Rev Inv Vet Perú 2022; 33(4): e23341
https://doi.org/10.15381/rivep.v33i4.23341

Seroprevalence and factors associated with bovine brucellosis in Pinar del Río province, Cuba

Seroprevalencia y factores asociados a la brucelosis bovina en la provincia de Pinar del Río, Cuba

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ABSTRACT

The objective of this study was to determine the seroprevalence of bovine brucellosis and identify risk factors for brucellosis in Pinar del Río province, Cuba. A cross-sectional study was performed based on the results of the epidemiological surveillance programme. Geospatial distribution was analyzed by calculating the standardized incidence ratio (SIR) at the municipality level. In addition, the association between diagnostic results and factors such as the productive system, sex and age groups (<12, 12-24, 25-36, >36 months) was assessed. A total of 12 760 blood samples were processed and 113 were positive; thus, the estimated seroprevalence in the province

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Recibido: 16 de noviembre de 2021
Aceptado para publicación: 4 de junio de 2022
Publicado: 31 de agosto de 2022

was 0.89% (95 % CI 0.74-1.06). The disease was present in three out of eight municipalities in the province (Los Palacios, Consolación del Sur and Pinar del Río), although only in Los Palacios the prevalence was significantly higher than that expected: 2.40 (1.30-3.28). Furthermore, the prevalence ratio in animals younger than 12 months was 8.33 (1.41-49.42) times higher than in animals between 12 and 24 months of age. In conclusion, the municipality of origin and age category are relevant factors that should be considered by the health authorities to design disease control strategies in the province.

Key words: *Brucella* spp, cattle, seroprevalence, risk factors, zoonoses

Resumen

El objetivo del estudio fue determinar la seroprevalencia de brucelosis bovina e identificar factores de riesgo para brucelosis en la provincia de Pinar del Río, Cuba. Se realizó un estudio transversal basado en los resultados del programa de vigilancia epidemiológica. La distribución geoespacial se analizó mediante el cálculo de la razón de incidencia estandarizada (SIR) a nivel de municipio. Además, se evaluó la asociación entre los resultados del diagnóstico y factores como el sistema productivo, el sexo y los grupos de edad (<12, 12-24, 25-36, >36 meses). Se procesaron 12 760 muestras de sangre y 113 resultaron positivas; así, la seroprevalencia estimada en la provincia fue de 0.89% (IC 95 % 0.74-1.06). La enfermedad estuvo presente en tres de los ocho municipios de la provincia (Los Palacios, Consolación del Sur y Pinar del Río), aunque solo en Los Palacios la prevalencia fue significativamente superior a la esperada: 2.40 (1.30-3.28). Además, la razón de prevalencia en animales menores de 12 meses fue 8.33 (1.41-49.42) veces mayor que en animales entre 12 y 24 meses. En conclusión, el municipio de procedencia y la categoría de edad son factores relevantes que deben ser considerados por las autoridades de salud para diseñar estrategias de control de enfermedades en la provincia.

Palabras clave: *Brucella* spp, bovinos, seroprevalencia, factores de riesgo, zoonosis

Introduction

Brucellosis is caused by a bacterium of the genus *Brucella*, which is formed by 11 species differentiated by their antigenic characteristics and their animal host (OIE, 2016). It is a zoonotic infectious disease with worldwide distribution (Beauvais *et al.*, 2016; OIE, 2016), causing reproductive disorders and productive and economic losses (Martínez *et al.*, 2018). The epidemiological situation of the disease varies (Adamu *et al.*, 2016) as it has been eradicated in most industrialized countries (Díaz-Aparicio, 2013); however, the disease is endemic in many developing countries (Márquez *et al.*, 2012; López, 2014; Dorneles *et al.*, 2015). In Latin America and the Caribbean, the prevalence of the disease varies considerably from one country to another (Tique *et al.*, 2016), partially depending on the control and eradication programmes entities (Tique *et al.*, 2009).

Brucellosis in domestic animals is associated with certain risk factors which favour the introduction and presence of the disease in a population (Martínez *et al.*, 2018; Cárdenas *et al.*, 2019). Characteristics such as age, breed, body condition, and sex are highlighted (Makita *et al.*, 2011). It is also associated with certain herd characteristics and production conditions, including abortion history, herd size, insemination method, and farm management practices (Anka *et al.*, 2014; Lindahl *et al.*, 2014).

In Cuba, the first case of brucellosis was detected in 1935 (Cotrina and Fernández, 1991), but the prevention and control programme for this disease was implemented in the 80s. A large part of the national territory was free of the disease due to the effectiveness of the epidemiological measures. However, there were difficulties in controlling the disease since the early 90s and therefore, it remains in some territories of the eastern provinces of the country and other western provinces such as Pinar del Río (Mendoza *et al.*, 2015).

In 2015, Cuba reported a total of 22 outbreaks of bovine brucellosis to the World Organization for Animal Health (OIE). In 2016, the number of reported outbreaks increased to 52 distributed in the provinces of Pinar del Río, Artemisa, Ciego de Ávila, Camagüey, Las Tunas, Holguín, and Granma (OIE, 2013), which indicated a trend towards the worsening of the epidemiological situation in the country. That year, nine outbreaks of bovine brucellosis occurred in Pinar del Río province, plus several cases of brucellosis in humans (ONEI, 2016), mostly associated with human contact with infected cattle or the consumption of contaminated food.

It is highly important to conduct epidemiological studies that help to understand and characterize the disease and thus contribute to its control and eradication. Therefore, the objective of this study was to determine the seroprevalence and geographical distribution of bovine brucellosis in Pinar del Río province and to evaluate its association with possible risk factors.

Materials and Methods

Study Area

A cross-sectional study was carried out in Pinar del Río province, the westernmost province of Cuba. The region is bordered by the waters of the Gulf of Mexico and the Caribbean Sea to the north, south, and west, while to the east, it limits with the Province of Artemisa (Figure 1).

Table 1. Area and bovine population per municipality of Pinar del Río province

Municipality	Bovine population (n)	Area (km^2)
Consolación del Sur	88,950	1,111.9
Guane	36,172	720.8
La Palma	35,487	636.2
Los Palacios	38,636	770.8
Mantua	25,391	914.6
Minas de Matahambre	33,733	857.9
Pinar del Río	190,337	726.7
San Juan y Martínez	44,969	408.2
San Luis	32,393	327.2
Sandino	37,891	1,714.1
Viñales	27,972	696.0

Source: IPF (2020)

Sampling and Diagnostic Methodology

Bovine blood samples were collected during 2019 in eight of the eleven municipalities of Pinar del Río Province (Table 1), as part of the Brucellosis control programme that establishes the monitoring of animals once a year and the slaughter of positive animals.

The blood samples were obtained from the jugular or the coccygeal vein of the animals. In all cases, the owners of the animals signed the informed consent form. A commercial vacuum tube system and disposable needles were used to collect the blood samples. Blood samples were sent in an icepack-cooled container to the provincial laboratory and received within 24 hours after

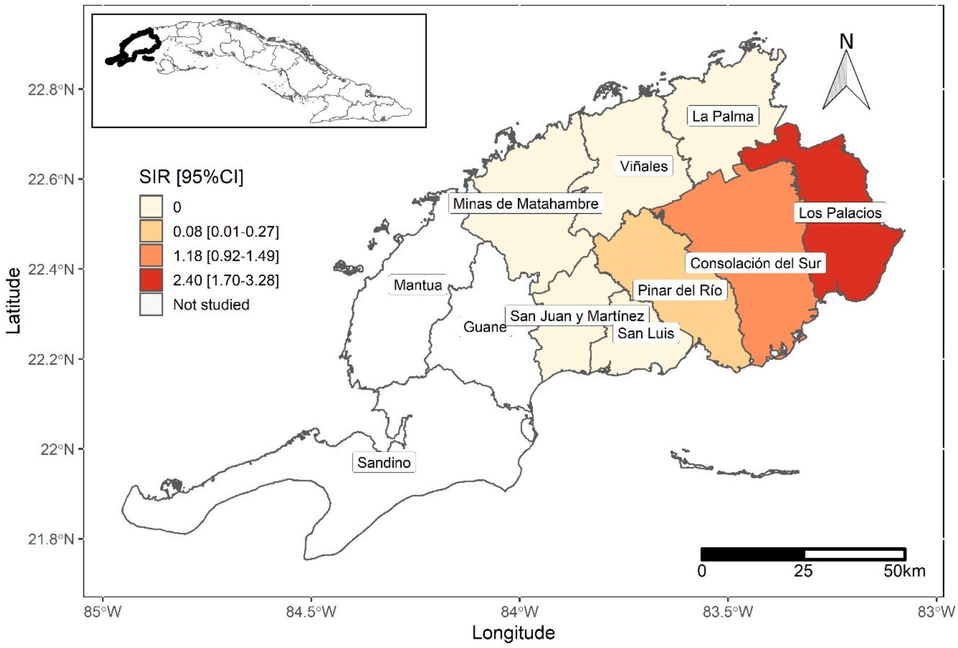

Figure 1. Map of the Pinar del Rio province (Cuba) showing the standardized incidence ratio per municipality

sampling. Samples were centrifuged at 1500 *g* for 6 min and the serum was transferred in 0.5 ml aliquots to labelled disposable microcentrifuge tubes and frozen at -20 °C.

Sample processing was carried out using the techniques recommended by the OIE (OIE, 2016). The Rose Bengal (RB) test was used as a screening test and Complement Fixation Reaction (CFR) was used as a confirmatory test for samples positive to RB test.

Statistical Analysis

The seroprevalence of brucellosis was calculated according to the formula SP = PA/ IA * 100, where SP = Seroprevalence, PA = Positive animals, and IA = Investigated animals.

The Standardized Incidence Ratio (SIR) was calculated to identify those municipalities with a more unfavourable situation. The number of expected cases (Ei) per municipality was calculated by multiplying the incidence of the disease in the province by the population per municipality. The number of observed cases (Oi) of brucellosis per municipality was divided by the expected cases. Thus, SIR was calculated as SIR= Oi / Ei.

The confidence interval of SIR per municipality was estimated using the *epi.conf* function of the «epiR» package of the R software (R_Development_Core_Team R, 2018). The hypothesis that SIR is equal to 1 was tested by *Byar's* method. Municipalities with an SIR significantly ($p<0.05$) higher than 1 were considered high risk, and those with an SIR lower than 1 were considered low risk. Geographical representation was carried out using the R statistical program (R_Development_Core_Team *R,* 2018).

The association between seroprevalence and animal and herd characteristics available in the accompanying information of the sera sent to the laboratory was assessed. Inde-

Table 2. Seroprevalence of bovine brucellosis per municipalities of Pinar del Río province, Cuba (2019)

Municipality	Cattle		Seroprevalence (%)	95% CI
	Sampled (n)	Positive (n)		
Los Palacios	1,838	39	2.12	1.56-2.89
Consolación del Sur	6,887	72	1.05	0.83-1.31
Pinar del Rio	2,989	2	0.07	0.02-0.24
San Juan y Martínez	400	0	0	0.00-0.95
Viñales	268	0	0	0.00-1.41
La Palma	243	0	0	0.00-1.56
San Luís	111	0	0	0.00-3.35
Minas de Matahambre	24	0	0	0.00-13.80
Total	12,760	113	0.89	0.74-1.06

pendent variables were, i) system (Semi-extensive / Intensive), ii) sex (F / M), iii) age (<12, 12-24, 25-36, >36 months). The prevalence ratio was calculated using a generalized linear model with a binomial distribution and log link in R (R_Development_Core_Team *R,* 2018). Univariate and multivariate analyses were performed.

Results

A total of 12 760 bovine samples were analysed and 113 of them were positive, showing a seroprevalence of 0.89% (95% CI 0.74-1.06). The positive cases were distributed in three out of eight municipalities (Pinar del Río, Los Palacios and Consolación del Sur) (Table 2).

Figure 1 shows the map of the study area and the standardized incidence ratio per municipality. Los Palacios municipality had 2.40 (95% CI 1.70-3.28) times more cases than those expected; hence, this ratio was significantly (p<0.05) higher than in the rest of the study area. This municipality can be considered a high-risk area for the occurrence of brucellosis in the bovine population. The incidence of the disease in Consolación del Sur was slightly but not significantly higher than that expected (1.18; 092-1.49). On the other hand, Pinar del Río municipality showed a significantly low SIR of 0.08 (0.01-0.27), and together with the rest of the municipalities studied having no cases, it was considered a low-risk territory.

Univariate analyses (Table 3) showed that sex of animals and farm production systems were not associated with *Brucella* spp seropositivity to and only age showed a significant association. Although the sex and production system conditions were not significant, both variables were included in the multivariate analysis to estimate the adjusted prevalence ratio. The age range variable showed an association with the dependent variable in both the univariate and multivariate analyses.

In the multivariate model, disease seroprevalence among animals under 12 months of age was 8.33 (1.41-49.42) times

Table 3. Univariate and multivariate analysis. Association between the prevalence of brucellosis and the potential risk factors

	Category	Cattle (n)	Positive (%)	Univariate models			Multivariate model		
				Prev. ratios	95% CI	p	Prev. ratios	95% CI	p
Production system	Semi-intensive / Extensive	6,314	46 (0.73)	Ref.			Ref.		
	Intensive	6,446	67 (1.04)	1.43	0.98 – 2.07	0.06	1.34	0.92 – 1.96	0.13
Age (months)	12-24	912	2 (0.22)	Ref.			Ref.		
	<12	165	3(1.82)	8.29	1.40 – 49.18	0.02	8.33	1.41 – 49.42	0.02
	25-36	3,082	26 (0.84)	3.85	0.92 – 16.15	0.07	3.17	0.75 – 13.49	0.12
	>36	8,601	82 (0.95)	4.35	1.07 – 17.62	0.04	3.77	0.92 – 15.41	0.06
Sex	F	8,770	85 (0.97)	Ref.			Ref.		
	M	3,990	28 (0.70)	0.72	0.47 – 1.11	0.14	0.78	0.50 – 1.21	0.26

higher than in animals between 12 and 24 months (reference category). In addition, the seroprevalence of brucellosis in animals aged between 25 and 36 months (3.17, 0.75-13.49) and animals older than 36 months (3.77, 0.92-15.41) was also higher, but not statistically significant ($p<0.05$); however, the latter showed a marginally significant p-value ($p=0.064$). Furthermore, it should be noted that the prevalence among animals older than 36 months was approximately 1%, while the prevalence in the reference category was only 0.22% (Table 3).

Discussion

The epidemiological situation of brucellosis has been worsening in Cuba since the 80s and 90s due to the aggravation of Cuba's economic problems, seriously affecting the disease control and eradication programme (Mendoza *et al*., 2015). The estimated seroprevalence was 0.89%, which, although low, it demonstrates the importance of the disease in the studied region. Similar results were obtained in a previous study conducted in the eastern provinces of the country (Mendoza *et al*., 2015), which also demonstrated that the number of outbreaks and prevalence of the disease tended to increase. On the other hand, other studies in Brazil (Gonçalves *et al*., 2009, Borba *et al*., 2013) reported seroprevalences of 0.16% and 2.5%, respectively.

It is widely recognized that multiple factors are associated with the presence of bovine brucellosis in herds (Cárdenas *et al*., 2019); however, in recent years, Cuba has shown no reports of studies where these factors are referred.

In this study, animals younger than 12 months old were found to have a higher risk of presenting the disease. These results differ from those reported by Apodaca (2004), in which higher seroprevalences were found in adults. Other authors (Walker, 1999; Asmare *et al.*, 2013) pointed out that young animals tend to be more resistant to infection or have less risk of exposure to it, but it is also noted that latent infections can occur (Walker, 1999). On the other hand, Kumar *et al.* (2016) in India also found higher seroprevalence in young animals.

In Cuba, vaccination is not used as a disease control measure. Hence, positive results in young animals are related to their infection, even directly or from infected mothers. This has been reported in other studies suggesting that in herds where brucellosis is present, there is a considerable risk of infection of young animals, either by consuming colostrum or during the birth of calves from infected mothers (Díaz-Aparicio, 2013). In general, serological diagnosis is only performed in adult animals (Fernández *et al*., 2018), which may explain the low number of calves examined during the study period. Fernández *et al*. (2018) recommend the testing of animals at earlier ages in those regions where the risk of infection is high.

The municipalities Los Palacios and Consolación del Sur showed a higher occurrence of the disease than that expected, which may be related to the environmental conditions in which livestock is developed. In these municipalities, located on the southern coast of the province, there are low and muddy soils with the presence of stagnant waters (Estévez *et al*., 2017) that can favour the transmission of *Brucella* (Agrocalidad, 2009; Zambrano Aguayo *et al*., 2016).

The ability of bacteria of the genus *Brucella* to survive in humid environments for long periods is well known, and climatic variables such as precipitation, temperature, humidity play an important role in the epidemiology of the disease (Aune *et al*., 2012). In this sense, a previous study carried out in Pinar del Río province in Cuba (Obregón *et al*., 2015) detected the presence of *Brucella* spp in the environment in regions where the disease is endemic.

Studies in Brazil have shown that the environmental conditions in breeding areas constitute a risk factor for the occurrence of brucellosis disease (Barbosa da Silva *et al*., 2014). Studies in African countries such as Ethiopia and Tanzania also demonstrated that the prevalence of brucellosis varies depending on the region and ecosystems (Yilma *et al*., 2016; Kedir Elemo and Minda, 2018; Sagamiko *et al*., 2018).

Conclusions

- The seroprevalence of cattle brucellosis at the Los Palacios municipality region was significantly higher than in other municipalities of Pinar del Río province, although Consolación del Sur municipality also deserves special attention.
- The age category was associated with the seroprevalence of brucellosis, as animals younger than 12 months showed higher seroprevalence.
- The municipality of origin and age category are relevant factors that should be considered by the health authorities to design disease control strategies in the province.

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