Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

Dias, Ricardo Augusto; Cunha Belchior, Ana Paula; de Souza Ferreira, Rodrigo; Coelho Gonçalves, Rita; Souza Costa Barão de Aguiar, Ricardo; da Rocha Sousa, Paola; Mastrangeli Amici Santos, Arianne; Amaku, Marcos; Ferreira, Fernando; Oliveira Telles, Evelise; Hildebrand Grisi-Filho, José Henrique; Heinemann, Marcos Bryan; Picão Gonçalves, Vitor Salvador; Soares Ferreira Neto, José

Controlling bovine brucellosis in the state of São Paulo, Brazil: results after ten years of a vaccination program

Semina: Ciências Agrárias, vol. 37, núm. 5, 2016, pp. 3505-3517
Universidade Estadual de Londrina
Londrina, Brasil

DOI: 10.5433/1679-0359.2016v37n5Supl2p3505

Controlling bovine brucellosis in the state of São Paulo, Brazil: results after ten years of a vaccination program

Controle da brucelose bovina no estado de São Paulo, Brasil: resultados após dez anos do programa de vacinação

Ricardo Augusto Dias[1*]; Ana Paula Cunha Belchior[2]; Rodrigo de Souza Ferreira[2]; Rita Coelho Gonçalves[2]; Ricardo Souza Costa Barão de Aguiar[2]; Paola da Rocha Sousa[2]; Arianne Mastrangeli Amici Santos[2]; Marcos Amaku[1]; Fernando Ferreira[1]; Evelise Oliveira Telles[1]; José Henrique Hildebrand Grisi-Filho[1]; Marcos Bryan Heinemann[1]; Vitor Salvador Picão Gonçalves[3]; José Soares Ferreira Neto[1]

Abstract

A cross- sectional study was carried out, between May and November 2011, to estimate the situation of the bovine brucellosis in São Paulo State, 10 years after the commencement of the vaccination of the heifers with the S19 strain. The State was divided into seven regions and in each of them, 300 farms with reproductive activity were randomly chosen and considered as primary sample units. A fixed number of cows was randomly selected and tested for antibodies against *Brucella* spp. A farm was considered infected if at least one female tested positive. In the selected farms, an epidemiological questionnaire was administered which focused on herd traits as well as husbandry and sanitary practices that could be associated with the risk of infection. The prevalence (percentile, [95% confidence interval]) of infected herds was 10.2% [8.8-11.8] for the State, and for the regions, it varied from 7.3% [4.7-11.2] to 12.3% [8.8-16.8], not showing significant difference between different regions. The apparent prevalence of positive farms in the State and regions remained similar to the prevalence observed 10 years before. The prevalence of positive animals was 2.4% [1.8-3.1] in the State and varied from 1.1% [0.6-2] to 3.5% [1.7-7.1] in the regions, not showing significant difference between regions. Again, there was no difference in the prevalence of positive animals after 10 years of the vaccination program. The risk factors (odds ratio, 95% confidence interval) associated with bovine brucellosis in the State included number of cows ≥ 24 (3.08, 2.22-4.27) and the acquisition of breeding animals (1.33, 0.95-1.87). The São Paulo State should conduct systematic vaccination coverage of above 80% of the eligible heifers with the S19 strain vaccine annually. Moreover, the State should emphatically use RB51 strain vaccine in females above 8 months of age not vaccinated with S19 strain vaccine. An efficient animal health education program to orientate farmers to test replacement animals for brucellosis prior to introduction in their herds should also be implemented.

Key words: Bovine brucellosis. PNCEBT. Prevalence. Risk factors. São Paulo. Brazil.

[1] Profs., Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, USP, São Paulo, SP, Brasil. E-mail: dias@vps.fmvz.usp.br; amaku@vps.fmvz.usp.br; fernando@vps.fmvz.usp.br; bufalo@usp.br; grisi@vps.fmvz.usp.br; marcosbryan@usp.br; jsoares@vps.fmvz.usp.br

[2] Discentes, Coordenadoria de Defesa Agropecuária do Estado de São Paulo, CDA, Campinas, SP, Brasil. E-mail: ana.belchior@cda.sp.gov.br; rodrigo.souza@cda.sp.gov.br; rita@cda.sp.gov.br; ricardo.barao@cda.sp.gov.br; paola@cda.sp.gov.br; arianne.mastrangi@cda.sp.gov.br

[3] Prof., Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, UnB, Brasília, DF, Brasil. E-mail: vitorspg@usb.br

[*] Author for correspondence

Received: Sept. 15, 2016 – Approved: Sept. 15, 2016

Resumo

Um estudo transversal foi realizado entre maio e novembro de 2011 para estimar a situação da brucelose bovina no estado de São Paulo, 10 anos após o início do programa de vacinação de fêmeas bovinas com a vacina B19. O Estado foi dividido em sete regiões e em cada uma delas, 300 propriedades com atividade reprodutiva foram aleatoriamente selecionadas e consideradas unidades primárias de amostragem. Um número fixo de vacas foi selecionado aleatoriamente e testado para anticorpos anti-*Brucella* spp. Uma propriedade foi considerada infectada se ao menos uma vaca resultou positiva. Nas propriedades selecionadas, um questionário epidemiológico foi aplicado, focando a caracterização do rebanho e as práticas de manejo e sanitárias que pudessem estar associadas com o risco de infecção. A prevalência (percentual, [intervalo de confiança de 95%]) dos rebanhos infectados foi 10,2% [8,8-11,8] para o Estado e, para as regiões, variou de 7,3% [4,7-11,2] a 12,3% [8,8-16,8], não apresentando diferença significativa entre as diferentes regiões. A prevalência aparente de propriedades positivas no Estado e regiões permaneceu similar à prevalência observada há 10 anos. A prevalência de fêmeas positivas foi 2,4% [1,8-3,1] no Estado e variou de 1,1% [0,6-2] a 3,5% [1,7-7,1] nas regiões, não apresentando diferenças significativas. Novamente, não houve diferença significativa na prevalência de animais positivos após 10 anos de programa de vacinação. Os fatores de risco (*odds ratio*; intervalo de confiança de 95%) associados à brucelose bovina no Estado incluíram o número de vacas ≥ 24 (3,08; 2,22-4,27) e a aquisição de reprodutores (1,33; 0,95-1,87). O estado de São Paulo deve promover uma cobertura vacinal sistemática superior a 80% do rebanho elegível de bezerras com a vacina B19, anualmente. Além disso, o Estado deve enfaticamente utilizar a vacina RB51 em fêmeas com idade superior a 8 meses de idade não vacinadas com a vacina B19. Um programa de educação em saúde animal eficiente deve ser implantado, a fim de orientar os proprietários a testar animais de reposição para brucelose antes da sua introdução no rebanho.

Palavras-chave: Brucelose bovina. Fatores de risco. PNCEBT. Prevalência. São Paulo. Brasil.

Introduction

Bovine brucellosis is a chronic zoonotic disease that causes economic losses in beef and milk production (ALVES et al., 2015) caused by *Brucella abortus*. Although the disease can also be transmitted by *B. melitensis*, this species has never been isolated in Brazil (POESTER et al., 2002). The main symptom of the disease is abortion in the second half of gestation, whereby the bacteria are transmitted through the aborted products and vaginal discharges (PAULIN; FERREIRA NETO, 2003). The disease is distributed worldwide, especially in poor countries (OIE, 2015). It is usually endemic and widespread in large territories connected by commercial relations, especially animal trade (MIKOLON et al., 1998; DIAS et al., 2009b).

In 2001, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) launched the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), aiming for the reduction in the negative impacts of bovine brucellosis and tuberculosis on human health, and for the promotion of competitive practices in the national livestock industry (LAGE et al., 2006).

The control measures of PNCEBT included compulsory vaccination against brucellosis in bovine and buffalo heifers aged between 3 and 8 months with the S19 vaccine throughout the country (excepting the state of Santa Catarina) and the voluntary certification of brucellosis-free farms (LAGE et al., 2006). Most of these measures are paid by the farmers, such as the vaccination of heifers, diagnostic tests, and veterinary services. The official veterinary service is only responsible for auditing the entire system.

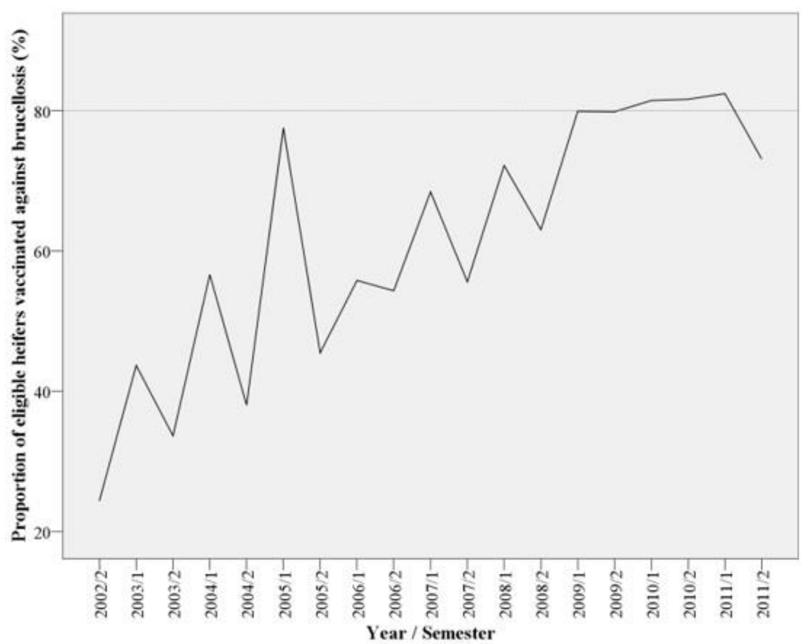
Along with the establishment of the PNCEBT, epidemiological surveys were conducted in Brazilian states using farms with reproductive activity as the primary sample units. The prevalence of brucellosis-infected herds varied from 0.32% in Santa Catarina to 41.5% in Mato Grosso do Sul (ALMEIDA et al., 2016; ALVES et al., 2009; AZEVEDO et al., 2009; BORBA et al., 2013; 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; SIKUSAWA et al., 2009; SILVA et al., 2009; VILLAR et al., 2009).

The states of Minas Gerais, Espirito Santo, Rondônia, Mato Grosso, Mato Grosso do Sul and Rio Grande do Sul carried out the second study on brucellosis prevalence. Of these, only in Mato Grosso, Mato Grosso do Sul, Minas Gerais and Rondônia a decreasing prevalence of infected herds was observed as a consequence of the vaccination program (BARDDAL 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, and had, additionally, prohibited vaccination and started the implementation of eradication strategies, also conducted a second study that did not indicate changes to the prevalence of infected herds (BAUMGARTEN et al., 2016).

In 2001, the São Paulo State reported an infected herd prevalence of 9.7% [95% CI, 7.8-11.6] and an animal-level prevalence of 3.8% [0.7-6.9] (DIAS et al., 2009b). At the time, it was recommended that the State should organize an effective vaccination program using the S19 strain vaccine, in order to ensure vaccination coverage of eligible heifers of > 80% annually (AMAKU et al., 2009). Moreover, in order to help with the increase in the vaccination coverage, the State was encouraged to stimulate the vaccination of adult bovines with the RB51 strain after 2007, supported by the Brazilian Ministry of Agriculture, Livestock and Food Supply regulation (Instrução Normativa SDA 33, of August 24, 2007).

After the implementation of the PNCEBT, São Paulo State has gradually increased the proportion of vaccinated, eligible heifers. Only after the first semester of 2009, did the State commence and maintain the vaccination coverage of eligible heifers against brucellosis above 80% (AGUIAR, 2012) (Figure 1).

Figure 1. Evolution of the proportion of heifers aged between 3-8 months vaccinated against brucellosis in the state of São Paulo, Brazil, between 2002 and 2011. Source: Aguiar (2012).



The present study aimed to conduct a repeat of the epidemiological survey in the state of São Paulo, 10 years after the beginning of the compulsory vaccination of heifers against brucellosis, in order to verify the efficacy of the vaccination program, and allow for the management and adjustments of the animal health policy.

Material and Methods

Sample design

The present study was planned by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), the Collaborator Centre in Animal Health of the School of Veterinary Medicine of the University of São Paulo (FMVZ-USP) and the Animal Health Service of the state of São Paulo (Coordenadoria de Defesa Agropecuária – CDA). The field work was performed by CDA staff from May to September, 2011, after being trained to standardize the procedures. The present work used the same methodology as that of a previous study, performed 10 years before (DIAS et al., 2009b).

In order to characterize regional differences in epidemiological parameters of bovine brucellosis, the State was divided into seven regions, each of which, were considered for their livestock production systems, managing practice, herd size and trade systems. This division also considered the operational and logistical capacity of the CDA to perform the fieldwork, based on its 40 regional offices. A map of the regions was made in ArcGIS 10.0. software.

In each region, a cross sectional study was performed to estimate the herd and animal-level prevalence of bovine brucellosis prevalence using a two-stage sampling method. In the first stage, a pre-determined number of farms with reproductive activity was randomly selected (primary sampling units). In the second stage, a pre-determined number of females, above two years of age, was randomly selected (secondary sampling units). Owing to their similar susceptibility to *Brucella abortus* (APARICIO, 2013), bovine and buffalo herds were also considered in the sampling. Moreover, both species were targeted in the official vaccination program. According to the Brazilian Ministry of Agriculture, the buffalo herd of Sao Paulo State was 74,925 or 0.7% of the bovine herd in 2011.

In farms where there were more than one individual herd, we have chosen the biggest, more economically important herd, in which the animals were subjected to the same management procedures, i.e. they were exposed to the same risk factors. The choice of the primary sampling units was based on the official farm registry database. If a selected farm could not be visited by CDA staff, a new one was randomly selected to replace it. The number of selected farms per region was estimated via a simple, random sample formula, proposed by Thrusfield (2007) and Noordhuizen et al. (1997):

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

where N denotes the sample size, Z_α is the normal distribution value for the confidence level of 95%, P is the expected prevalence (20%) and d is the absolute error (5%).

The choice of the secondary sampling units aimed for the appropriate classification of a farm as either positive or negative. To achieve this, we have considered herd sensitivity and specificity (DOHOO et al., 2003). The values for sensitivity and specificity for the test protocol were 95% and 99.5%, respectively (FLETCHER et al., 1988), and 20% for the expected prevalence. The calculations were made using the Herdacc version 3 and the selected sample size allowed for herd sensitivities and specificities greater than 90%. Thus, in farms with at least 99 females above two years of age, 10 animals were randomly sampled, and in farms with more than 99 females above two years of age, 15 animals were tested. If the selected herd was smaller than the required sample, all animals would be sampled.

A farm was considered positive when at least one animal was detected as positive.

Test protocol

The test protocol consisted of a screening procedure using the Rose Bengal test, followed by a retest of those with positive results via the complement fixation test, in accordance to Alton et al. (1988). The samples were collected by CDA staff and the laboratory tests were performed at the Instituto Biológico de São Paulo.

The blood was collected through a jugular vein puncture with a sterile needle in a vacuum tube. Sera were stored in plastic microtubes, maintained at –20°C until testing.

Prevalence estimation

The sample design allowed us to determine herd and animal-level prevalence in the state of Sao Paulo and in the regions as well. The apparent prevalence and the respective confidence intervals for each region were estimated according to Dean et al. (1996). All calculations were weighted (DOHOO et al., 2003). The formula for the weight of each farm (W_f) factored into the calculation of the positive herd prevalence for the whole state was:

$$W_f = \textit{number of farms in the region / number of sampled farms in the region}$$

The weight of each bovine female above two years of age (W_a) in the calculation of animal-level prevalence in the whole state was:

$$W_a = \textit{females} \geq 2 \textit{ years in the farm / sampled females} \geq 2 \textit{ years in the farm} * \textit{females} \geq \textit{ years in the region / females} \geq 2 \textit{ years in the sampled farms of the region}$$

In the above expression, the first term refers to the weight of each sampled animal in the farm and the second term refers to the weight of each farm in the region.

The prevalence estimates with their respective 95% confidence intervals (95% CI) were calculated using EpiInfo 6.0 and SPSS version 9.0. The prevalence of the disease indicated in the 2001 and 2011 surveys were compared by the proportion comparison test, using SPSS 9.0 software.

Risk factor analysis

In each sampled farm, a questionnaire was administered in order to generate data about its managing practices. All information generated in the field and in the laboratory was inserted into a database.

In this cross sectional study, the risk factors that were assessed included: the production system (meat, milk, mixed), raising system (extensive, any degree of confinement), artificial insemination, cattle breeds, number of cows above two years of age, total herd size, presence of other domesticated species, presence of wild species, destination of the placenta and aborted fetuses, animal trade, vaccination against brucellosis, slaughter in the farm, pasture sharing, indirect contact between farms, flooded pastures, breeding paddock and veterinary assistance.

These variables were organized in accordance to increasing risk scale. If it was deemed necessary, the variables were re-categorized. The lowest risk category was always considered as the baseline for comparisons with the other categories. The quantitative variables were categorized using quartiles as cut points.

An exploratory univariate analysis using chi-square (χ^2) or the Fischer exact test was made with all variables, which considered the entirety of the state. Those which were below the significance level of 0.20 were selected for a multivariate analysis using logistic regression, accordingly to Hosmer and Lameshow (1989). All calculations were made in SPSS 9.0 and EpiInfo 7.0 computer software.

In order to check for correlation between the variables, a Spearman correlation test was performed. In the scenario where two variables were correlated, the variable that was less associated with the dependent variable was excluded from the multivariate analysis.

Results and Discussion

The state of São Paulo was divided into seven regions (Figure 2), following the same manner as the study conducted in 2001 (DIAS et al., 2009b).

The apparent prevalence of bovine brucellosis positive herds in the state of São Paulo was estimated at 10.2% [95% CI, 8.8-11.8], varying from 7.3% [95% CI, 4.7-11.2] in region 3 to 12.3% [95% CI, 8.8-16.8] in region 2, whereby no differences among the regions were observed (Table 1).

Moreover, there were no significant differences observed in the apparent prevalence of brucellosis positive herds between the farm enterprises (beef, mixed and dairy), and for each farm enterprise category between the regions (Table 2). This indicated that the disease was evenly distributed among beef, dairy and mixed farms within and between regions.

The apparent prevalence of brucellosis positive cows in the state of São Paulo was estimated at 2.4% [95% CI, 1.8-3.1], varying from 1.1% [95% CI, 0.6-2.0] in region 7 to 3.5% [95% CI, 1.7-7.1] in region 4, whereby no differences between the regions were observed (Table 3).

No significant difference in the prevalence of brucellosis infected herds for each region, or for the state, was observed between the 2001 (DIAS et al., 2009b) and 2011 serological surveys in the present study. As for the animal-level prevalence, no significant differences were observed as well. A comparison of brucellosis positive herds, and the positive animals between the 2001 (DIAS et al., 2009b) and the 2011 surveys is shown in Figure 3. The states of Espírito Santo (ANZAI et al., 2016) and Rio Grande do Sul (SILVA et al., 2016) also failed to lower the prevalence of brucellosis with their immunization programs.

Figure 2. Map of the state of São Paulo showing the division of regions.

Table 1. Apparent prevalence of bovine brucellosis infected herds in the State of São Paulo, 2011.

Region	Farms with reproductive activities	Sampled farms	Positive farms	Prevalence (%)	95% CI (%)
1	28,943	247	30	12.1	8.6 – 16.9
2	25,343	269	33	12.3	8.8 – 16.8
3	23,599	260	19	7.3	4.7 – 11.2
4	9,732	237	26	11.0	7.6 – 15.6
5	15,881	251	28	11.2	7.8 – 15.7
6	17,976	230	18	7.8	5.0 – 12.1
7	13,037	249	21	8.4	5.6 – 12.6
Total	134,511	1,743	175	10.2	8.8 – 11.8

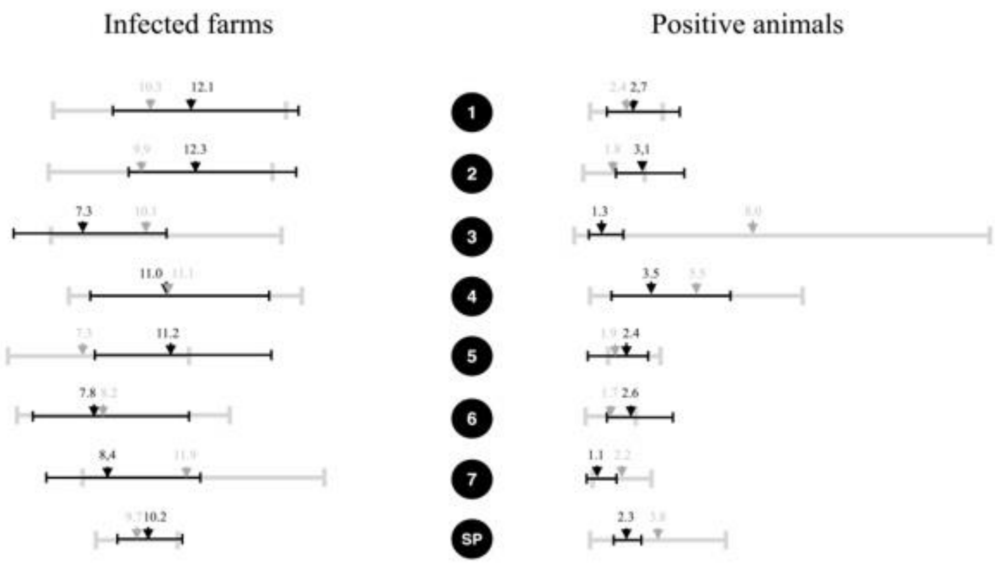
Table 2. Apparent prevalence of bovine brucellosis infected herds according to the farm enterprises in the state of São Paulo, 2011.

Region	Beef farms			Dairy farms			Mixed farms		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
1	20/115	17.4	11.5-25.5	7/95	7.4	3.5-14.7	3/37	12.1	8.6-16.9
2	10/93	10.8	5.9-18.9	16/113	14.2	8.8-21.9	7/63	12.3	8.8-16.8
3	9/111	8.1	4.3-14.9	9/92	9.8	5.2-17.8	1/57	7.3	4.7-11.2
4	9/112	8.0	4.2-14.8	11/67	16.4	9.3-27.3	6/58	11.0	7.6-15.6
5	9/73	12.3	6.5-22.1	6/84	7.1	3.2-15.0	13/94	11.2	7.8-15.7
6	9/75	12.0	6.4-21.5	2/41	4.9	1.2-17.6	7/114	7.8	5.0-12.1
7	3/43	7.0	2.3-19.6	12/144	8.3	4.8-14.1	6/62	8.4	5.6-12.6

Table 3. Apparent prevalence of bovine brucellosis positive females above two years of age in the state of São Paulo, 2011.

Region	Females ≥ 2 years of age	Sampled females ≥ 2 years of age	Positive animals	Prevalence (%)	95% CI (%)
1	1,193,467	2,177	34	2.7	1.5 – 4.8
2	823,073	2,072	46	3.1	1.9 – 5.0
3	984,312	1,957	27	1.3	0.7 – 2.3
4	264,744	1,627	44	3.5	1.7 – 7.1
5	364,848	1,693	31	2.4	1.5 – 4.0
6	392,063	1,570	19	2.6	1.5 – 4.5
7	308,948	1,824	26	1.1	0.6 – 2.0
Total	4,331,455	12,920	227	2.4	1.8 – 3.1

Figure 3. Comparison of the prevalence of bovine brucellosis positive-farms and positive-animals between the 2001 and 2011 serological surveys conducted in the state of São Paulo. Numbers in the center of the figure indicate survey regions, gray lines indicate point estimates and 95% confidence intervals of the 2001 survey (DIAS et al., 2009b) and the black lines indicate the results of the 2011 survey analyzed in the present study.



The main reason for these results is the fact that the state of São Paulo only managed to reach a consistent annual vaccination coverage of over 80% from 2009 onwards, achieving 89.04% in 2014, as informed by Klaus Saldanha Hellwig[4]. At the beginning of the vaccination program, the State had experienced an inconsistent distribution in the availability of the S19 strain vaccine. Currently, the state of São Paulo has a constant supply of the S19 strain vaccine, and 2,806 licensed veterinarians to perform vaccination in 514 (out of 645) municipalities, as informed by Klaus Saldanha Hellwig[5].

In 2007, MAPA approved the use of the RB51 strain vaccine, which was recommended to heifers above 8 months of age that had not yet been vaccinated with the S19 strain between 3 to 8 months of age. This was an attempt to increase the herd vaccination coverage. In 2008, the vaccination distribution reached its peak, but in 2010, this was halted due to technical problems in the production methods. Currently, the distribution of the RB51 strain vaccine is marginal in the state of São Paulo.

Table 4 presents the results of the univariate analysis, and the multivariate model. The final multivariate model indicated two risk factors (odds ratio, 95% confidence interval): number of cows ≥ 24 (3.08, 2.22-4.27) and the acquisition of breeding animals (1.33, 0.95-1.87) (Table 5).

[4] Coordinator of PECEBT in Sao Paulo State during a meeting at FMVZ-USP in April 9, 2015.

[5] Coordinator of PECEBT in Sao Paulo State during a meeting at FMVZ-USP in April 9, 2015.

Table 4. Univariate analysis of the possible risk factors for bovine brucellosis in the state of São Paulo, 2011.

Variable	Proportion of infected herds	Proportion (%)	p
Total number of bovines			< 0.001
1 – 56*	100/1,304	7.7	
≥57	75/439	17.1	
Number of females ≥ 2 years			< 0.001
1 – 23*	88/1,293	6.8	
≥ 24	87/450	19.3	
Acquisition of breeding animals			0.001
No	108/1,262	8.6	
Yes	67/481	13.9	
Raising system			0.020
Extensive	140/1,496	9.4	
Any degree of confinement	35/247	14.2	
Numberof milking females			0.023
1 – 9*	66/776	8.5	
≥ 10	38/289	13.1	
Presence of flooded areas			0.117
No	110/1,187	9.3	
Yes	65/556	11.7	
Veterinary assistance			0.140
No	107/1,153	9.3	
Yes	68/590	11.5	

*Third quartile. **Fischer exact test.

Table 5. Final multivariate model of the risk factors for bovine brucellosis in the state of São Paulo, 2011.

Variable	Odds ratio	Confidence interval (95%)	p
Number of females ≥ 2 years≥ 24	3.08	2.22 – 4.27	< 0.001
Acquisition of breeding animals	1.33	0.95 – 1.87	0.100

r^2 = 6.4%

The association between the herd size and brucellosis has already been reported by many international authors (KELLAR et al., 1976; NICOLETTI, 1980; SALMAN; MEYER, 1984), and also in the Brazilian states of Mato Grosso, Mato Grosso do Sul, Rio de Janeiro, Sergipe, Tocantins and in the previous study carried out in São Paulo in 2001 (NEGREIROS et al., 2009; CHATE et al., 2009; KLEIN-GUNNEWIEK et al., 2009; SILVA et al., 2009; OGATA et al., 2009; DIAS et al., 2009b). Characteristics such as a higher frequency of animal replacement, and a greater difficulty in implementing disease control strategies, in addition to the dynamics of the disease itself, may all be attributable to the biggest herds that facilitate brucellosis transmission (CRAWFORD et al., 1990).

Christie (1969) observed that an increased herd size resulted in an increased probability of occurrence, infection persistence and an increased difficulty in eradicating the disease. Therefore, a larger herd size was associated with an increased risk of brucellosis introduction, in addition to a higher probability for intra-herd dissemination.

The purchase of infected animals is largely reported as the main risk factor for the introduction of brucellosis into free herds (VAN WAVERN, 1960; NICOLETTI, 1980). Regarding this variable, some factors may act independently or in combination, such as purchase frequency, origin of animals and serological tests for brucellosis (CRAWFORD et al., 1990). Kellar et al. (1976) reported that infected herds acquired more replacement animals than free ones. In Brazil, this variable also emerged as a risk factor for brucellosis in the states of Bahia, Goias, Minas Gerais, Paraná and in a previous study carried out in São Paulo in 2001 (ALVES et al., 2009; ROCHA et al., 2009; GONÇALVES et al., 2009a; DIAS et al., 2009a; KLEIN-GUNNEWIEK et al., 2009; DIAS et al., 2009b). The real problem is not in the introduction of animals, which is routine in cattle herds, but rather, in the acquisition of animals without testing or without knowledge of the health status of the farm of origin.

Conclusion

São Paulo State should aim for systematic vaccination coverage of > 80% of the eligible heifers with the S19 strain vaccine annually. Moreover, the State should use the RB51 strain vaccine in females above 8 months of age not vaccinated with the S19 strain vaccine in order to accelerates the reduction of the disease prevalence, as described by Souza et al. (2016). An efficient animal health education program to orientate farmers to test replacement animals for bovine brucellosis prior to introduction into their herds should also be implemented.

Acknowledgements

The authors acknowledge the logistical and financial support from MAPA, CDA-SP, CNPq and FAPESP.

References

AGUIAR, R. S. C. B. *Caracterização epidemiológica da brucelose e tuberculose bovinas na região de Campinas, Piracicaba, Bragança Paulista, Limeira, Mogi Mirim e São João da Boa Vista, Estado de São Paulo.* 2012. Dissertação (Mestrado). Faculdade de Medicina Veterinária e Zootecnia, Univesidade de São Paulo, São Paulo. Available at: <http://www.teses.usp.br/teses/disponiveis/10/10134/tde-01072013-102513/es.php>. Accessed at: 5 ago. 2015.

ALMEIDA, E. C.; FREITAS, A. A.; PONTUAL, K. A. Q.; SOUZA, M. M. A.; AMAKU, M.; DIAS, R. A.; FERREIRA, F.; TELLES, E. O.; HEINEMANN, M. B.; GONÇALVES, V. S. P.; EVÊNCIO NETO, J.; MARVULO, M. F. V.; GRISI-FILHO, J. H. H.; FERREIRA NETO, J. S.; SILVA, J. C. R. Prevalence and associated risk factors for bovine brucellosis in the state of Pernambuco, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3413-3424, 2016. Suplemento 2.

ALTON, G. G.; JONES, L. M.; ANGUS, R. D.; VERGER, J. M. *Techniques for the brucellosis laboratory.* Paris: French National Institute for Agricultural Research. 1988. 190 p.

ALVES, A. J. S.; GONÇALVES, V. P. S.; FIGUEIREDO, V. C. F.; LÔBO, J. R.; BAHIENSE, L.; AMAKU, M.; FERNANDO, F.; FERREIRA NETO, J. S.; DIAS, R. A. Situação epidemiológica da brucelose bovina no Estado da Bahia. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 6-13, 2009. Suplemento 1.

AMAKU, M.; DIAS, R. A.; FERREIRA NETO, J. S.; FERREIRA, F. Mathematical modelling of bovine brucellosis control by vaccination. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 135-141, 2009. Suplemento 1.

ANZAI, E. K.; COSTA, D.; SAID, A. L. P. R.; GRISI-FILHO, J. H. H.; AMAKU, M.; DIAS, R. A.; FERREIRA, F.; GALVIS, J. O. A.; GONÇALVES, V. S. P.; HEINEMANN, M. B.; TELLES, E. O.; FERREIRA NETO, J. S. An update on the epidemiological situation of bovine brucellosis in the state of Espírito Santo, Brazil. *Semina: Ciências Agrárias,* Londrina, v. 37, n. 5, p. 3437-3448, 2016. Suplemento 2.

APARICIO, E. D. Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus. *Revue Scientifique et Technique Office Internationale de Epizooties*, Paris, v. 32, p. 53-60, 2013. Suplemento 1.

AZEVEDO, S. S.; FERREIRA NETO, J. S.; DIAS, R. A.; FERREIRA, F.; AMAKU, M.; FIGUEIREDO, V. C. F.; LOBO, J. R.; GONÇALVES, V. S. P.; SOUZA, A. C.; VASCONCELLOS, S. A. Situação epidemiológica da brucelose bovina no Estado do Espírito Santo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 19-26, 2009. Suplemento 1.

BARDDAL, J. E. I.; SANTOS, J. C. Q.; LOPES, I. F.; FERREIRA NETO, J. S.; FERREIRA, F.; AMAKU, M.; DIAS, R. A.; TELLES, E. O.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; GONÇALVES, V. S. P.; AGUIAR, D. M. Effect of vaccination in lowering the prevalence of bovine brucellosis in the state of Mato Grosso, Brazil. *Semina: Ciências Agrárias,* Londrina, v. 37, n. 5, p. 3479-3492, 2016. Suplemento 2.

BAUMGARTEN, K. D.; VELOSO, F. P.; GRISI-FILHO, J. H. H.; FERREIRA, F.; AMAKU, M.; DIAS, R. A.; TELLES, E. O.; HEINEMANN, M. B.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Prevalence and risk factors for bovine brucellosis in Santa Catarina state, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3425-3436, 2016. Suplemento 2.

BORBA, M. R.; STEVENSON, M. A.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S.; FERREIRA, F.; AMAKU, M.; TELLES, E. O.; SANTANA, S. S.; FERREIRA, J. C. A.; LÔBO, J. R.; FIGUEIREDO, V. C. F.; DIAS, R. A. Prevalence and risk-mapping of bovine brucellosis in Maranhão State, Brazil. *Preventive Veterinary Medicine*, Amsterdam, v. 110, n. 2, p. 169-176, 2013.

CHATE, S. C.; DIAS, R. A.; AMAKU, M.; FERREIRA, F.; MORAES, G. M.; COSTA NETO, A. A.; MONTEIRO, L. A. R. C.; LÔBO, J. R.; FIGUEIREDO, V. C. F.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado do Mato Grosso do Sul. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 46-55, 2009. Suplemento 1.

CHRISTIE, T. E. Eradication of brucellosis in Northern Ireland: field problems and experiences. *Veterinary Record*, London, v. 85, n. 10, p. 268-269, 1969.

CLEMENTINO, I. J.; DIAS, R. A.; AMAKU, M.; FERREIRA, F.; TELLES, E. O.; HEINEMANN, M. B.; GONÇALVES, V. S. P.; GRISI-FILHO, J. H. H.; FERREIRA NETO, J. S.; ALVES, C. J.; SANTOS, C. S. A. B.; AZEVEDO, S. S. Epidemiological situation of bovine brucellosis in the state of Paraiba, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3403-3412, 2016. Suplemento 2.

CRAWFORD, R. P.; HUBER, J. D.; ADAMS, B. S. Epidemiology and surveillance. In: NIELSEN, K.; DUNCAN, J. R. (Ed.). *Animal brucellosis*. Boca Raton: CRC Press, 1990. p. 131-151.

DEAN, A. G.; DEAN, J. A.; COULOMBIER, D.; BRENDEL, K. A.; SMITH, D. C.; BURTON, A. H.; DICKER, R. C.; SULLIVAN, K.; FAGAN, R. F.; ARNER, T. G. *Epi Info, version 6*: a word processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta: Centers for Disease Control and Prevention, 1996. 595 p.

DIAS, J. A.; MÜLLER, E. E.; DIAS, R. A.; FREITAS, J. C.; AMAKU, M.; FERREIRA, F.; SILVA, M. C. P.; LÔBO, J. R.; FIGUEIREDO, V. C. F.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado do Paraná. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 66-76, 2009a. Suplemento 1.

DIAS, R. A.; GONÇALVES, V. S. P.; FIGUEIREDO, V. C. F.; LÔBO, J. R.; LIMA, Z. M. B.; PAULIN, L. M. S.; GUNNEWIEK, M. F. K.; AMAKU, M.; FERREIRA NETO, J. S.; FERREIRA, F. Situação epidemiológica da brucelose bovina no Estado de São Paulo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 118-125, 2009b. Suplemento 1.

DOHOO, I.; MARTIN, W.; STRIYHN, H. *Veterinary epidemiologic research.* Charlottetown: Atlantic Veterinary College, 2003. 704 p.

FLETCHER, R. H.; FLETCHER, S. W.; WAGNER, E. H. *Clinical epidemiology:* the essentials. 2th ed. Baltimore: Willians; Wilkkins, 1988. 260 p.

GONÇALVES, V. S. P.; DELPHINO, M. K. V. C.; DIAS, R. A.; FERREIRA, F.; AMAKU, M.; FERREIRA NETO, J. S.; PORTO, T. B.; ALVES, C. M.; FIGUEIREDO, V. C. F.; LOBO, J. R. Situação epidemiológica da brucelose bovina no Estado de Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 35-45, 2009a. Suplemento 1.

GONÇALVES, V. S. P.; RIBEIRO, L. A.; CALDAS, R. A.; FRANCISCO, P. F. C.; DIAS, R. A.; FERREIRA, F.; AMAKU, M.; FERREIRA NETO, J. S.; FIGUEIREDO, V. C. F.; LOBO, J. R.; BORGES, J. R. J. Situação epidemiológica da brucelose bovina no Distrito Federal. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 14-18, 2009b. Suplemento 1.

HOSMER, D. W.; LAMESHOW, S. *Applied logistic regression*. New York: John Wiley and Sons, 1989. 307 p.

INLAMEA, O. F.; ROCHA, A. B.; FERREIRA, F.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; DIAS, R. A.; TELLES, E. O.; GONÇALVES, V. S. P.; AMAKU, M.; FERREIRA NETO, J. S. Effect of vaccination in lowering bovine brucellosis in the state of Rondônia, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3493-3506, 2016. Suplemento 2.

KELLAR, J.; MARRA, R.; MARTIN, W. Brucellosis in Ontario: a case control study. *Canadian Journal of Comparative Medicine*, Ottawa, v. 40, n. 2, p. 119-128, 1976.

KLEIN-GUNNEWIEK, M. F. C.; AMAKU, M.; DIAS, R. A.; FERREIRA, F.; GITTI, C. B.; PEREIRA, L. A.; FIGUEIREDO, V. C. F.; LOBO, J. R.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado do Rio de Janeiro. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 77-84, 2009. Suplemento 1.

LAGE, A. P.; ROXO, E.; MÜLLER, E.; POESTER, F.; CAVALLÉRO, J. C. M.; FERREIRA NETO, J. S.; MOTA, P. M. P. C.; GONÇALVES, V. S. P. *Programa nacional de controle e erradicação da brucelose e da tuberculose animal (PNCEBT).* Brasília: Ministério da Agricultura, Pecuária e Abastecimento, 2006. 188 p. Disponível em: <http://www.agricultura.gov.br>. Acesso em: 5 ago. 2015.

LEAL FILHO, J. M.; BOTTENE, I. F. N.; MONTEIRO, L. A. R. C.; PELLEGRIN, A. O.; GONÇALVES, V. S. P.; FERREIRA, F.; DIAS, R. A.; AMAKU, M.; TELLES, E. O.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; FERREIRA NETO, J. S Control of bovine brucellosis from 1998 to 2009 in the state of Mato Grosso do Sul, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3467-3478, 2016. Suplemento 2.

MARVULO, M. F. V.; FERREIRA, F.; DIAS, R. A.; AMAKU, M.; GROFF, A. C. M.; GONÇALVES, V. S. P.; FIGUEIREDO, V. C. F.; LOBO, J. R.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado do Rio Grande do Sul. *Arquivo Brasileiro de Medicina Veterinária e Zootec*nia, Belo Horizonte, v. 61, p. 93-102, 2009. Suplemento 1.

MIKOLON, A. B.; GARDNER, I. A.; HERNANDEZ DE ANDA, J.; HIETALA, S. K. Risk factors for brucellosis seropositivity of goat herds in the Mexicali Valley of Baja California, Mexico. *Preventive Veterinary Medicine*, Amsterdam, v. 37, n. 1-4, p. 185-195, 1998.

NEGREIROS, R. L.; DIAS, R. A.; FERREIRA, F.; FERREIRA NETO, J. S.; GONÇALVES, V. S. P.; SILVA, M. C. P.; FIGUEIREDO, V. C. F.; LOBO, J. R.; FREITAS, J.; AMAKU, M. Situação epidemiológica da brucelose bovina no Estado do Mato Grosso. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 56-65, 2009. Suplemento 1.

NICOLETTI, P. The epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine*, New York, v. 24, p. 69-98, 1980.

NOORDHUIZEN, J. P. T. M.; FRANKENA, K.; HOOFD, C. M. *Application of quantitative methods in veterinary epidemiology*. Wageningen: Wageninggen Press, 1997. 445 p.

OGATA, R. A.; GONÇALVES, V. S. P.; FIGUEIREDO, V. C. F.; LOBO, J. R.; RODRIGUES, A. L.; AMAKU, M.; FERREIRA, F.; FERREIRA NETO, J. S.; DIAS, R. A. Situação epidemiológica da brucelose bovina no Estado do Tocantins. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 126-134, 2009. Suplemento 1.

OLIVEIRA, L. F.; DORNELES, E. M. S.; MOTA, A. L. A. A.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S.; FERREIRA, F.; DIAS, R. A.; TELLES, E. O.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; AMAKU, M.; LAGE, A. P. Seroprevalence and risk factors for bovine brucellosis in the State of Minas Gerais, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3449-3446, 2016. Suplemento 2.

PAULIN, L.; FERREIRA NETO, J. *O Combate à brucelose bovina*: situação brasileira. Jaboticabal: Fundação de Estudos e pesquisas em Agronomia, Medicina Veterinária e Zootecnia, 2003. 154 p.

POESTER, F. P.; GONÇALVES, V. S. P.; LAGE, A. P. Brucellosis in Brazil. *Veterinary Microbiology*, Amsterdam, v. 90, n. 1-4, p. 55-62, 2002.

ROCHA, W. V.; GONÇALVES, V. S. P.; COELHO, C. G. N. F. L.; BRITO, W. M. E. D.; DIAS, R. A.; DELPHINO, M. K. V. C.; FERREIRA, F.; AMAKU, M.; FERREIRA NETO, J. S.; FIGUEIREDO, V. C. F.; LÔBO, J. R.; BRITO, L. A. B. Situação epidemiológica da brucelose bovina no Estado de Goiás. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 27-34, 2009. Suplemento 1.

SALMAN, M. D.; MEYER, M. E. Epidemiology of bovine brucellosis in the Mexicali Valley, Mexico: literature review of disease-associated factors. *American Journal of Veterinary Research*, Chicago, v. 45, n. 8, p. 1557-1560, 1984.

SIKUSAWA, S.; AMAKU, M.; DIAS, R. A.; FERREIRA NETO, J. S.; MARTINS, C.; GONÇALVES, V. S. P.; FIGUEIREDO, V. C. F.; LÔBO, J. R.; FERREIRA, F. Situação epidemiológica da brucelose bovina no estado de Santa Catarina. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 103-108, 2009. Suplemento 1.

SILVA, N. S.; GROFF, A. C. M.; VIDOR, A. C. M.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; DIAS, R. A.; TELLES, E. O.; GONÇALVES, V. S. P.; AMAKU, M.; FERREIRA, F.; FERREIRA NETO, J. S. Epidemiological situation of brucellosis after implementation of the vaccination program in Rio Grande do Sul State, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3519-3530, 2016. Suplemento 2.

SILVA, V. G. S. O.; DIAS, R. A.; FERREIRA, F.; AMAKU, M.; COSTA, E. L. S.; LOBO, J. R.; FIGUEIREDO, V. C. F.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado de Sergipe. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 109-117, 2009. Suplemento 1.

SOUZA, V. A. F.; FERREIRA NETO, J. S.; AMAKU, M.; DIAS, R. A.; TELLES, E. O.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; FERREIRA, F. Mathematical modeling of bovine brucellosis control using the RB51 vaccine. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 5, p. 3767-3776, 2016. Suplemento 2.

THRUSFIELD, M. Veterinary epidemiology. 3th ed. Oxford: Blackwell Science, 2007. 610 p.

VAN WAVERN, G. M. The control of brucellosis in the Netherlands. *Veterinary Record*, London, v. 72, p. 928, 1960.

VILLAR, K. S.; AMAKU, M.; DIAS, R. A.; FERREIRA NETO, J. S.; BENITEZ, F.; GONÇALVES, V. S. P.; FIGUEIREDO, V. C. F.; LOBO, J. R.; FERREIRA, F. Situação epidemiológica da brucelose bovina no Estado de Rondônia. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, p. 85-92, 2009. Suplemento 1.

WORLD ORGANIZATION FOR ANIMAL HEALTH – OIE. World animal health information database interface. Paris: OIE, 2015. Available at: <http://www.oie.int/wahis/public.php?page=home>. Accessed at: 5 aug. 2015.