



Seroprevalence and risk factors for bovine brucellosis in the state of Paraná, Brazil: an analysis after 18 years of ongoing control measures

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Abstract

Seroprevalence and risk factors of bovine brucellosis (*Brucella abortus*) in herds and cattle were estimated by a cross-sectional study in the state of Paraná, Brazil. The state was divided into seven regions and a random, two-stage sampling was performed on properties and cattle from each region between 2018 and 2019. Serum samples were collected from 11,592 cows over 24 months from 1,757 properties and a questionnaire was applied to identify potential risk factors. As recommended by the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), serological testing for the detection of anti-*Brucella* antibodies included the buffered plate agglutination test (screening test) and the fluorescence polarization assay (confirmatory test). The seroprevalence of bovine brucellosis on properties and in cattle was 4.87% (95% confidence interval [CI]: 3.98–5.93%) and 2.24% (95% CI: 1.47–3.41%), respectively. Multiple logistic regression analysis identified larger herd size and failure to test for brucellosis as risk factors for the presence of anti-*B. abortus* antibodies. These results demonstrate no change in the prevalence when comparing initial studies conducted in 2002. Given our findings, it is recommended that policies for brucellosis control include a widespread vaccination program for higher prevalence areas and eradication approach to lower prevalence areas. All steps related to correct immunization of the herds should be verified and improved by training and education. Health education action must be carried out informing farmers about the risks of introducing animals not tested for brucellosis into their herds and the benefits of testing their herds regularly.

Keywords Cattle · *Brucella abortus* · Prevalence · Risk factors · Brazil

Introduction

Bovine brucellosis is a zoonosis caused by *Brucella abortus*. The main clinical signs of bovine brucellosis are related to reproductive disorders in males and females (Neta et al., 2010; Poester et al., 2013). Other signs include interstitial mastitis and arthritis (Lage et al., 2008; Poester et al., 2013).

The disease has a worldwide distribution, even though several countries in Western and Northern Europe, Canada, Japan, Australia, and New Zealand are believed to be free from the causal agent (OIE 2020). This was achieved by

animal health programs using different combinations of strategies (Zhang et al., 2018).

In Brazil, the mean economic impact of bovine brucellosis was estimated at US\$206 per dairy cow infected and \$108 per beef cow infected, taking into account costs associated with reproductive losses, decreased milk and beef production, and costs of veterinary interventions (Santos et al., 2013). According to that study, every 1% increase or decrease in prevalence in Brazil is expected to increase or decrease the economic burden of brucellosis by approximately \$76 million per year. Economic impact of brucellosis has been described in detail by McDermott et al. (2013).

The Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal — PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) was developed in 2001 in

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Brazil by the national animal health authority, Ministério da Agricultura Pecuária e Abastecimento — MAPA (Ministry of Agriculture, Livestock and Supply) (Brasil, 2001). The main objective of the PNCEBT is to reduce the prevalence and incidence of both diseases. To achieve this objective, sound evidence is necessary and thus many states performed brucellosis prevalence studies with a standard methodological approach. Reported prevalence of herds in the different states of Brazil varied between 0.32 and 41.5% and heterogeneity was evident throughout the country (Alves et al., 2009; Marvulo et al., 2009; Negreiros et al., 2009; Ogata et al., 2009; Rocha et al., 2009; Sikusawa et al., 2009; Silva et al., 2009; Villar et al., 2009; Azevedo et al., 2009; Chate et al., 2009; Dias et al., 2009; Gonçalves et al. 2009a; Gonçalves et al. 2009b; Klein-Gunnewiek et al., 2009; Borba et al., 2013; Almeida et al., 2016; Clementino et al., 2016).

Seven states of Brazil reported a second prevalence study. Of those, only Mato Grosso, Mato Grosso do Sul, and Rondônia described reduction of prevalence of seropositive herds due to vaccination (Anzai et al., 2016; Barddal et al., 2016; Baumgartem et al., 2016; Dias et al., 2016; Guedes et al., 2016; Inlamea et al., 2016; Silva et al., 2016). Herd

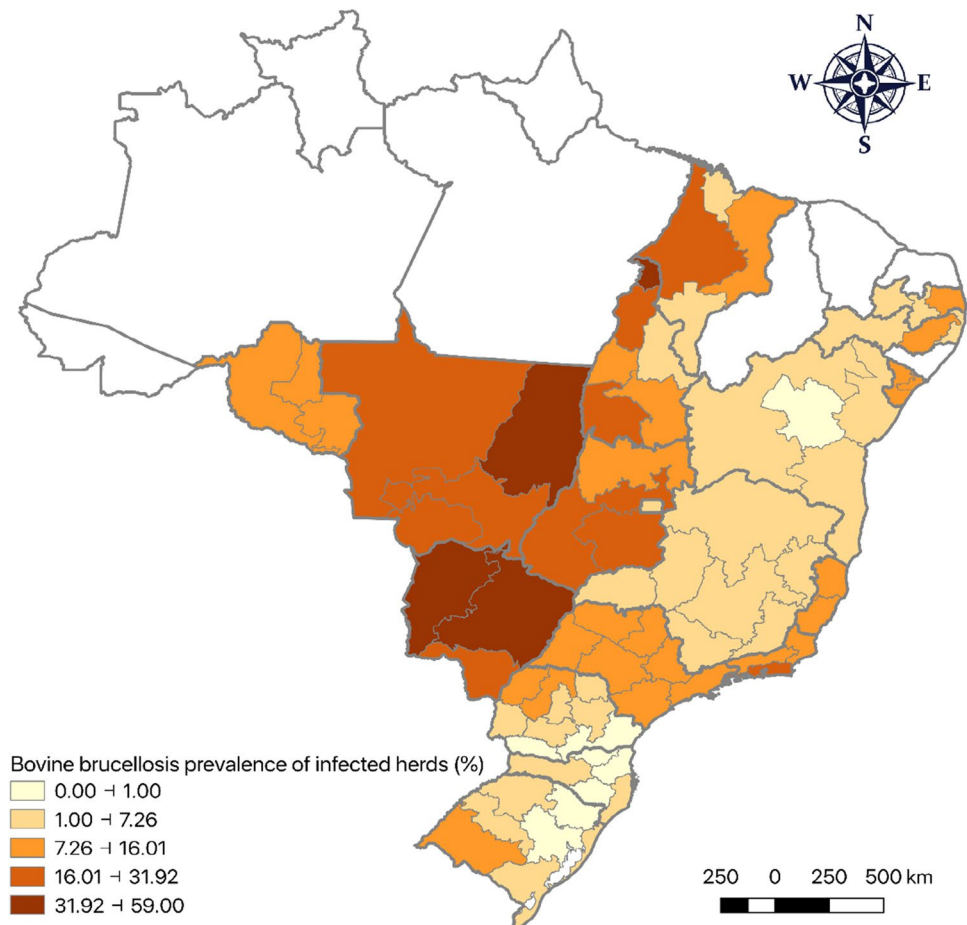
prevalence in several states of Brazil was obtained between 2003 and 2014 and can be seen in Fig. 1.

Dias et al. (2009) estimated brucellosis prevalence in Paraná in 2002. They reported a herd prevalence of 4% [CI 3.2–4.8%] and animal prevalence of 1.7% [CI 1.1–2.4%] and identified the purchase of breeding cattle and sharing of pastures as risk factors.

As per PNCEBT, control of brucellosis in Brazil is based on vaccination of female calves between 3 and 8 months of age, testing of cattle prior to their movements when animals are destined for reproduction or agglomeration, elimination of all positive animals in abattoirs, voluntary certification of free herds, and surveillance and culling in areas with low prevalence. Female calves are mainly vaccinated with *B. abortus* strain S19, although strain RB51 may be used instead according to PNCEBT (Brasil, 2017b). Also, the latter can be used in a revaccination program at the farmer will.

Considering the importance of understanding the results achieved under the PNCEBT actions in the state of Paraná, Brazil, we aimed in this study to assess current risk factors and the variation in prevalence of brucellosis in cattle herds in Paraná, Brazil. Furthermore, data obtained would allow

Fig. 1 Prevalence of herds infected for bovine brucellosis carried out in the Brazilian states between 2003 and 2014. Data not available in white areas



the evaluation of the efficacy of control measures implemented since the inception of PNCEBT 18 years ago.

Materials and methods

Study area and population

This epidemiological study was performed in the state of Paraná, positioned in the south region of Brazil. The target population was all its domestic female bovines and buffalos (in this text generically referenced as cattle) over 24 months of age. The region has a geographical area of 199.305 km² and a cattle population of 8,397,219 individuals (IBGE 2017), which represents 4.8% of the national herd. Its bovine population is the eighth largest in the nation. Paraná has the third highest milk production in the country (IBGE 2017).

The state is divided into seven regions to characterize the different regional production profiles. This regional partitioning is the same as was used in a previous study (Dias et al., 2009), to allow comparisons (Fig. 2).

Sampling

A two-level sampling was applied to estimate seroprevalence of herds and cattle seropositive for anti-Brucella antibodies. The first step consisted to choose a defined number of farms, followed by the random selection of cows over 24 months of age within those farms. We limited the scope of the study to this category in order to focus on reproductive age and

reduce false positive due to immunization, considering that female calves are vaccinated between 3 and 8 months of age in Brazil, according to PNCEBT (Brasil, 2017b). Collection of samples and their analysis in the laboratory were accomplished from September 2018 to April 2019.

The sample size of properties to be accessed in each of the seven regions was estimated in accordance with the formula for simple random samples (Thrusfield, 2018):

$$n = \frac{Z_{\alpha}^2 * P * (1 - P)}{d^2}$$

where n denotes the sample size, Z is the normal distribution value at α level of confidence, P is expected prevalence, and d is the absolute error.

Thus, we defined the parameters to define sample size of properties as degree of confidence of 0.95, expected apparent prevalence of 5%, and absolute error of 0.03.

An exhaustive alphabetically sorted list of primary units was used to apply a systematic random sampling. When the selected unit needed to be replaced, it was substituted by the next available propriety in the list. To select the number of animals to be tested in each farm, we calculated the minimum number of cows that should have been tested to reveal the brucellosis status of the herd, considering the sensitivity and specificity of the test protocols (animal level) as 95% and 99.5%, respectively (Gall and Nielsen, 2004), 20% of intra-herd prevalence, and a sensitivity and specificity greater than or equal to 90% (herd level). Thus, 10 animals were sampled in properties with up to 99 cows older than

Fig. 2 Map of the state of Paraná, showing the regions defined in the current study. The state was divided into seven regions: 1. Noroeste; 2. Centro-Oeste-Norte; 3. Norte pioneiro; 4. Centro-Sul; 5. Oeste; 6. Leste-Sul; and 7. Sudoeste

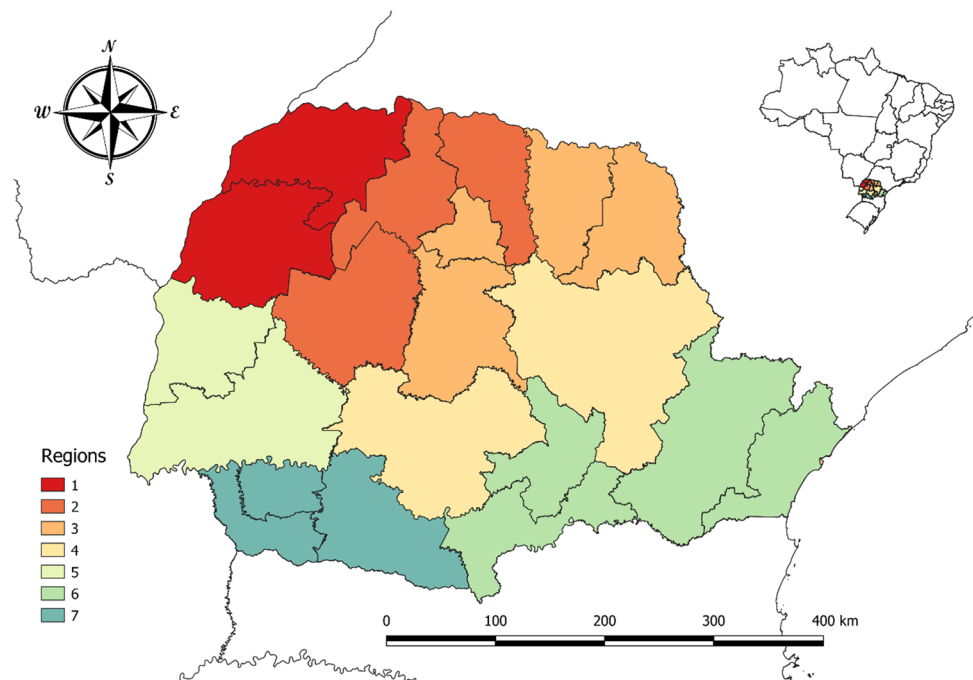


Table 1 Population and sample data in the study per region and total

Region	No. of municipalities	Total herds with reproductive activity	Total cows	Herds sampled	Sampled cows
1. Noroeste	57	18,875	763,643	251	2035
2. Centro-Oeste-Norte	78	15,533	488,045	250	1766
3. Norte Pioneiro	80	28,133	795,964	253	1702
4. Centro-Sul	32	26,011	729,383	251	1702
5. Oeste	52	23,153	583,813	251	1528
6. Leste-Sul	56	18,697	182,131	251	1105
7. Sudoeste	44	32,786	583,796	250	1755
Total	399	163,188	4,126,775	1757	11,592

24 months and 15 animals were sampled in properties with 100 or more cows older than 24 months. Table 1 provides information regarding the target population and samples per region.

Within the properties, cows were selected randomly. Cows in the peripartum period, i.e., approximately 15 days before and after delivery, were excluded from the selection, since it can affect diagnosis results.

Serologic tests

A serial testing scheme was performed as described in national regulation (Brasil, 2017a). Each serum sample was submitted to buffered plate agglutination test (BPAT) and reactors (positive samples) were subsequently submitted for fluorescence polarization assay (FPA), both tests accomplished as described in OIE standards (OIE 2019). BPAT has sensitivity of 95.4% and 98.4%, while those values are 97.5% and 98.9% for FPA (Gall and Nielsen, 2004). Samples that reacted to both assays were considered seropositive. All tests were performed by the Centro de Diagnóstico Marcos Enrietti (Diagnosis Centre Marcos Enrietti) in 2019.

Apparent prevalence in herds and animals

Apparent prevalence in herds was calculated for each region as the direct proportion of tested herds that were seropositive. A herd was considered positive when one or more of its cows tested positive.

Herd prevalence in the state of Paraná was based on the relative weight of each region (HWS) as follows:

$$HWS = \frac{\text{properties in the region}}{\text{properties sampled in the region}}$$

Thus, when calculating herd prevalence in Paraná, each farm weight was applied based on what region this farm is located and regions with larger total number of properties contributed more to state level herd prevalence.

The weight of each animal in the calculation of the prevalence of infected animals within the regions (AWR) was given by the following equation:

$$AWR = \frac{\text{cows} \geq 24 \text{ months in the property}}{\text{cows} \geq 24 \text{ months sampled in the property}}$$

To compute apparent animal prevalence within the state of Paraná, each sampled cow had its weight (AWS) calculated by the following expression (DOHOO et al., 2003):

$$AWS = \frac{\text{cows} \geq 24 \text{ months in the property}}{\text{cows} \geq 24 \text{ months sampled in the property}} * \frac{\text{cows} \geq 24 \text{ months in the region}}{\text{cows} \geq 24 \text{ months sampled in the region}}$$

Therefore, herds contributed according to their size to animal prevalence in each region and in the state level.

The final weight of each farm and animal can be conferred in the data made available below in “Availability of data and material” section.

Also, a spatial analysis using a kernel density estimate map was produced to represent clustering of positive herds. The model was design to consider and correct the differences of sampling density across the state of Paraná.

Study of risk factors

A questionnaire to access potential risk factors was fulfilled based solely on the owner or farm manager answers. All herds sampled provided one set of answers each. Data were collected in loco, in a face-to-face interview during the same visit to collect samples, using an electronic device, and then uploaded into an electronic spreadsheet.

Questions included information regarding the number of bovines and buffalos reared by age and sex category, presence of other domestic and wild species, management and operation type, breeds, trade of animals, placentar waste disposal, veterinary attention, vaccination, and history of abortions on the property (Appendix I).

Statistical analysis

Statistical analyses were performed using R Core Team (2018). Confidence intervals (CIs) were obtained by binomial logistic regression model.

Variables with more than two possible answers (not dichotomic) were regrouped in some cases for a more meaningful analysis. The number of cows was used as a measure of herd size and was categorized based on percentiles.

All potential risk factors were submitted to univariate analysis regarding whether the respective farm was classified as brucellosis infected herd or not. Techniques used to achieve this were the chi-squared test or Fischer test. A conservative p value of ≤ 0.2 was used as criteria to select which variables would be used in a multivariate logistic regression (Silva Abreu et al., 2009). The construction of a statistical model was based in a backward elimination of variables until all the variables were statistically significant in the model ($p < 0.05$). When necessary, models fitting was compared using Akaike information criterion (AIC).

Results

Apparent herd prevalence per region and for the entire state is presented in Table 2. Of 1,757 herds, 95 had one or more positive cows. Prevalence at state level was calculated applying weight as described in “Materials and methods.”

Table 3 shows the apparent prevalence in cows aged over 24 months. Of 11,592 animals, 137 were seropositive (2.24%).

Table 4 shows the apparent prevalence in herds stratified by type of production. Beef herds had higher prevalence, except in region 4, while mixed-type herds had lower levels, except in region 7; when considering the 95% confidence intervals, the only difference observed was between mixed-type and beef herds in region 2.

Based in the geolocation of each of the farms sampled, a kernel density estimate was produced for positive herds in Paraná (Fig. 3). It shows that seropositive farms are unevenly distributed across the area, with higher density in the north and northwest regions. The model considers the sampling density of the different areas of the state.

Table 2 Apparent prevalence of herds seropositive for brucellosis in cattle in seven regions and in the state of Paraná, 2019

Region	Herds sampled	Herds positive	Prevalence (%)	CI (95%)
1. Noroeste	251	33	13.14	8.95–17.33
2. Centro-Oeste-Norte	250	24	9.6	5.94–13.25
3. Norte Pioneiro	253	9	3.55	1.27–5.84
4. Centro-Sul	251	10	3.98	1.55–6.40
5. Oeste	251	10	3.98	1.55–6.40
6. Leste-Sul	251	3	1.19	0–2.54
7. Sudoeste	250	6	2.4	0.4–4.3
Paraná	1757	95	4.87†	3.98–5.93†

†Calculated using weights as described.

Table 3 Apparent prevalence of cattle seropositive for brucellosis in seven regions and in the state of Paraná, 2019

Region	Cows sampled	Cows positive	Prevalence (%)	CI (95%)
1. Noroeste	2035	47	4.28	1.73–6.84
2. Centro-Oeste-Norte	1766	28	1.38	0.52–2.23
3. Norte Pioneiro	1701	22	1.15	0–2.68
4. Centro-Sul	1702	12	1.43	0.28–2.58
5. Oeste	1528	15	1.54	0.34–2.73
6. Leste-Sul	1105	5	1.14	0–2.65
7. Sudoeste	1755	8	2.06	0–4.63
Paraná	11,592	137	2.24	1.47–3.41

Note: Calculated using weights as described.

Table 4 Apparent prevalence of herds seropositive for brucellosis in cattle in seven regions stratified by type of production (beef, dairy, and mixed), 2019

Region	Beef		Dairy		Mixed	
	Prevalence %	CI 95% (%)	Prevalence %	CI 95% (%)	Prevalence %	CI 95% (%)
1	20.79 (21/101)	12.86–28.72	8.69 (8/92)	2.90–14.48	6.89 (4/58)	0.31–13.47
2	20.00 (15/75)	10.88–29.11*	6.73 (7/104)	1.89–11.56	2.81 (2/71)	0–6.69*
3	8.00 (8/100)	2.65–13.34	1.04 (1/96)	0–3.08	0 (0/57)	0–6.26†
4	4.10 (3/73)	0–8.69	5.94 (6/101)	1.30–10.57	1.29 (1/77)	0–3.84
5	11.36 (5/44)	1.96–20.75	3.20 (4/125)	0.10–6.29	1.21 (1/82)	0–3.60
6	4.05 (3/74)	0–8.57	0 (0/72)	0–4.99†	0 (0/105)	0–6.26†
7	5.26 (2/38)	0–12.45	1.11 (2/179)	0–2.66	6.06 (2/33)	0–14.32

*Statistically different ($p < 0.05$).

†Calculated by exact binomial method.

Fig. 3 Kernel density of herd prevalence across the state of Paraná, 2019

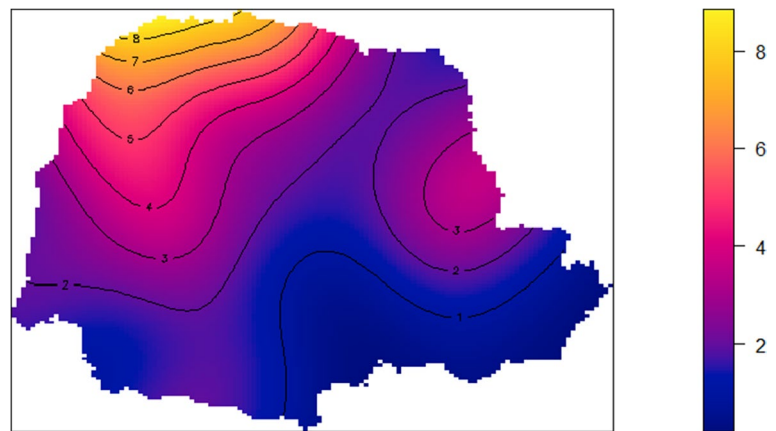
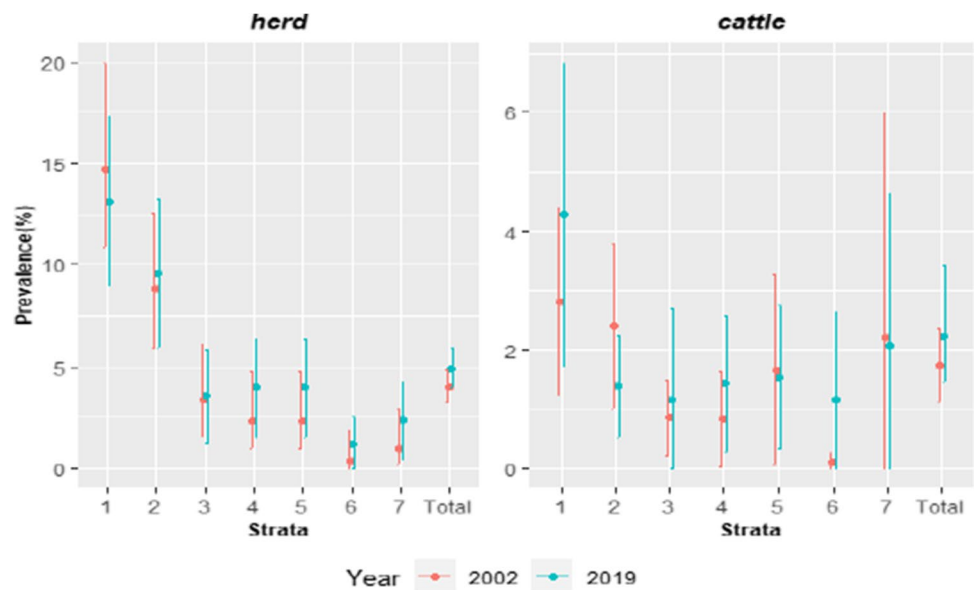


Fig. 4 **a** Herd apparent prevalence in seven regions of state of Paraná in 2002 and 2019. **b** Cattle apparent prevalence in seven regions of state of Paraná in 2002 and 2019



Herd and cattle prevalence obtained in a similar cross-sectional study developed in 2002 (Dias et al., 2009) was compared to the present study (Fig. 4). No evident differences in terms of prevalence were noticed.

As result of univariate analysis, the following variables had $p \leq 0.2$ when confronted with the herd outcome for brucellosis and were included in a multivariate logistic regression analysis: purchase ($p < 0.001$) and sale ($p < 0.001$) of cattle, introduction of breeding cattle ($p = 0.02$), number of

cows aged over 24 months ($p < 0.001$), brucellosis vaccination ($p = 0.01$), correct administration of vaccine ($p < 0.001$), slaughter of animals on the farm ($p < 0.001$), presence of equines ($p < 0.001$), breed ($p < 0.001$), presence of domestic birds ($p = 0.01$), presence of domestic feline ($p = 0.04$), presence of tapir ($p = 0.05$), presence of swine ($p = 0.05$), presence of wild feline ($p = 0.08$), presence of flooded areas on the farm ($p = 0.08$), presence of wild canine ($p = 0.12$), concentration of cattle in some areas of the farm ($p = 0.14$), presence of resting areas to other herds within the farm ($p = 0.15$), system intensity — confined, semi-confined, or extensive ($p = 0.15$) — and presence of caprine or ovine ($p = 0.18$).

In the multivariate logistic regression, only two variables had a significant result ($p \leq 0.05$) and were included in the final model: number of cows aged over 24 months (3 levels: small herds (≤ 8 cows — 50th percentile), medium herds (≥ 9 to ≤ 88 cows — 51st to 95th percentiles), and large herds (≥ 89 cows — 96th to 100th percentiles)) and if cattle were tested for brucellosis (Table 5).

Discussion

The results revealed an overall herd prevalence of 4.87% (CI 3.98–6.00), distributed heterogeneously in the state of Paraná. Regions 1. Noroeste (13.14%) and 2. Centro-Oeste-Norte (9.6%) had the highest prevalence in the area while regions 6. Leste-Sul (1.19%) and 7. Sudoeste (2.4%) had the lowest (Table 2). The differences can be partially attributed to different production systems and practices within each region, as discussed below. Also, a kernel density model (Fig. 3) has shown an intense variation of infected farms across the areas of the state of Paraná.

The geographical pattern of higher herd prevalence of brucellosis in the northern areas of the state of Paraná when compared to its southern areas mirrors the prevalence of their bordering states, that is, São Paulo and Santa Catarina, respectively. In São Paulo, the bordering state in the north, herd prevalence was estimated at 10.2% (Dias et al., 2016), comparable to regions 1. Noroeste and 2. Centro-Oeste-Norte of Paraná, while a herd prevalence of 1.2% was

reported by the bordering state in the south, Santa Catarina (Baumgartem et al., 2016), comparable to regions 6. Leste-Sul and 7. Sudoeste of Paraná. Bordering regions share some features as same climate and types of production and hold a higher rate of animals' trade within its area, which may explain those spatial patterns. Argentina and Paraguay reported similar overall cattle prevalence — 2.1% and 3.15%, respectively (Aznar et al., 2012), compared with 2.24% in Paraná.

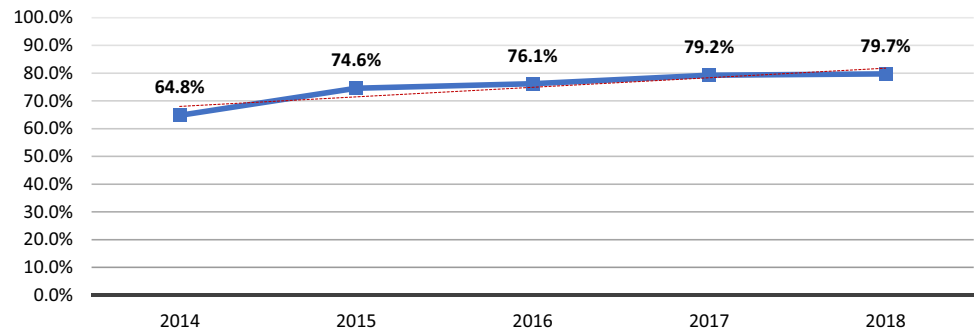
Animal prevalence (Table 3) tends to follow the same distribution seen in herd prevalence (Table 2). However, its lower values do not allow confirmation that differences observed between regions are statistically significant.

Herd prevalence at the state level was 4.87 (CI 95% 3.98–5.93) and animal prevalence was 2.24 (CI 95% 1.47–3.41). Thus, no major change was detected when comparing that figures to those of a similar study conducted in 2002 (Dias et al., 2009) when those authors reported herd prevalence being 4.02 (CI 95% 3.23–4.80) and animal prevalence 1.73 (CI 95% 1.10–2.36), as shown in Fig. 4. Since 2001, the PNCEBT has introduced a range of measures to control cattle brucellosis in Brazil (Brasil, 2001), including mandatory vaccination of all female calves. Regardless of this, the vaccination coverage has grown slowly in most states of Brazil (Brasil, 2020). In Paraná, vaccination levels in female calves increased from 64.8 to 79.7% between 2014 and 2018 (Fig. 5). Even considering this increasing pattern, limited immunization at the population level may partially explain why no decrease in brucellosis prevalence was observed in this period. Different levels of vaccination will lead to different intensity in herd prevalence decrease (Amaku et al., 2009). This is of particular relevance to areas with high prevalence, in which successful immunoprotection tends to reduce prevalence levels (Barddal et al., 2016; Inlamea et al., 2016). To better understand the role of vaccination, further analysis of prevalence of brucellosis and vaccination coverage in each area of the state is recommended. Also, it is worthwhile to consider that higher levels of vaccination may produce a slightly higher number of false positive when using BPAT and FPA testing protocol (Nielsen et al., 1996; Gall and Nielsen, 2004).

Table 5 Final multivariate logistic regression model of the association of risk factors with bovine brucellosis in herds from the state of Paraná, 2019

Variable and levels	Estimate	Odds ratio	95% CI	<i>p</i>
Herd size (cows aged over 24 months)				
Herd ≤ 8 cows (50th percentile)	Base category			
Herd ≥ 9 to ≤ 88 cows	1.51	4.56	2.54–8.19	<0.001
Herd > 88 cows (95th percentile)	2.87	17.62	8.69–37.76	<0.001
Diagnosis				
Brucellosis regularly tested	Base category			
Brucellosis not regularly tested	0.82	2.28	1.40–3.69	<0.001

Fig. 5 Brucellosis vaccination coverage of female calves in the state of Paraná between 2014 and 2018 (Brasil, 2020)



Conversely, the southern area of Paraná (regions 6 and 7) will benefit much less from a reliable vaccination program due its low prevalence of brucellosis. In these areas, prevalence is as low as that observed in the state of Santa Catarina (Baumgartem et al., 2016), where brucellosis vaccines are forbidden as a general rule and a surveillance program aimed at eradicating the disease is the current strategy. The network surveillance in this state includes slaughterhouse serum sampling, milk pool sampling in every dairy herd, and investigation of epidemiological links of infected herds. Each infected herd is fully tested twice, and positive animals are eliminated before restrictions may be lift. A test-and-slaughter strategy has been used worldwide in countries that successfully eradicated the disease (Zhang et al., 2018).

Risk factors identified in multivariate logistic regression were herd size and frequency of testing animals for brucellosis (Table 4). Herd size seems to play an important role in brucellosis transmission as larger herds are concentrated in areas with higher prevalence. While herd size is a relative concept, its association with brucellosis has been demonstrated over a diverse range of situations (Crawford et al., 1990). Although animals within large herds are not intrinsically more susceptible to infection, there are aspects associated with large-sized herds which may facilitate the transmission of brucellosis. Farms running larger herds are more likely to source replacement animals externally and from different origin, which increases the risk of introducing *B. abortus*. Also, more complex and intense relationship between animals tends to occur within larger herd size, resulting in wider opportunities to transmission events occur (Ossada et al., 2013). Herd size has been described as a major risk factor in many other states in Brazil. Using massive datasets from different areas of Brazil, de Alencar Mota et al. (2016) concluded that larger herds that purchased replacement stock pose greater risk regarding brucellosis.

Routine testing of animals for brucellosis allows assessment of herd status and control of the disease. Our data indicated that of the 778 farms where brucellosis is tested regularly, 33 (4.29%) were classified as positive, while in the 979 farms where testing was not regularly performed, 60 (6.12%) were classified as positive. The state of Paraná accounts for

30% of all brucellosis tests in Brazil, which corresponds to roughly 3 million tests performed between 2014 and 2018 (Brasil, 2020). Nevertheless, it is recommended that further analysis is undertaken to ascertain how the current testing protocol impacts on disease control. The adequate identification of positive animals and their culling should be followed by a consistent surveillance program in their farm of origin. All dairy herds must be annually tested for brucellosis following a policy enforced by authorities (PARANA, 2017) and in our sample, 572/769 (74.38%) dairy herds reported regular animal testing, while only 54/505 (10.69%) beef herds reported regular testing for brucellosis. Accordingly, beef cattle had a higher herd prevalence (Table 4) and this is in line with reports from Argentina (Caione et al., 2010) and other states of Brazil (de Alencar Mota et al., 2016).

In a previous study of risk factors for brucellosis in Paraná, purchase of breeding cattle and pasture rental were described as risk factors (Dias et al., 2009), and in their univariate analysis, the authors reported that the herd size had a high correlation ($p < 0.001$) with the disease. However, the influence of brucellosis testing was not assessed in this previous study. Our data did not show any relationship to prevalence of brucellosis for the variable pasture rental ($p = 0.78$).

Given the limitation of cross-sectional studies to detect risk factors (DOHOO et al., 2003), it is our recommendation that longitudinal studies are conducted to further explore risk and causal effects. As well, consideration should be given to the fact that transversal studies will present limitations in detecting eventual reduction of brucellosis prevalence in regions 3, 4, 5, 6, and 7, due to their low level of disease prevalence and their respective confidence intervals.

In conclusion, it is recommended that policies for brucellosis control include a consistent vaccination program, especially in areas of high risk and/or higher prevalence, thus including beef and larger herds. All steps related to immunization of the herds should be verified and improved by training and education, from the adequate maintenance of cold chain to the correct inoculation of cattle. Furthermore, a strong health education action must be implemented, informing farmers about the risks of introducing animals into their

herds without testing. In areas exhibiting low levels of disease prevalence (regions 6 and 7), the state should assess the possibility of implementing eradication strategies. New approaches should be adapted to access eventual changes in the pattern of this, including those based on surveillance system.

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Author contribution Conceptualization: M.M., O.S.B., J.H.H.G.F., R.A.D., M.B.H., E.O.T., V.S.P.G., J.S.F.N., and F.F.; analyzed data: D.L.R.; performed research: D.L.R. and E.A.A.; wrote paper: D.L.R.; review and editing: J.S.F.N., V.S.P.G., and C.H. All authors have read and agreed to the published version of the manuscript.

Data availability The following raw data are available online at <https://data.mendeley.com/datasets/3ntpkdvsc/2>, Table S1: Brucellosis_Parana_Animal_Prevalence, Table S2: Brucellosis_Parana_Herd_Prevalence, Table S3: Risk Factors Brucellosis—NoGeo.xlsx, and Table S4: Variables key.pdf.

Declarations

Ethics approval The manuscript does not contain clinical studies or patient data and the research was approved by the Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science under protocol number CEUA 3253011018.

Conflict of interest The authors declare no competing interests.


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