

Effect of vaccination in lowering the prevalence of bovine brucellosis in the state of Mato Grosso, Brazil

Efeito da vacinação no rebaixamento da prevalência da brucelose bovina no estado de Mato Grosso, Brasil

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Abstract

The study was conducted to verify the efficacy of the bovine brucellosis vaccination program implemented by the state of Mato Grosso, using prevalence as an indicator. The state was divided into four regions: Pantanal, Milk, Fattening, and Breeding. For each region, a predetermined number of properties were selected and blood samples were taken from randomly chosen female animals, aged 24 months or greater. Sera from the animals were initially screened with a buffered acidified plate antigen, and confirmed using complement fixation. In each property, a questionnaire was used in order to identify the risk factors associated with the disease. In the state, the prevalence rate of infected herds was 24.0% [21.3; 26.8] and the prevalence rate of infected animals was 5.1% [3.5; 7.2]. The prevalence rates of infected herds and animals in each region were as follows: 21.2% and 6.4% in the Pantanal region, 17.2% and 3.7% in the Milk region, 34.0% and 7.2% in the Fattening region, and 24.3% and 4.4% in the Breeding region. Bovine brucellosis in the state is associated with the introduction of breeding, herd size, pasture sharing, and farms used for mixed and beef purposes. Therefore, in order to improve control of bovine brucellosis, it is proposed that the state of Mato Grosso intensify its prophylactic program, primarily its vaccination strategy. There has been a reduction in the prevalence of infected herds since 2002; however, there remains a high prevalence of infected herds and animals throughout the state. Additionally, the use of non-inducing antibodies vaccine should be encouraged, especially in the Pantanal region where the management of the animals is complicated by the flood and ebb cycle. The state should make greater efforts to educate producers on how to assess the breeding animals for brucellosis before introducing them into their properties as well as avoid shared grazing among herds of unknown health conditions.

Key words: Bovine brucellosis. Vaccination. Prevalence. Risk factors. Mato Grosso. Brazil.

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Resumo

O estudo foi realizado para se verificar a eficácia do programa de vacinação contra brucelose bovina implementado pelo estado de Mato Grosso, utilizando-se a prevalência como indicador. O Estado foi dividido em quatro regiões: Pantanal, Leite, Engorda e Cria. Para cada região, um número preestabelecido de propriedades foi selecionado e em cada uma delas foram colhidas amostras de sangue de fêmeas com idade igual ou superior a 24 meses, aleatoriamente escolhidas. Os soros dos animais foram submetidos a um protocolo de testes em série, com triagem pelo teste com Antígeno Acidificado Tamponado e confirmação pela Fixação do Complemento. Em cada propriedade foi aplicado um questionário para individualizar os fatores de riscos associados à doença. No estado, a prevalência de focos foi 24,0% [21,3; 26,8] e a de animais 5,1% [3,5; 7,2]. As prevalências de focos e de animais nas regiões foram 21,2% e 6,4% no Pantanal, 17,2% e 3,7% na região Leite, 34,0% e 7,2% na região de Engorda e 24,3% e 4,4% na região de Cria. A brucelose bovina no estado está associada à introdução de reprodutores, ao tamanho do rebanho, ao compartilhamento de pastagem e ao tipo de exploração corte e mista. Propõe-se assim que o estado de Mato Grosso intensifique seu programa profilático principalmente a estratégia de vacinação, buscando maior eficácia do controle da brucelose bovina. Houve redução na prevalência de focos desde 2002, entretanto, ainda são altas as prevalências de focos e de animais em todo o estado. Adicionalmente a utilização da vacina não indutora de anticorpos, especialmente no Pantanal deve ser estimulada, onde o manejo dos animais é dificultado pelo ciclo de cheia e vazante. Além disso, o estado deve realizar maiores esforços no sentido de conscientizar os produtores para que avaliem os animais de reprodução para brucelose antes de introduzi-los nas propriedades e evitem o compartilhamento de pastagens entre rebanhos de condição sanitária desconhecida.

Palavras-chave: Brucelose bovina. Vacinação. Prevalência. Fatores de risco. Mato Grosso. Brasil.

Introduction

Mato Grosso is home to 3 million inhabitants and is a state that exports meat with an effective beef production of approximately 28 million heads. In 2014, meat export generated R \$1.2 billion in revenue for the state, representing 19% of all export of Brazilian beef (ACRIMAT, 2015). The Mato Grosso Institute of Agricultural Economics and the Federation of Agriculture and Livestock of Mato Grosso reported that agribusiness is the largest generator of jobs in the state, accounting for 27% of employees hired in 2012.

Cattle diseases, especially those that affect reproduction, represent a major obstacle to the development of the livestock industry. In Brazil, brucellosis is caused by the bacterium *Brucella abortus* and the main disease that affects the reproduction of cattle. Every 1% variation in the prevalence of bovine brucellosis corresponds to an estimated R\$155 million damage that is caused by bovine brucellosis in Brazil (SANTOS et al., 2013).

In cattle, the main signs of the disease are

miscarriage in the late trimester, placenta retention, stillbirth, and birth of weak calves (PAULIN; FERREIRA NETO, 2003, 2002). This disease generates economic losses due to the increase in the calving interval, a drop in the birth of calves, decreased production of meat and milk, and an increase in animal replacement rate (FARIA, 1984). The disease hinders the marketing of animals and animal products and compromises the country's competitiveness in the international market (LAGE, 2006). This also involves losses related to the cost of human treatment and the absence from work during convalescence (PAULIN; FERREIRA NETO, 2003). Humans can be exposed by contact with infected animals or consumption of unpasteurized milk. Cowboys, handlers, veterinarians, milkers, and slaughterers are in high-risk occupations where one can become infected through contact with the fetus, attachments, or fetal fluids of the infected female and accidents with live vaccines (PAULIN; FERREIRA NETO, 2003).

In 2001, the Ministry of Agriculture, Livestock and Supply (MAPA), with awareness for the need to

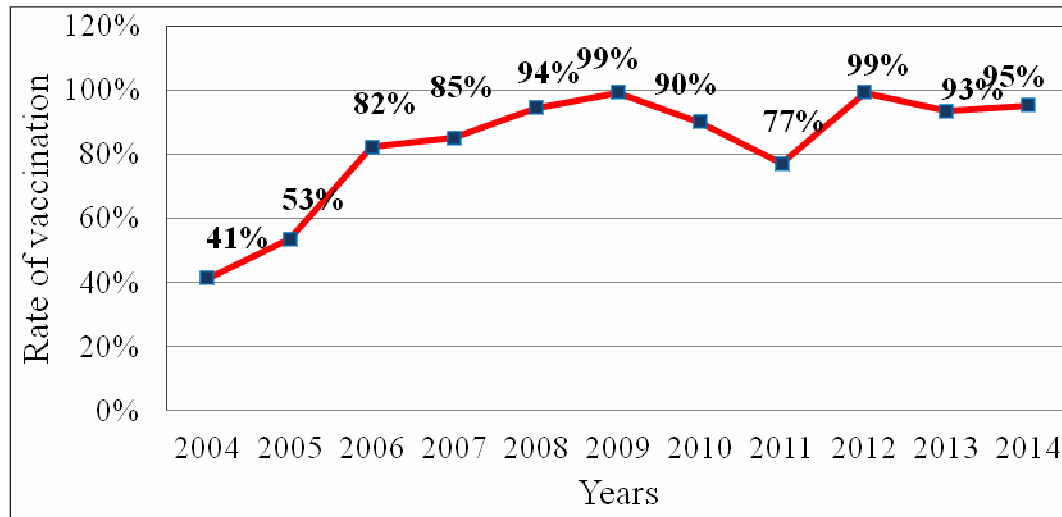
properly control the disease, developed and launched the National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) (LAGE, 2006). The Collaborating Center for Animal Health of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo was instructed to standardize methodology and perform cross-sectional studies in all Federal Units in order to choose the most appropriate strategies and perform management processes (POESTER et al., 2009). Initial studies have been conducted in 18 Federative Units, representing 85% of the actual Brazilian cattle population. The lowest prevalence of infected herds was detected in the state of Santa Catarina (0.32%) and the highest in Mato Grosso do Sul (41.5%). Mato Grosso (41.2%) was among the group of states with very high prevalence rates of infected herds (SIKUSAWA et al., 2009; ALVES et al., 2009; AZEVEDO et al., 2009; CHATE et al., 2009; DIAS et al., 2009a, 2009b; GONÇALVES et al., 2009a, 2009b; KLEIN-GUNNEWIEK et al., 2009; MARVULO et al., 2009; OGATA et al., 2009; ROCHA et al., 2009; SILVA et al., 2009; VILLAR et al., 2009; NEGREIROS et al., 2009; BORBA et al., 2013; ALMEIDA et al., 2016; CLEMENTINO et al., 2016). After approximately ten years, the states of São Paulo, Minas Gerais, Espírito Santo, Rondônia, Mato Grosso do Sul, and Rio Grande do Sul conducted the second brucellosis prevalence study to verify the effectiveness of their immunization programs. However, the prevalence of infected herds only decreased in Mato Grosso do Sul, Minas Gerais and Rondônia (DIAS et al., 2016; ANZAI et al., 2016; INLAMEA et al., 2016; LEAL FILHO et al., 2016; OLIVEIRA et al., 2016; SILVA et al., 2016). The state of Santa Catarina, which had the lowest prevalence of infected herds and animals, prohibited vaccination and began the implementation of eradication strategies. They also

conducted a second study, which did not indicate changes in prevalence (BAUMGARTEN et al., 2016).

Due to the high prevalence previously observed in Mato Grosso, it was recommended that the state form an effective vaccination program with B19 and target an annual vaccination coverage of 80% of calves from 3 to 8 months of age (AMAKU et al., 2009). In addition, in order to increase vaccination coverage, the state was encouraged to vaccinate adults with no inducing antibodies vaccine from 2007. Their implementation was regulated by SDA Normative Instruction Number 33 on August 24, 2007.

Thus, the state of Mato Grosso made vaccination with B19 mandatory in 2003 and established the mandatory vaccination against brucellosis for issuing the animal transport formulary in 2005. This significantly contributed to the increase in vaccination rates, which increased from 54% in 2005 to 82% in 2006. They remained above 80% until 2014, with the exception of 2011 due to a shortage of inputs (Figure 1). Vaccination rates in the Pantanal, Fattening, Milk and Breeding regions proved to be homogeneous. In 2013, the state made vaccination with the non-antibody inducing vaccine mandatory in the default properties.

In 2013, in the Pantanal region, where the handling of the animals is complicated by the flood and ebb cycle, the state allowed the use of non-antibody inducing vaccines to replace B19 (INDEA, 2013), but the accession of producers has been low due to the availability of the vaccine and its cost. Therefore, this study aimed to evaluate the effectiveness, using prevalence as an indicator, of the vaccination program implemented in the state. This study also aimed to individualize the risk factors associated with bovine brucellosis.

Figure 1. Rate of annual vaccination of calves with B19 in the state of Mato Grosso.

Methods and Materials

The study was designed by staff personnel from MAPA, the Collaborating Center for Animal Health (Faculty of Veterinary Medicine and Animal Science at the University of São Paulo [FMVZ-USP]), the Federal University of Mato Grosso (UFMT) and the State Agricultural Defense Institute of Mato Grosso (INDEA). INDEA veterinarians carried out the fieldwork from September to December of 2014.

Initially, the state was divided into regions, taking into consideration the different production systems, management practices, types of operation, average herd sizes, animal commercialization system, and the operational capacity of the animal health protection service. Within each of these regions, a predetermined number of properties with reproductive activity (primary sampling units) were randomly chosen. This process was based on property records maintained by INDEA. Within each selected property, a predetermined number of cows aged over 24 months were drawn at random (secondary sampling units).

In rural properties with more than one flock, the herd with greater economic importance was chosen as the target of the study and the animals were subjected to the same type of management (i.e., under the same risk factors). The choice of the

primary sampling unit was random and based on the registration of farms with reproductive activity of cattle. Properties that were drawn but could not be visited for various reasons were replaced by another nearby property that had the same production characteristics (THRUSFIELD, 2007). The parameters designated for the calculation were as follows: a confidence level of 0.95, estimated prevalence of 0.20, and 0.05 error.

For secondary units, a minimum number of animals to be examined within each property was estimated in order to determine if it would be classified as a herd that was infected or not infected by brucellosis. We used the concepts of sensitivity and specificity aggregates (DOHOO et al., 2003). For purposes of calculation, values of 95% and 99.5% were adopted for sensitivity and specificity of the test protocols, respectively, (FLETCHER et al., 1998) and 20% for the estimated intra-herd prevalence. This was processed using the Herdacc program version 3 and the sample size was chosen to enable a sensitivity and specificity greater than or equal to 90% for the herd. Thus, in properties with up to 99 female bovine older than 24 months, 10 animals were sampled; 15 animals were sampled in properties with 100 or more female animals older than 24 months. The choice of female animals within the properties was casual systematic. Cows

in the peripartum period or abortion, i.e., about 15 days before and after delivery, were excluded from the selection.

The blood (10 ml) was collected by puncturing the jugular vein with a sterile disposable needle in a previously identified vacuum tube. The sera stored in plastic wells were kept at -20°C until testing. The serodiagnosis protocol consisted of screening with the buffered acidified antigen test and positivity was confirmed by a subsequent complement fixation test. These tests were conducted by the Laboratory of Animal Health Support INDEA (LASA-MT) and National Agricultural Laboratory (LANAGRO-MG). The property was considered positive when at least one positive animal was detected.

The sample design allowed us to determine the prevalence of infected herds and adult female animals (≥ 24 months) seropositive for brucellosis in the state and in the regions. Estimates of the prevalence and confidence intervals were performed as recommended by Dean et al. (1994). Estimates of the prevalence of infected herds and animals in the state and prevalence of animals in the regions were made in a balanced manner based on the method of Dohoo et al. (2003).

The weight of each property in calculating the prevalence of infected herds in the state was given by the following equation:

$$P_1 = \frac{\text{properties in the region}}{\text{properties sampled in the region}}$$

The weight of each animal in the calculation of the prevalence of animals in the state was given by the following equation:

$$P_2 = \frac{\text{female bovine } \geq 24 \text{ months in the property}}{\text{Female animals } \geq 24 \text{ months sampled in the property}} \times \frac{\text{Female bovine } \geq 24 \text{ months in the region}}{\text{Female animals } \geq 24 \text{ months sampled in the region}}$$

In the above expression, the first term refers to the weight of each animal in the calculation of animal prevalence within regions. Estimates of the prevalence and confidence intervals of 95% were made through EpiInfo 6.0 and SPSS software, version 9.0.

In each sampled property, during the blood samples drawn, an epidemiological questionnaire designed to obtain information on the type of farming and management practices employed was conducted.

The following variables were analyzed: type of operating system (beef, dairy and mixed), type of breeding (confined, semi-confined, extensive), use of artificial insemination, predominant breeds, number of animals, the presence of other domestic and wild species, occurrence of abortion in the last two years, the destination of placenta and aborted fetuses, purchase of animals, vaccination against bovine brucellosis, pastures sharing with other properties, occurrence of flooding in the grasslands, existence of picket lambing, and veterinary care.

The variables were arranged in ascending scale of risk. When necessary, they were re-categorized. The lower risk category was used as a basis for comparison to the other categories. Quantitative variables were categorized into percentiles. An exploratory analysis (univariate) for selection of those with $p < 0.20$ for the χ^2 test (chi-square) or Fisher's exact test and subsequent logistic regression was conducted, as recommended by Hosmer and Lameshaw (1989). The calculations were performed with SPSS. All information generated by field and laboratory work was included in a specific database used in epidemiological studies.

Results

The state was divided into four regions (Figure 2). Table 1 provides a summary of census and sample data. Table 2 shows the prevalence of infected herds and animals in the region and the

state. The prevalence of infected herds by type of farming in the regions is shown in Table 3. Table 4 shows the comparison of this study with that conducted in 2002 by Negreiros et al. (2009). Table

5 shows the results of the univariate analysis and Table 6 shows the final logistic regression model for risk factors associated with herds infected with bovine brucellosis in Mato Grosso.

Figure 2. Map of the State of Mato Grosso divided into regions.

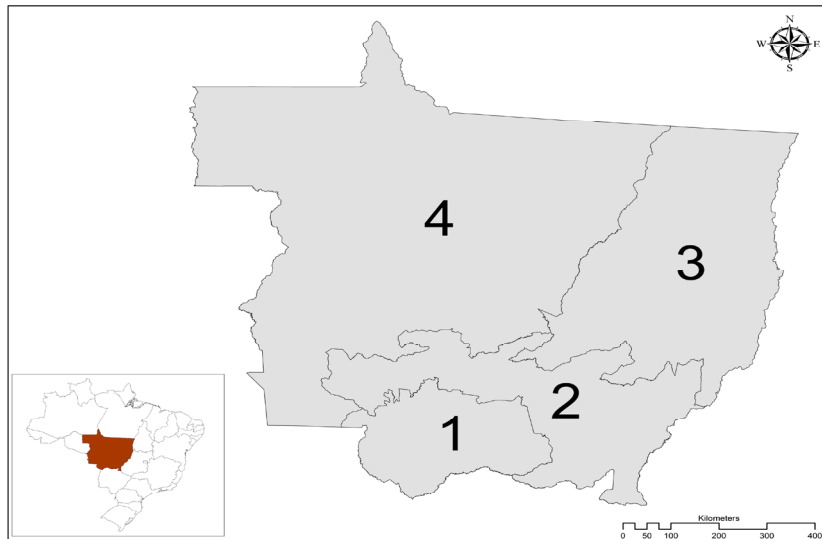


Table 1. Census and sample data in the state of Mato Grosso.

Region	Number of municipalities	Properties with reproductive activity	Sampled properties	Females aged ≥ 24 months	Sampled females aged ≥ 24 months
1. Pantanal	07	7778	344	1021623	3204
2. Milk	50	26924	296	2365639	2831
3. Fattening	27	18485	300	2533207	3193
4. Breeding	57	50930	313	5299557	3207
Total	141	104117	1253	11220026	12435

Table 2. Prevalence of herds and animals infected with bovine brucellosis in the state of Mato Grosso.

Region	Proprieties			Females ≥ 24 months		
	Positive/sampled	Prevalence (%)	CI95% (%)	Positive/sampled	Prevalence (%)	CI95% (%)
1. Pantanal	73/344	21.2	17.2 – 25.9	98/3204	6.4	3.1 – 12.8
2. Milk	51/296	17.2	13.3 – 22.0	74/2831	3.7	2.1 – 6.5
3. Fattening	102/300	34.0	28.8 – 39.6	163/3193	7.2	3.2 – 15.2
4. Breeding	76/313	24.3	19.8 – 29.4	129/3207	4.4	2.6 – 7.3
Total	302/1253	24.0	21.3 – 26.8	464/12435	5.1	3.5 – 7.2

Table 3. Prevalence of herds infected with bovine brucellosis, stratified by type of farming in the regions in the state of Mato Grosso.

Region	Beef		Dairy		Mixed	
	Prevalence % (p/e*) ¹	CI 95% (%)	Prevalence % (p/e)	CI 95% (%)	Prevalence % (p/e)	CI 95% (%)
1. Pantanal	24.6 (46/187)	18.8 – 31.1	7.8 (6/77)	3.2 – 15.5	26.2 (21/80)	17.7 – 36.7
2. Milk	24.5 (35/143)	18.0 – 32.0	9.6 (11/114)	5.2 – 16.1	12.8 (5/39)	4.8 – 26.2
3. Fattening	41.7 (83/199)	35.0 – 48.7	13.8 (8/58)	6.6 – 24.5	25.6 (11/43)	14.2 – 40.1
4. Breeding	30.5 (64/210)	24.5 – 37.0	8.9 (7/79)	3.9 – 16.7	20.8 (5/24)	8.1 – 40.3

* positive/ examined.

Table 4. Comparison of the prevalence rates (P) of infected herds and animals with bovine brucellosis between the present study (2014) and that carried out in 2002 in Mato Grosso.

Region	properties				animals			
	2002		2014		2002		2014	
	P (%)	CI 95% (%)	P (%)	CI 95% (%)	P (%)	CI 95% (%)	P (%)	CI 95% (%)
1. Pantanal	36.9	29.2 – 45.2	21.2	17.2 – 25.9	7.9	3.0 – 12.9	6.4	3.1 – 12.8
2. Milk	27.2	22.8 – 32.1	17.2	13.3 – 22.0	4.1	2.8 – 5.4	3.7	2.1 – 6.5
3. Fattening	40.4	38.8 – 46.2	34.0	28.8 – 39.6	8.1	5.2 – 11.1	7.2	3.2 – 15.2
4. Breeding	50.3	44.5 – 56.1	24.3	19.8 – 29.4	15.3	9.2 – 21.3	4.4	2.6 – 7.3
Total	41.2	38.0 – 44.4	24.0	21.3 – 26.8	10.2	7.4 – 13.1	5.1	3.5 – 7.2

Table 5. Results of univariate analysis of potential risk factors for bovine brucellosis (p <0.05) in the state of Mato Grosso.

Variable	Total no. of properties	Number of properties with infected herds	%	P value
Number of female animals aged ≥ 24 months				< 0.0001
≤ 85*	945	179	18.9	
≥ 86	308	123	39.9	
Type of usage				< 0.0001
Beef	739	228	30.9	
Dairy	328	32	9.8	
Mixed	186	42	22.6	
Introduction of reproductive animals				0.006
No	601	124	20.6	
Yes	652	178	27.3	
Picket farrowing				0.002
No	837	180	21.5	
Yes	416	122	29.3	
Sharing of grazing				0.037
No	1056	243	23.0	
Yes	197	59	29.3	

continue

continuation

Introduction of new cattle				0.05
No	838	188	22.4	
Yes	415	114	27.5	
Presence of wild animals				0.004
No	479	94	19.6	
Yes	774	208	26.9	
Presence of horses				0.001
No	240	38	15.8	
Yes	1013	264	26.1	
Presence of sheep and goats				0.033
No	1083	250	23.1	
Yes	170	52	30.6	

* 3rd quarter.**Table 6.** Final model of multivariate logistic regression for risk factors for bovine brucellosis in the state of Mato Grosso.

Variable	OR	CI 95%
Number of female animals aged ≥ 24 months		
$\leq 85^*$		
≥ 86	2.15	1.6 – 2.9
Type of use		
Dairy		
Mixed	2.95	1.2 – 3.5
Beef	3.26	2.7 – 4.9
Sharing of pasture		
No		
Yes	1.56	1.1 – 2.2
Introduction of reproductive animals		
No		
Yes	1.32	1.002 – 1.7

* 3rd quarter.

Discussion

The results in Table 2 show that the prevalence of infected herds remains high throughout the state, but with a significant decrease compared to the 2002 study by Negreiros et al. (2009) (Table 4). The decrease in the prevalence of infected herds due to vaccination was also found in Mato Grosso do Sul, Minas Gerais, and Rondônia (DIAS et al., 2016; ANZAI et al., 2016; INLAMEA et al., 2016; LEAL FILHO et al., 2016; OLIVEIRA et al., 2016; SILVA et al., 2016).

In Mato Grosso State, there was a remarkable decrease in the prevalence of infected herds in all evaluated regions, except in the Fattening region (3), where the results were at the limit of statistical significance (Table 4). The greatest decrease was observed in the Breeding region (4). Since the rates of vaccination of calves with B19 were homogeneous for all regions, this decrease is likely due to development of a higher-quality vaccination process, from the purchase of the vaccine to its application.

Table 3 shows a trend of higher prevalence of infected herds in beef, and lower in dairy farms, which corroborates the results obtained in 2002 by Negreiros et al. (2009). It should be noted that, compared to 2002, the prevalence of infected herds in dairy properties decreased significantly in all regions except the Fattening region (3).

Compared to the 2002 study, there was a significant decrease in the prevalence of animals in the state as well as the Breeding region (4) (Tables 2 and 4), following what has already been outlined in the previous paragraph. In other regions, there was a decrease in the point value of prevalence in animals, but without statistical differences.

Therefore, the state should continue its vaccination program with emphasis on the quality of the process, from the purchase of the vaccine to its application. Furthermore, it should also encourage the use of non-antibody inducing vaccines in adult animals, since this practice helps to lower prevalence rates (SOUZA et al., 2016). In Pantanal, since there was little compliance from the producer with the use of non-antibody inducing vaccines to replace B19, as prescribed by state law, the state must develop further strategies to encourage this practice.

The final model of logistical regression showed that the introduction of breeding, herd size, pasture sharing, and farms used for mixed and beef purposes are associated with *B. abortus* infected herds (Table 6).

As reported in international literature, the introduction of breeding animals is the classic risk factor for bovine brucellosis (KELLAR et al., 1976; NICOLETTI, 1980; CRAWFORD et al., 1990). In Brazil, this variable was also seen as a risk factor for bovine brucellosis in the states of Bahia, Goiás, Minas Gerais, Paraná, São Paulo, and Mato Grosso do Sul (ALVES et al., 2009; ROCHA et al., 2009; GONÇALVES et al., 2009; DIAS et al., 2009a, 2009b; KLEIN-GUNNEWIEK et al., 2009; LEAL FILHO, 2016). The introduction or replacement of breeding is routinely conducted without prior testing

of animals or knowledge of the health conditions of their herd of origin.

Numerous international authors (KELLAR et al., 1976; NICOLETTI, 1980; SALMAN; MEYER, 1984) have reported an association between herd size and brucellosis. In Brazil, this association was also found in the states of Rondônia, Rio de Janeiro, Sergipe, Tocantins, São Paulo, Mato Grosso do Sul, and Mato Grosso in 2002 (AGUIAR et al., 2007; KLEIN-GUNNEWIEK et al., 2009; SILVA et al., 2009; OGATA et al., 2009; DIAS et al., 2009b; CHATE et al., 2009; NEGREIROS et al., 2009). The dynamics of brucellosis and some characteristics of the largest herds, such as the greater need to replace animals and difficulty in implementing disease control measures, facilitate the transmission of brucellosis (CRAWFORD et al., 1990). Christie (1969) observed that increasing the size of the herd results in increased probability of occurrence, persistence of infection, and difficulty in eradicating brucellosis. Therefore, the larger the herd, the greater the risk of introducing brucellosis and likelihood of its intra-herd spread.

Mixed and especially beef properties have presented higher risks of containing herds infected with brucellosis when compared to dairy properties. This is most likely attributed to their large herd size. In the state, the median number of cows in beef properties was 50, 35 in mixed, and 19.5 in dairy.

The sharing of grazing land allows animals from different properties to coexist in the same environment and increases the likelihood of contact between those infected and those susceptible. Since the health conditions of the properties from which those animals originate can be distinct, this is an indirect form of contact between properties. Pasture rental, another indirect form of contact between properties, has been reported as a risk factor for bovine brucellosis in the states of Paraná and Rio de Janeiro (DIAS et al., 2009a; KLEIN-GUNNEWIEK et al., 2009). The practice of pasture rental may favor the contact of animals with previously contaminated

environments. According to Wray (1975), the main risk of infection with *B. abortus* is related to environmental contamination by abortion products. Depending on the environmental conditions, abortion products can maintain the viability of the *B. abortus* for up to 180 days (CRAWFORD et al., 1990).

Conclusions

There has been a reduction in the prevalence of infected herds since 2002; however, a high prevalence of infected herds and animals throughout the state still exists. Additionally, the use of non-inducing antibodies vaccine should be encouraged, especially in the Pantanal region where the management of the animals is complicated by the flood and ebb cycle. Furthermore, the state should make greater efforts towards prevention of shared grazing between herds with unknown health conditions and education of the producers on methods of assessing the breeding animals for brucellosis before introducing them into their properties.

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