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Large-scale study of herd-level risk factors for bovine brucellosis in Brazil

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ABSTRACT

Bovine brucellosis is an important zoonosis caused by *Brucella abortus* that negatively impacts livestock productivity. In 2001, Brazil launched a new national program aimed at eradicating animal brucellosis that included large-scale studies of the prevalence and risk factors to support strategic decision-making. These studies were implemented by the animal health authorities and were underpinned by the scientific coordination of the University of São Paulo and the University of Brasília. The state-level results were published and revealed important differences in herd prevalence among regions. The risk factors varied across states and did not clearly explain the observed spatial disease spread. This study used a consolidated herd-level database of 14 states and 17,100 herds, from the prevalence surveys' data, to gain insights into herd profiles and cattle production practices that might be associated with the risk of brucellosis. At the time of data collection, the study area comprised just over 56 million bovine females aged over 24 months and approximately 1.8 million herds. After an exploratory univariable analysis, all factors with $p \leq 0.20$ were included in a multiple logistic regression model, using the design-based method in order to take herd sampling weights into account. The number of females in the herd markedly increased the risk of infection; compared with smaller herds (less than 30 females), the odds ratio was 3.42 [CI 95% 2.98–3.91] for herds with 31 to 100 females, 5.68 [4.92–6.55] for herds with 101 to 400 females, and 13.14 [10.94–15.78] for herds with more than 400 females. The risk was higher for extensive cattle production farms (OR = 1.23 [1.07–1.42]) and for farms that purchased replacement stock from cattle traders (OR = 1.27 [1.08–1.47]) or directly from other farms (OR = 1.19 [1.07–1.32]). The exclusive use of artificial insemination (OR = 0.57 [0.4–0.81]) and regular veterinary support (OR = 0.68 [0.6–0.77]) appeared to be protective factors. These findings are consistent with the regional prevalence trends observed in the study and provide key guidance for the planning of the national effort to control and eradicate brucellosis. High vaccination coverage of heifers is recommended, especially when targeted to areas where large-scale extensive cattle production predominates. The smaller, more intensive herds, are good candidates for disease accreditation schemes.

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1. Introduction

Bovine brucellosis is an infectious disease caused by *Brucella abortus*, which has developed mechanisms to live intracellularly for prolonged periods in its hosts, including cattle. In cattle, the disease is largely associated with reproductive problems, such as abortions, birth of weak calves and low fertility, and often causes serious economic and livestock losses (Jones et al., 2000; Silva et al., 2005).

Since the beginning of the twentieth century, many countries have adopted stringent measures for the control and eradication of brucellosis in animal populations to mitigate production losses and risks to human health (Poester et al., 2009). Most recorded cases of infection and clinical disease caused by bovine brucellosis worldwide are observed in the Middle East, the Mediterranean, sub-Saharan Africa, Latin America and some zones of China and

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India (WAHID, 2012). The countries or zones considered to be free of the disease are concentrated in areas of greater socio-economic development, such as North America and Europe, and in east Asian countries and Oceania, such as Japan, Australia and New Zealand (OIE, 2014). Previous studies on the factors associated with *Brucella* spp. infection dynamics highlighted the importance of a high population density (Salman and Meyer, 1984; Matope et al., 2010), large herd size (Kellar et al., 1976; Salman and Meyer, 1984; Kadohira et al., 1997; McDermott and Arimi, 2002; Muma et al., 2006; Tun, 2007; Lee et al., 2009; Al-Majali et al., 2009; Matope et al., 2010), extensive production (Kadohira et al., 1997; Silva et al., 2000; McDermott and Arimi, 2002; Renukaradhya et al., 2002; Blasco, 2004; Martins et al., 2009) and intense animal purchase or transportation (Kadohira et al., 1997; Renukaradhya et al., 2002; Muma et al., 2006; Berhe et al., 2007; Stringer et al., 2008; Jergefa et al., 2009; Kaoud et al., 2010). When large herds are affected by bovine brucellosis, a greater proportion of the animals are infected, and the disease tends to persist longer in the herd, making eradication difficult. Adequate production and sanitary management practices, such as the use of calving paddocks and use of veterinary assistance, are cited as protective factors that cause a marked reduction in the level of infection in herds (Van Waveren, 1960; O'Connor, 1972; Vanderwagen et al., 1978; Luna-Martínez and Mejía-Terán, 2002; Rosales et al., 2002; Aricapa et al., 2008; Al-Majali et al., 2009; Jergefa et al., 2009).

In Brazil, the first attempts to control bovine brucellosis date back to the 1940s and 1950s with the establishment of serological examinations for cows that aborted, the segregation of positive animals, voluntary vaccinations of animals and guidelines for animal transportation (Poester et al., 2002; Lage et al., 2005). In 1976, Ministerial Decree no. 23 regulated the diagnostic testing protocol based on rapid or slow seroagglutination and required the slaughter of animals that tested positive and voluntary vaccination with B19 of heifers between three and eight months of age (Brazil, 1976). However, these measures alone without the implementation of a structured national animal health program resulted in little change in the Brazilian situation at that time (Paulin and Ferreira Neto, 2002).

A national study conducted in 1975 revealed a prevalence of affected animals of 4.1% in the northern region, 2.5% in the northeast, 6.8% in the central west, 7.5% in the southeast and 4.0% in the south (Brazil, 1977). In the following years, studies carried out in some states revealed changes in the epidemiological situation of bovine brucellosis that were primarily associated with the effectiveness of the vaccination programs. In the state of Rio Grande do Sul, a 2.0% reduction in disease prevalence in animals was reported in 1975, and a 0.3% reduction was reported in 1986. In Minas Gerais, the prevalence decreased from 7.6% in 1975 to 6.7% in 1980. In Paraná, the estimated prevalence in 1975 was 9.6% and decreased to 4.6% in 1989 (Poester et al., 2002; Brazil, 2006).

In 2001, the National Program for the Control and Eradication of Brucellosis and Tuberculosis (*Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose* – PNCEBT) from the Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento* – MAPA) established a new regulatory framework and a renewed political will to control and eradicate these diseases. This program proposed harmonized measures of prevention and control for the whole country (Lage et al., 2005).

The PNCEBT was developed with the objective of lowering the prevalence and incidence of bovine brucellosis based on compulsory vaccination of calves between three and eight months of age with the B19 vaccine, control of animal transportation and certification of brucellosis-free or monitored properties. The program seeks to increase the supply of low-risk food products to benefit public health and the productivity and competitiveness of livestock products in domestic and foreign markets, thereby offering consumers a safer and higher value product (Brazil, 2006; Lage et al., 2005).

Because knowledge of the epidemiological situation of the disease is extremely important at the onset of a disease control program and the last nationwide study on brucellosis was conducted in the 1970s (Brazil, 1977), in 2001, MAPA's Department of Animal Health demanded new studies on the prevalence and risk factors of bovine brucellosis in all Brazilian states. The studies are being conducted since then, as a first step of the onset of PNCEBT in each state. Field work is carried out by trained veterinary officers of the state animal health services, with the technical support of the University of São Paulo (*Universidade de São Paulo* – USP) and the University of Brasilia (*Universidade de Brasília* – UnB) (Poester et al., 2009). Studies of the following 16 Federative Units (FUs) have been published to date: Bahia (BA), Federal District (DF), Espírito Santo (ES), Goiás (GO), Maranhão (MA), Mato Grosso (MT), Mato Grosso do Sul (MS), Minas Gerais (MG), Paraná (PR), Rio de Janeiro (RJ), Rio Grande do Sul (RS), Rondônia (RO), Santa Catarina (SC), São Paulo (SP), Sergipe (SE) and Tocantins (TO).

These cross-sectional random surveys were carried out between October 2001 and December 2004 using a standardized sampling method, questionnaire and database, except for MS, where a state survey had been previously carried out in 1998 (Poester et al., 2009), and for MA, where data collection ended in March 2009 (Borba et al., 2013). Each state was split into regions based on distinct cattle production characteristics, such as average herd size, predominant production purpose (dairy, beef, dual-purpose) and feeding systems (extensive grazing, confined, mixed). A simple random sample of herds was conducted in each region. Within each selected herd, there was a random sample of cows aged 24 months or older. The prevalence for each state was estimated assuming a stratified sampling procedure, where each region was a stratum whose sampling weights were taken into account in the calculation. State-level results are detailed in Table 1 and the geographical location of each state is displayed in Fig. 1.

The prevalence studies revealed a very heterogeneous situation between states (Fig. 1) and between regions within the same state (Fig. 2). The areas traditionally known for having extensive farming systems for beef production, which coincide with the areas containing the largest herds, form a broad swath of high disease prevalence that encompasses the central western states of the country as well as the northern states of RO and TO (Figs. 1 and 2). Conversely, the southern states, where small-scale farming predominates, have lower herd prevalence. Prevalence was also variable within some states, such as RS, where the most southwestern region, along the border to Uruguay, and with high concentration of extensive beef farming, showed a prevalence of approximately 7.7% (Fig. 2), whereas the prevalence in the northern regions of the state, characterized by small dairy herds, was below 1% (Marvulo et al., 2009).

The analysis of risk factors yielded heterogeneous results across states. Although the observed spatial distribution of herd-prevalence of bovine brucellosis suggests an association between certain production typologies and health management practices (e.g., the highest prevalence occurs in regions dominated by larger extensive herds), no variable/factor has been clearly and systematically identified in the states studied.

Based on the hypothesis that an assessment of risk factors using data obtained from the state-based prevalence surveys would be limited in statistical power by the number of herds sampled and epidemiologically restricted to intrastate analysis, the main objective of this study was to evaluate the association of bovine brucellosis with possible risk factors in all previously sampled regions of Brazil using a consolidated database with information from 13 contiguous states and the Federal District. In the region studied, there were 1,852,872 herds with female cattle with ages greater than or equal to 24 months at the time of data collection, accounting

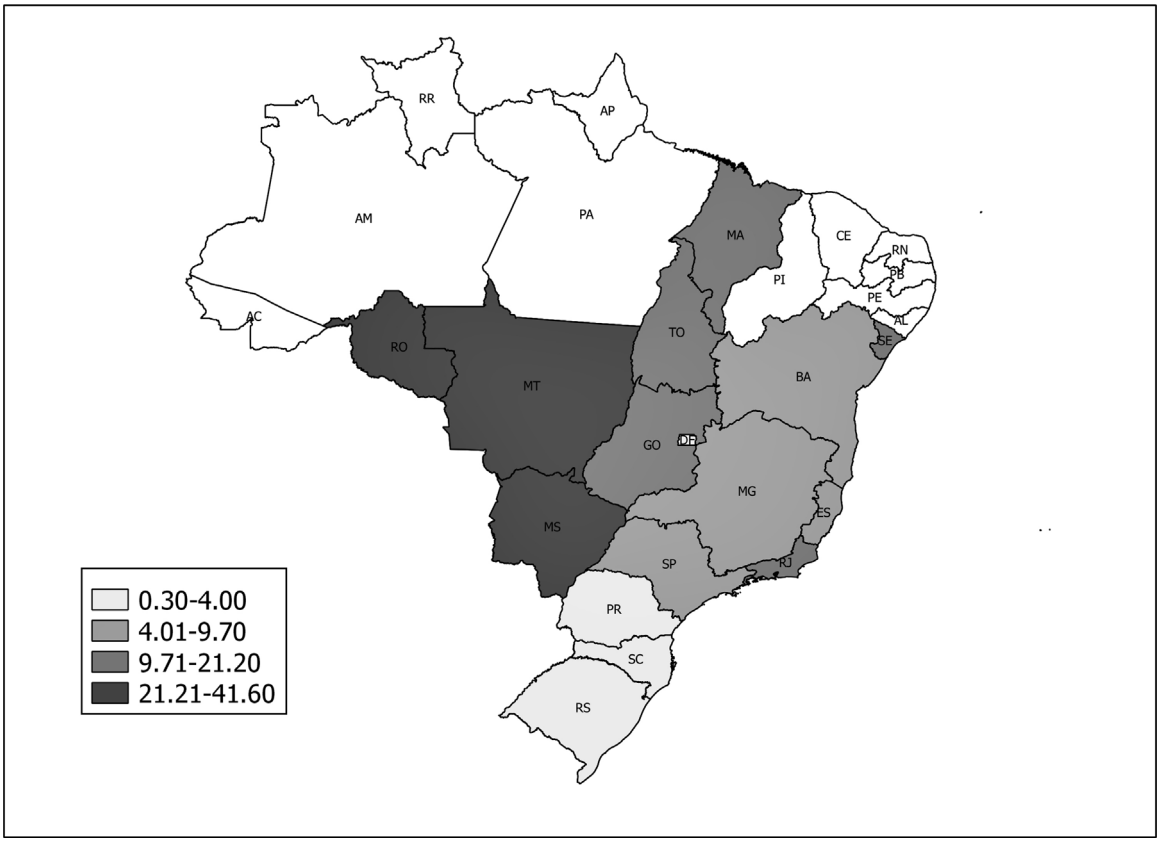

Fig. 1. Apparent herd prevalence for *Brucella abortus* at the state level, indicating the state location (prevalence levels divided in quartiles).

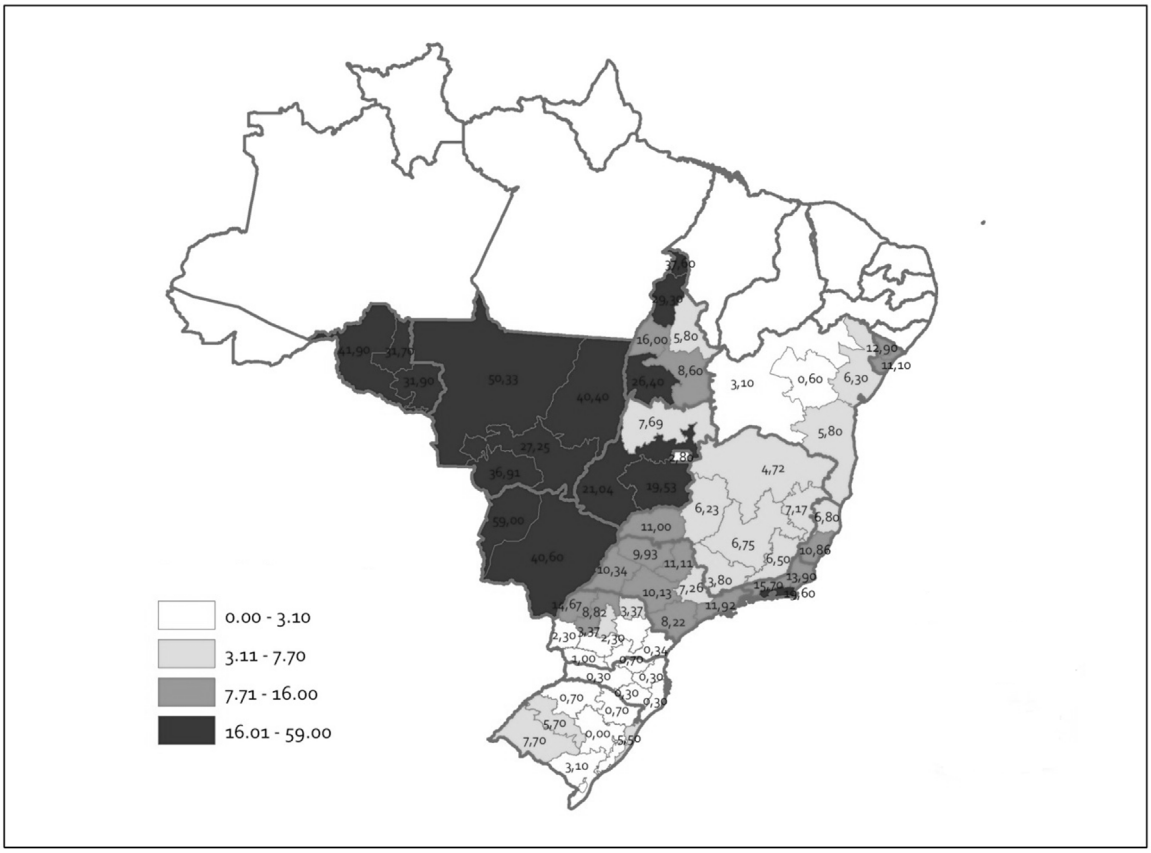

Fig. 2. Apparent herd prevalence for *Brucella abortus* per cattle production region, showing the state boundaries (prevalence levels divided in quartiles).

Table 1
Demographics, sample size and apparent herd prevalence of bovine brucellosis in 15 States and the Federal District.

States	Existing herds(with cows aged ≥24 months)	Sampledherds	Positiveherds	Apparent herd Prevalence (%)	95% CI (%)
BA	228,843	1413	57	4.20	[3.10; 5.30]
DF	3314	278	7	2.52	[1.02; 5.12]
ES	23,255	622	55	9.00	[6.97; 11.55]
GO	121,245	895	145	17.54	[14.91; 20.17]
MA	100,466	749	76	11.40	[9.20–14.00]
MG	300,730	2204	145	6.04	[4.98; 7.10]
MT	82,474	1152	428	41.20	[38.00; 44.40]
MS	45,374	1004	426	41.50	[36.50; 44.70]
PR	210,273	2094	99	4.02	[3.23; 4.80]
SC	168,909	1586	1	0.32	[0.10; 0.69]
RJ	34,124	945	155	15.42	[12.91; 17.91]
RS	392,987	1957	63	2.06	[1.50; 2.63]
RO	49,648	921	324	35.18	[32.09; 38.36]
SP	159,999	1073	105	9.70	[7.80; 11.60]
SE	20,604	588	70	12.60	[9.19; 16.01]
TO	56,605	1842	377	21.22	[19.33; 23.11]

Source: Villar et al. (2009); Klein-Gunnewiek et al. (2009); Dias et al. (2009b); Dias et al. (2009a); Gonçalves et al. (2009a); Azevedo et al. (2009); Negreiros et al. (2009); Ogata et al. (2009); Rocha et al. (2009); Gonçalves et al., 2009b; Sikusawa et al. (2009); Silva et al. (2009); Marvulo et al. (2009); Chate et al. (2009); Alves et al. (2009); and Borba et al. (2013).

for approximately 56 million females in this age group. Our aim was to perform a large-scale study to find associations between typologies and cattle production systems with the occurrence of bovine brucellosis that might explain the geographic spread of disease in the regions studied and provide information for the planning of strategic measures by the PNCEBT.

2. Materials and methods

The analysis of risk factors for bovine brucellosis was conducted using the statistical program Stata 12® (Statacorp, 2011) using a consolidated database of prevalence studies from the states of BA, SC, ES, DF, GO, MT, MG, PR, RJ, RS, RO, SP, SE and TO. The objective was to associate the presence of test-positive animals in the herd with the main production and sanitary characteristics of each sampled herd. The standardized epidemiological questionnaire and the methodologies applied for the selection of the sampled herds were similar in all states under study. Data from MS was not included because it was not collected using the same methodology and would not fit into a single database. Data from MA was not available for analysis in the consolidated database at the time of the data analysis.

The initial sample included 17,534 herds distributed among 61 cattle production regions. The division of the states into regions was performed during the prevalence surveys for the stratification of the state sample. Approximately 300 herds were tested in each region except for the state of São Paulo, where approximately 150 herds were sampled per region (USP, 2006; Dias et al., 2009b). The region sample sizes were calculated using the formula described by Petrie and Watson (2009) with the simple random sampling method. The parameters adopted for the calculation were as follows: confidence level (CL) 0.95, estimated prevalence 0.25 and absolute error 0.05. Within each herd, a pre-established number of adult females (≥24 months) were randomly selected and went through the diagnostic testing protocol so that the aggregate sensitivity and specificity values of the herd reached at least 90%. The herd was considered positive when at least one animal tested positive in the buffered plate agglutination test (BAPAT) and 2-mercaptoethanol tests, following the PNCEBT recommendations. These 14 cross-sectional studies were performed between 2001 and 2004 with an average duration of data collection per state of seven months (USP, 2006; Brazil, 2006).

During the exploratory analysis, we noted that some questionnaires in the database were filled out incompletely or inconsistently. To ensure credibility of the results, the decision was made to discard these cases. Herds that contained zero or only one female per herd (i.e., properties that did not have well-characterized reproductive activity) were also omitted from the study, resulting in a final sample of 17,100 herds. The 434 omitted observations were distributed among 12 states and 53 cattle production regions and did not significantly alter the geographic dispersion of the sample.

The variables obtained through the epidemiological questionnaire included in the analysis were the type of operation (beef, dairy and dual-purpose), type of farming (confined, semi-confined, and extensive), use of artificial insemination, number of females in the herd, presence of other domestic species, presence of wild animals, destination of the placenta and aborted fetuses, purchase and sale of animals for breeding purposes, rental of pastures, use of common pastures shared with other properties, and the presence of flooded pastures, calving paddocks and veterinary assistance.

The quantitative variable "number of females" was categorized to represent the various herd sizes. The cutoff points for these categories were based on descriptive statistics of the variable based on its percentiles and empirical knowledge of the mean number of animals per herd type. The total number of females was categorized as follows: small herd, up to 30 females; median herd, ranging from 31 to 100 females; large herd, ranging from 101 to 400 females; and extra-large herd, over 400 females. These cutoff points cumulatively represented the 50th, 78th and 95th percentiles.

In most of the sampled regions, data were collected at the onset of the implementation of mandatory vaccination of heifers with B19 in the country. Thus, the effect of the vaccine was not taken into account in the analysis of risk factors. It should be noted that vaccination is unlikely to have introduced any bias in the results because: (1) the PNCEBT states that heifers must be vaccinated between 3 and 8 months of age and only females older than 24 months were tested in the prevalence studies; and (2) the estimated herd prevalence per state was not positively correlated with vaccination coverage.

Possible risk factors were initially screened using an exploratory data analysis. Only variables with $p \leq 0.20$ in the χ^2 test were included in a multiple logistic regression model. The lower risk category was used for comparison with the other categories. The analytical parameters (α and β's) were estimated using the maxi-

Table 2
Results of the *design-based* logistic regression model for bovine brucellosis risk factors in Brazil.

Variable	Odds Ratio	CI (95%)	p-value
Exclusive use of artificial insemination	0.57	[0.40–0.81]	0.002
Presence of veterinary assistance	0.68	[0.60–0.77]	≤0.001
Extensive farming	1.23	[1.07–1.42]	0.003
Purchase of breeding stock			
From cattle dealers	1.27	[1.08–1.47]	0.002
From other farms	1.19	[1.07–1.32]	0.001
Total number of females in the herd			
$x \leq 30$	base category		
$30 < x \leq 100$	3.42	[2.98–3.91]	≤0.001
$100 < x \leq 400$	5.68	[4.92–6.55]	≤0.001
$x > 400$	13.14	[10.94–15.78]	≤0.001

mum likelihood method. The construction strategy for the chosen model was hierarchical backward elimination, which consists of the gradual removal of variables with no statistical significance from the complete initial model.

Because data from a cross-sectional sampling study were used, the model was developed in a design-based format as suggested by Hosmer et al. (2013). The results obtained from the design-based logistic regression model are more suitable for population inference because they consider the weights of the herds per region sampled (Hosmer et al., 2013). In the present study, each sampled herd represented from 11 herd in region 1 of the state of Sergipe to 462 herds in region 4 of Rio Grande do Sul, which reinforced the importance of considering the sample weights in the analytical model for the internal validation of the results. The command sequence performed in STATA® version 12 was as follows: (module: Statistics → Binary outcomes → Logistic Regression (reporting odds ratios) → SE/Robust → *Survey data estimation* → *Survey settings*. . . → [aba] *Main* → *Number of stages* → *Stage 1:* properties → *Strata:* regions → [aba] *Weights* → *Sampling weight variable*: weight assigned to each property by region → [aba] *Model: Dependent variable* → *Independent variables*).

The results of the risk factor analysis are expressed as point and interval values (95% CI) of the odds ratios. Only variables that showed $p \leq 0.05$ in the logistic regression are shown in the final model.

3. Results

In the univariate analysis, nearly all tested variables obtained significant χ^2 results with $p \leq 0.01$ due to the very large sample size. The only exceptions (which were subsequently excluded from the multivariable analysis) were the presence of birds ($p = 0.356$), dogs ($p = 0.949$) and cats ($p = 0.287$) on the farm, the use of common pastures ($p = 0.453$), and the fate of aborted animals ($p = 0.795$).

The final logistic model revealed that extensive cattle farming, the purchase of breeding stock and the total number of females in the herd were risk factors for bovine brucellosis. The presence of veterinary assistance and reproductive management based exclusively on artificial insemination were protective factors for bovine brucellosis (Table 2).

Herds that purchased breeding stock from cattle traders or directly from other farms had a higher risk of testing positive for bovine brucellosis compared to farms that did not purchase animals or those that acquired cattle from auctions and fairs.

The herd size (represented by the total number of females) showed the strongest association with herd prevalence; mean values of the odds ratios of each category increased as herd size increased, without overlapping of the 95% confidence intervals (Table 2).

4. Discussion and conclusions

The risk variables associated with the presence of bovine brucellosis in the 14 states under study were consistent with the regional trends observed in the state prevalence surveys, notably with the higher prevalence observed in regions where large-scale extensive cattle production is predominant. The statistical power of the final model based on 17,100 observations ensures a high reliability of the obtained estimates. The use of the logistic model with the relative weights from each sampled herd (design-based) allowed the results to be inferred to the scale of the target population sampled.

The association between the presence of bovine brucellosis and the herd size, represented in this study by the total number of females, corroborated the results of studies in the scientific literature (Kellar et al., 1976; Salman and Meyer, 1984; Kadohira et al., 1997; McDermott and Arimi, 2002; Muma et al., 2006; Tun, 2007; Al-Majali et al., 2009; Matope et al., 2010). Certain characteristics of large herds, such as increased frequency of animal replacement, difficulty of individualized handling of animals and problems related to sanitary control when many animals are in contact (Crawford et al., 1990), may facilitate the transmission of brucellosis and influence the disease dynamics. Lee et al. (2009) observed that increasing the size of the herd resulted in an increased likelihood of disease and persistence of infection.

An analysis conducted in 2007 by Cipullo et al. (in preparation) with data from the state of Mato Grosso showed an association between the size of a herd on a specific date and the number of animals traded by that herd during the year, with larger herds buying and selling more intensively ($p < 0.001$). This analysis indicates that the presence of bovine brucellosis in larger herds may also be associated with a higher frequency of animal trade, and strengthens the case for testing animals prior to movement.

The importance of the number of females per herd also highlights the role of the cow as the main source of infection and responsible for maintaining brucellosis in the herd (Jones et al., 2000; Brazil, 2006). During the calving season, seropositive cows eliminate large amounts of the etiological agent during abortion or parturition, thereby contaminating pastures, water and food; when younger, these cows may become residual sources for *Brucella abortus*, maintained the incidence of the disease during the eradication stages (Salman and Meyer, 1984).

The purchase of animals is correlated with the practice of replacing and trading animals without proper sanitary control (Alves et al., 2009) and is also described as a factor for the introduction or further spread of bovine brucellosis in disease-free herds (Kellar et al., 1976; Luna-Martínez and Mejía-Terán, 2002; Renukaradhya et al., 2002; Muma et al., 2006; Stringer et al., 2008; Jergefa et al., 2009; Kaoud et al., 2010). The acquisition of animals from cattle dealers or directly from other farms (especially the purchase of females from these sources) demands greater attention from producers as a risk factor for bovine brucellosis and highlights the need for health certification. While the number of accredited free herds is still small, cattle farmers must seek assurances regarding the health status of the originating herd or may separate and retest purchased animals. At livestock events such as auctions and fairs, sanitary control is often part of the requirements for the entry and participation of animals. In other situations, buyers should require negative diagnostic tests for brucellosis, as recommended by the PNCEBT, and be aware of the risks that their herd may be exposed to in the absence of this practice.

The higher risk associated with extensive farming complements the two factors discussed above. According to Salman and Meyer

(1984), no factor should be studied in isolation because variables are usually a set of characteristics and production practices common to larger herds that are responsible for the higher frequency of the disease. The extensive production system is characterized by more intense transportation of animals and the possibility of contact with a greater number of sources of infection and contaminated sites (Silva et al., 2000; McDermott and Arimi, 2002; Tun, 2007; Martins et al., 2009). Kadohira et al. (1997) and Renukaradhya et al. (2002) also cite the difficulty of controlling bovine brucellosis in this type of farming, which favors the survival of the disease for long periods in the herd. It should be stressed that among the herds sampled in this study, approximately 91.4% of those with more than 400 females were under extensive grazing, and 77.8% of these herds were characterized as beef operations.

Thus, the encountered risk factors explain the higher disease prevalence observed in regions characterized by the predominance of large extensive grazing herds of predominantly beef cattle where the flow of animals through the purchase and sale of breeding stock is more intense.

The presence of veterinary assistance and the exclusive use of artificial insemination in the reproductive management of herds were indicated as protective factors by the analytical model (Table 2). The lower probability of infection by bovine brucellosis in herds under the supervision of a veterinarian is likely due to the better monitoring and adequacy of preventive health measures for the disease, as indicated by Aricapa et al. (2008) in a district in Colombia, Al-Majali et al. (2009) in Jordan, and Kaoud et al. (2010) in Egypt. In contrast to the findings of this study, the use of artificial insemination is often cited as a risk factor for *B. abortus* infection when assuming the use of semen from infected bulls with no sanitary control (Renukaradhya et al., 2002; Jergefa et al., 2009; D'Pool et al., 2004; Chate et al., 2009). When deposited directly into the uterus, contaminated semen can infect the female even in small doses, making this process an efficient transmission route and source for the spread of bovine brucellosis (Paulin and Ferreira Neto, 2003). However, given that in Brazil semen comes from artificial insemination centers, with quality control standards, artificial insemination is a consolidated and safe reproductive procedure. It should be stressed that the exclusive use of artificial insemination for reproductive management might be a proxy variable for herds that have better production standards and therefore greater concern over health issues, given the quality of the genetic material in the herd.

The findings of this study provide important epidemiological information for the development of risk-based surveillance systems and disease control. We recommend that the PNCEBT intensify the compulsory vaccination of calves with the B19 vaccine, especially in regions of large-scale extensive cattle production. In such regions, the use of RB51 to vaccinate adult animals, not previously vaccinated, could accelerate the reduction of the incidence and prevalence of bovine brucellosis (Rivera et al., 2002; Martins et al., 2009; Caetano et al., 2014).

Smaller herds that use modern production technologies are good candidates for the integration of health certification projects, especially dairy farms, because the prevalence of the disease is lower in this segment of the cattle industry. The compliance with test requirements for cattle movement is of paramount importance across all production segments of the cattle industry and calls for efforts to educate and inform producers about the risks of brucellosis to human health and cattle productivity.

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