez, J., Jimenez, M., Gutierrez, J., Cruz, J., and Nahet, J. (2020). Seroprevalence of abortus diseases that commit the bovine reproductive efficiency in two dairy areas of chiapas. *ESPACIO I+D, INOVACIÓN MÁS DESARROLLO*, (10).

Sussex, W. (2016). *Veterinary Microbiology*. World Organization for Animal Health.

Vhoko, K., Iannetti, S., Burumu, J., Ippoliti, C., Bhebhe, B., and De Massis, F. (2018). Estimating the prevalence of brucellosis in cattle in zimbabwe from samples submitted to the central veterinary laboratory between 2010 and 2014. *Vet Ital*, 54(1):21–27. Online:https://n9.cl/q8pak.

Viveros, J. (2019). Prevalencia y factores de riesgo de la brucelosis bovina en ganaderías de imbabura que proveen leche a floralp s.a. Master's thesis, Pontificia Universidad Católica del Ecuador.

Zambrano, M., Pérez, M., and Rodríguez, X. (2016). Brucelosis bovina en la provincia manabí, ecuador: Estudio de los factores de riesgo. *Revista de Investigaciones Veterinarias del Perú*, 27(3):607–617. Online:https://n9.cl/3wzbe.

Zheng, R., Xie, S., Lu, X., Sun, L., Zhou, Y., Zhang, Y., and Wang, K. (2018). A systematic review and meta-analysis of epidemiology and clinical manifestations of human brucellosis in china. *BioMed research international*, 2018. Online:https://bit.ly/3KGWeJG.