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Effect of vaccination in lowering bovine brucellosis in the state of Rondônia, Brazil

Efeito da vacinação no rebaixamento da brucelose bovina no estado de Rondônia, Brasil

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Abstract

The study was carried out to evaluate the effectiveness of the vaccination program against bovine brucellosis, implemented by the State of Rondônia, using prevalence rates as an indicator. The State was divided into three regions. For each region, a pre-determined number of herds were selected and at each herds, blood samples were collected from randomly chosen females with 24 months age or more. Sera from animals were submitted to a serial testing protocol, with screening performed using the buffered acidified antigen test and confirmation by the complement fixation test. A epidemiological questionnaire was administered to each farm property to identify the risk factors associated with the disease. The prevalence rates of infected herds and infected animal at the state were 12.3% [10.3-14.6] and was 1.9% [1.4-2.5], respectively. Between the regions, the prevalence of infected herds varied from 11.6% to 12.8% and the prevalence of infected animals ranged between 1.4% and 2.6%. Bovine brucellosis in Rondônia State is associated with the beef herd, a larger number of cows and the presence of flooded pastures. There was an important reduction in prevalence rates of infected herds and infected animals since 2004, however further reduction could still be achieved with vaccination. Thus, the State should continue its vaccination program, placing emphasis on ensuring the quality of the process. In addition, it should also promote the use of a vaccine that does not induce antibody formation. Additionally, the State must carry out a great effort of education so that producers test breeding animals for brucellosis before introducing them onto their properties and, where possible, prevent calved cows from having access to areas that are flooded.

Key words: Bovine brucellosis. Vaccination. Prevalence. Risk factors. Rondônia. Brazil.

Resumo

O estudo foi realizado para se verificar a eficácia do programa de vacinação contra brucelose bovina implementado pelo estado de Rondônia, utilizando-se a prevalência como indicador. O Estado

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foi dividido em três regiões. Para cada região, um número preestabelecido de propriedades foram selecionadas 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 12,3% [10,3 – 14,6] e a de animais 1,9% [1,4 – 2,5]. Entre as regiões, a prevalência de focos variou entre 11,6% a 12,8% e a de animais entre 1,4% e 2,6%. A brucelose bovina no estado está associada à exploração de corte, ao maior número de vacas e a presença de áreas alagadas. Houve importante redução nas prevalências de focos e de animais desde 2004, entretanto ainda podem ser rebaixadas com a vacinação. Assim, o estado deve continuar seu programa de vacinação, dando ênfase para a qualidade do processo. Além disso, deve também estimular a utilização da vacina não indutora de anticorpos. Adicionalmente, o estado deve realizar um grande esforço de educação para que os produtores testem os animais de reprodução para brucelose antes de introduzi-los em suas propriedades e, se possível, evitem que vacas a termo tenham acesso a áreas alagadas.
Palavras-chave: Brucelose bovina. Vacinação. Prevalência. Fatores de risco. Rondônia. Brasil.

Introduction

Bovine brucellosis is a highly prevalent anthropozoonosis with a worldwide distribution, caused by *Brucella abortus*. In cattle, the main features include abortion in the final third of gestation, retention of the placenta, incidence of stillbirths and the birth of weak calves (PAULIN; FERREIRA NETO, 2003, 2002). This disease has negative economic implications as it increases the calving interval, leading to a fall in the birth rate of calves, decreased production of meat and milk, and an increase in the replacement rate of animals (PAULIN; FERREIRA NETO, 2003). The disease hampers the commercialization of animals and their products and subsequently undermines the competitiveness of the country in the international market (LAGE, 2006).

Humans can be exposed to brucella through contact with infected animals or consumption of unpasteurized milk. farmers, handlers, veterinarians, milkers, and slaughterers are considered high-risk occupations, and individuals working in these fields may become infected by contact with the fetus, fetal fluids and vaginal discharges of infected cows and by accidents with live vaccines (PAULIN; FERREIRA NETO, 2003). Therefore, losses are also incurred due to the costs of human treatment and the period of absence at work during convalescence (PAULIN; FERREIRA NETO, 2003).

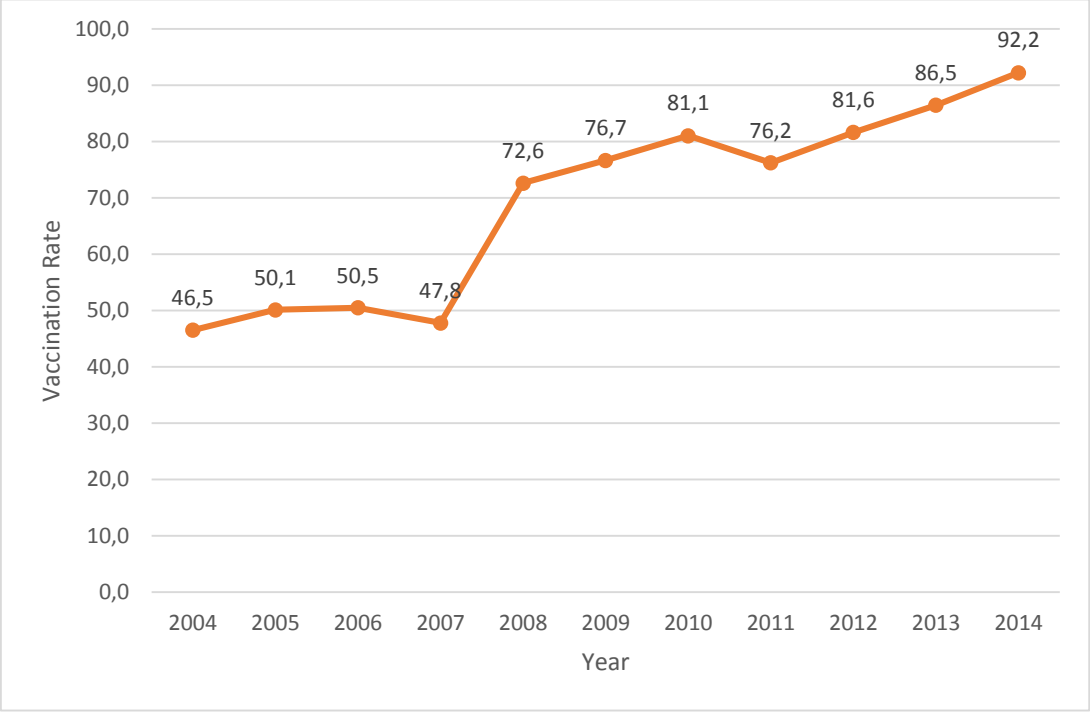
The Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) launched the Program for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) in 2001, whose objective is to reduce the incidence and prevalence of newly infected herds and subsequently create a significant number of farms free from brucellosis and tuberculosis (LAGE, 2006). Thus, in order to establish rational strategies for combating bovine brucellosis, its epidemiological status was studied in 18 Federative Units, which revealed that the prevalence of infected herds ranged from 0.32% in Santa Catarina to 41.5% in Mato Grosso do Sul (ALMEIDA et al., 2016; ALVES et al., 2009; AZEVEDO et al., 2009; BORBA et al., 2013; SIKUSAWA et al., 2009; CHATE et al., 2009; CLEMENTINO et al., 2016; DIAS et al., 2009a, 2009b; GONÇALVES et al., 2009a, 2009b; KLEIN-GUNNEWIEK et al., 2009; MARVULO et al., 2009; NEGREIROS et al., 2009; OGATA et al., 2009; ROCHA et al., 2009; SILVA et al., 2009; VILLAR et al., 2009).

The States of São Paulo, Minas Gerais, Espírito Santo, Mato Grosso, Mato Grosso do Sul and Rio Grande do Sul have conducted a second study of the prevalence of brucellosis to verify the effectiveness of their vaccination programs, however, a decrease in prevalence of infected herds was only verified in Mato Grosso, Mato Grosso do Sul and Minas Gerais

(BARDDAL et al., 2016; DIAS et al., 2016; ANZAI 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, and prohibited vaccination and initiated the implementation of eradication strategies, also conducted a second prevalence study that revealed no change (BAUMGARTEN et al., 2016).

Located in the northern region of Brazil, the State of Rondônia has a bovine herd estimated at around 13 million cattle, distributed over approximately 80 thousand properties within 52 municipalities (IBGE, 2014). In 2004, the State had a high prevalence of infected herds and animals, of 35.2% and 6.2% respectively (VILLAR et al., 2009). On that occasion, it was recommended that the State should concentrate efforts to vaccinate, on an annual basis, a minimum of 80 % of the calves between three and eight months of age with the B19 vaccine (VILLAR et al., 2009). Thus, from the moment at which the State of Rondônia instated mandatory vaccination with B19 for the issuing of a GTA (Guide of Animal Transit), good vaccine coverage was obtained (Figure 1).

Figure 1. Vaccination of calves with the B19 vaccine in the State of Rondônia (2004-2014).



Thus, the objective of the present study was to evaluate the effectiveness of the vaccination program implemented by the State, using the prevalence of infected herds and animals as indicators. Furthermore, an additional objective was to identify risk factors for bovine brucellosis in the State.

Material and Methods

The present study was planned by the Ministry of Agriculture, Livestock and Supply (MAPA) and the Collaborating Center in Animal Health of the MAPA, located in the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo, in collaboration with the Agency for

Agrosilvopastoral Sanitary Defense of the State of Rondônia (IDARON). The fieldwork was carried out by veterinarians of IDARON from February to July 2014.

In order to better characterize and evaluate the epidemiological status of bovine brucellosis after the implementation of vaccination, the State of Rondônia was divided into regions according to a system of livestock production, husbandry practice, purpose of the exploitation, average size of herds and the marketing system. The operational capacity and logistics of the official veterinary service of the State to conduct field activities were also taken into account.

For each region, a cross-sectional study was carried out in order to estimate the prevalence of brucellosis. The sample design was carried out in two stages: in the first phase a pre-determined number of properties with reproductive activity (primary sample units) were randomly selected and in the second phase a pre-determined number of cows with age equal to or greater than 24 months (secondary units of sampling) were randomly selected.

In the farms with more than one herd, the herd of greater economic importance was chosen amongst those in which the animals were submitted to the same management, i.e. under the same risks of exposure to infection.

The choice of the primary sampling unit was based on the land registry of properties with reproductive activity. If for some reason the selected property could not be visited, a new lot replaced it. The number of properties selected for each livestock region was estimated on the basis of the simple random sampling formula proposed by Thrusfield (2007) and Noordhuizen et al. (1997). The parameters adopted for the calculation were a confidence level of 0.95, an estimated prevalence of 0.20, and an error of 0.05.

The sample planning for the secondary units aimed at estimating the minimum number of animals that needed to be examined within each property to allow the farm's classification as either infected with brucellosis or non-infected using the concepts of sensitivity and specificity (DOHOO et al., 2003). For the purposes of calculation, the values 0.95 and 0.995 were adopted for the sensitivity and specificity of the test protocol respectively (FLETCHER et al., 1998), and 0.20 for the estimated prevalence. The *epitools* (Ausvet: Canberra, ACT, Australia) program was used to select the sample size that allowed values of the sensitivity and specificity obtained from the herd to be equal to or greater than 90%. Thus, in the properties with up to 99 female with an age equal to or greater than 24 months, 10 animals were sampled whilst in those with 100 or more female with ages greater than or equal to 24 months, 15 animals were sampled. Cows in the peri-partum period, i.e. approximately 15 days before and after delivery, were excluded from the selection. The choice of cows within the properties was systematic casual.

Blood samples of all selected animals were collected by puncture of the jugular vein with a sterile disposable needle and into a previously identified vacuum tube. The sera obtained from the samples were stored at –20°C in polypropylene microtubes until completion of the tests. The protocol for serodiagnosis consisted of screening with the buffered acidified antigen test, followed by retesting of positive samples with the complement fixation test. IDARON (SEAPES) and the National Laboratory of Agriculture (LANAGRO-MG) performed these tests. The property, from which the sample was initially obtained, was considered infected when at least one positive animal was detected.

The sample planning enabled the determination of the prevalence of infected herds and adult cows (≥24 months) seropositive for bovine brucellosis in the State as well as the various regions respectively. The calculations of prevalence and the respective confidence intervals were performed as recommended by Dean et al. (1994). The estimates

of prevalence of infected herds and animals in the State and prevalence of infected animals within the regions were performed in a weighted manner, according to Dohoo et al. (2003).

The weight of each individual farms in calculation of the prevalence in the State was given by:

$$W_1 = \frac{Farms\ in\ the\ region}{Farms\ sampled\ in\ the\ region}$$

The weight of each animal in calculation of the prevalence of infected animals in the State was given by:

$$W_2 = \frac{Female \geq 24\ months\ in\ the\ farm}{female \geq 24\ months\ sampled\ in\ the\ farm}\ X\ \frac{female \geq 24\ months\ in\ the\ region}{Female \geq 24\ months\ sampled\ in\ the\ region}$$

The first term in the expression above refers to the weight of each animal in the calculation of the prevalence of infected animals within the regions.

The estimates of prevalence ratios and their respective 95% confidence intervals were carried out using EpiInfo 6.0 and SPSS, version 20.0 programs.

In each of the sampled properties, in addition to sampling of blood for serological examination, an epidemiological questionnaire was also administered. It was designed to collect information on the type of exploitation and the management practices employed.

The variables analyzed were: type of production system (beef, dairy and mixed), husbandry type (confined, semi-confined, extended), the use of artificial insemination, predominant breeds, number of animals, presence of other domestic and wild species, incidence of abortions in the past 2 years, destination of the placenta and aborted fetuses, trade animals, vaccination against bovine brucellosis, sharing of pastures with other properties, occurrence of floods in pastures, existence of calving paddocks and levels of veterinary care.

The variables were organized in a scale by increasing risk and when necessary variables were re-categorized. The low risk categories were considered as a basis for comparison with other categories. The quantitative variables were categorized in percentiles. An exploratory analysis (univariate analysis) was performed to select those variables with $p<0.10$ for the $\chi 2$ test (chi-square) or Fisher exact test, and subsequent analysis by logistic regression. The calculations were performed with the aid of the SPSS program.

All of the information generated through the work carried out in the field and laboratory was inserted into a specific database used in epidemiological analyses.

Results

The State was divided into 3 regions (Figure 2). Table 1 presents the census data and the sample studied by regions. Table 2 shows the prevalence of infected herds and animals in the regions and in the State as a whole. Table 3 shows the stratification of the prevalence of infected herds by type of exploitation. The comparison between the prevalences obtained in the present study and those obtained in the study performed in 2004 (VILLAR et al., 2009) is shown in Tables 4 and 5.

Table 6 shows the final logistic regression model for the risk factors associated with an infected of bovine brucellosis in Rondônia.

Figure 2. Map of the State of Rondônia with the division into regions

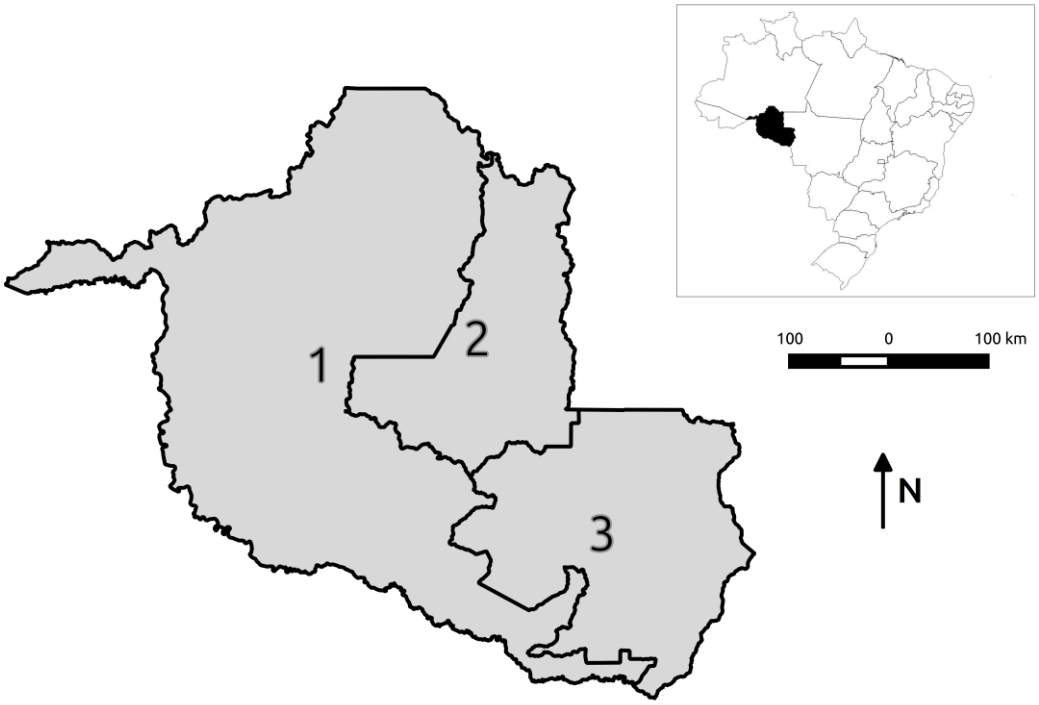


Table 1. Census data and of the sample of the State of Rondônia.

Region	Farms with reproductive activities	Farms sampled	Female with ages ≥24 months	Female with ages ≥24 months sampled
1	29.100	309	2.548.656	3.019
2	26.707	309	1.826.330	3.067
3	24.665	312	1.869.071	2.997
Total	80.472	930	6.244.057	9.083

Table 2. Prevalence of herds and animals infected with of bovine brucellosis (P) in the State of Rondônia.

Region	Farms			Female ≥ 24 months		
	Positive/ Sampled	P (%)	95% CI (%)	Positive/ Sampled	P (%)	95% CI (%)
1	39/309	12.8	9.5-17.0	55/3019	2.6	1.7-3.9
2	35/309	11.6	8.5-15.8	41/3067	1.4	0.9-2.2
3	39/312	12.5	9.3-16.7	48/2997	1.4	0.9-2.3
Total	113/930	12.3	10.3-14.6	144/9083	1.9	1.4-2.5

Table 3. Prevalence of herds infected with of bovine brucellosis (P) stratified by type of activity, according to the regions of the State of Rondônia.

Region	Beef			Dairy			Mixed		
	p/s*	P (%)	95% CI (%)	p/s	P (%)	95% CI (%)	p/s	P (%)	95% CI (%)
1	21/106	19.8	13.0-28.2	12/142	8.5	4.7-13.9	5/59	8.5	3.2-17.8
2	9/45	20.0	10.2-33.5	16/197	8.1	4.9-12.6	9/64	14.1	7.1-24.2
3	25/116	21.6	14.8-29.7	10/154	6.5	3.3-11.3	4/41	9.8	3.18-21.9

*positive/sampled.

Table 4. Comparison of the prevalence of herds infected with of bovine brucellosis (P) between the present study (2014) and the previous one (2004) conducted in the State of Rondônia.

Region	2004			2014		
	p/s*	P (%)	95% CI (%)	p/s	P (%)	95% CI (%)
1	129/308	41.88	36.3-47.6	39/309	12.80	9.5-17.0
2	97/306	31.70	26.5-37.2	35/309	11.60	8.5-15.8
3	98/307	31.92	26.7-37.5	39/312	12.50	9.3-16.7
Total	324/921	35.18	32.1-38.4	113/930	12.30	10.3-14.6

*positive/sampled.

Table 5. Comparison of the prevalence of seropositive animals (P) with bovine brucellosis between the present study (2014) and the previous one (2004) conducted in the State of Rondônia.

Region	2004			2014		
	p/s*	P (%)	95% CI (%)	p/s*	P (%)	95% CI (%)
1	216/3293	8.33	5.9-10.8	55/3019	2.6	1.7-3.9
2	171/3149	5.99	4.3-7.7	41/3067	1.4	0.9-2.2
3	173/3261	4.58	2.5-6.6	48/2997	1.4	0.9-2.3
Total	560/9703	6.22	4.9-7.6	144/9083	1.9	1.4-2.5

*positive/sampled.

Table 6. Final multivariate logistic regression model for the risk factors associated with bovine brucellosis in the State of Rondônia.

Variable	OR	95% CI
Number of cows with age ≥ 24 months		
≤ 26*		
≥ 27	1.62	1.07 – 2.46
Production system		
Beef	2.6	1.72 – 3.92
Presence of flooded areas	1.66	1.11 – 2.49

*3rd quartile.

Discussion

The results presented in Table 2 indicate a persistently high prevalence of infected herds in the entire State (12.3%) and marked homogeneity between the regions. Although without statistical significance, the point values prevalence of infected herds was higher for the beef herd in all regions (Table 3). The prevalence of seropositive animals in the State was estimated at 1.9%, with no statistical differences between the values found in each of the 3 regions.

In comparison with the study of 2004 (VILLAR et al., 2009), the present data shows that there was a significant reduction in the prevalence of both infected herds and animals (Tables 4 and 5). This was the result of the vaccination program implemented by the State, which attained good coverage from 2008 (Figure 1). As already stated previously, Mato Grosso, Mato Grosso do Sul and Minas Gerais also confirmed a decrease in prevalence of infected herds due to vaccination (BARDALL et al., 2016; LEAL FILHO et al., 2016; OLIVEIRA et al., 2016).

Thus, it is recommended that Rondônia should proceed with its vaccination program, placing emphasis on the quality of the process, from the purchasing of vaccines to the inoculation of livestock. In addition, it should also promote the use of a vaccine that does not induce antibodies in animals over 8 months of age, not already vaccinated with the B19 vaccine, since this practice accelerates the reduction of the disease prevalence (SOUZA et al., 2016).

Table 6 shows that, in the State of Rondônia, the risk factors for bovine brucellosis included having 27 or more cows, beef herds and having had areas of flooding.

The association between brucellosis and larger cattle herds had already been reported previously in Rondônia (AGUIAR et al., 2007). In Brazil, this association was also observed in the states of Rio de Janeiro, Sergipe, Tocantins, São Paulo, Mato Grosso do Sul and Mato Grosso (CHATE et al., 2009; DIAS et al., 2009b; KLEIN-GUNNEWIEK et al., 2009; OGATA et al., 2009; NEGREIROS et al., 2009; SILVA et al., 2009). Furthermore, this association was also noted by numerous international authors (KELLAR et al., 1976; NICOLETTI, 1980; SALMAN; MEYER, 1984). Some characteristics of larger herds facilitate the transmission of brucellosis, namely: the increased replacement rate of animals, greater difficulty in implementing measures to control the disease and the dynamics of brucellosis itself (CRAWFORD et al., 1990). Christie (1969) observed that an increase in herd size results in an increased probability of occurrence, persistence of infection and difficulty in eradicating brucellosis. Thus, the larger the herd, the greater the risk of brucellosis being introduced and the greater the likelihood of its dissemination within the herd.

The fact that beef herds were at a higher risk of having infection of brucellosis could most likely be attributed to the fact that these are larger herds. In the State, the median number of cows was 47 in beef herds, 30 in the mixed herds, and 23 in dairy herds. This result is corroborated by the data in Table 3 and the study performed in the State in 2004 (VILLAR et al., 2009).

The survival rate of *Brucella abortus* in the environment is further increased by the presence of humidity, and the greater the survival in the environment the larger the likelihood of this agent infecting a new susceptible animal (PAULIN, FERREIRA NETO, 2003). Thus, the presence of flooded areas in a property can promote the dissemination of disease. In three states of the Brazilian northeast – Bahia, Pernambuco and Maranhão – and also in Santa Catarina, this variable has emerged as a risk factor associated with bovine brucellosis infection (ALMEIDA et al., 2016; ALVES et al., 2009; BAUMGARTEN et al., 2016; BORBA et al., 2013). It is worth pointing out that this factor is related to the persistence of disease within an infected property rather than as a form of transmission between herds (CRAWFORD et al., 1990).

Conclusion

There has been a reduction in the prevalence of infected herds and animals since 2004 as a result of the vaccination program implemented by the State. Nonetheless, there is high prevalence of infected herds and animals in all regions. Thus, the State should continue its vaccination program, placing emphasis on the quality of the process. In addition, it should also promote the use of a vaccine that does not induce the formation of antibodies in cows, especially in beef herds. Furthermore, the State must carry out a significant effort of education so that producers test breeding animals for brucellosis before introducing them to their properties and, where possible, prevent cows from having access to areas that are flooded.

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