ORIGINAL ARTICLE

PREVALENCE AND RISK FACTORS FOR BRUCELLOSIS IN TOCANTINS AND BRAZILIAN NATIONAL PROGRAM TO FIGHT THIS DISEASE

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ABSTRACT

In 2010 1,818 sera obtained from cows with 24 or more months of age were analyzed. These animals were distributed over 102 herds in 16 of the 17 Araguaína micro-region municipalities, in the State of Tocantins. Immunoglobulins against *Brucella abortus* were investigated. The farmers or farm holders responded a questionnaire on risk factors for brucellosis. Positive sera to the Rose Bengal Test (RBT) were examined by 2-Mercaptoethanol test (2-ME). The prevalence in herds and animals was 43.5% (42.3 to 44.8%) and 6.2% (6.1 to 6.2%), respectively. Except for a small region in the North of the studied area, the herd prevalence observed in this study was higher (p <0.05) than that found seven years before in all other regions of the State. There was no difference in the prevalence of disease in vaccinated and unvaccinated cows (OR=1.52; [0.73 to 3.54]). Efforts to eliminate the sources of infection from herds and health management practices are needed. More consistent standards for performing and monitoring vaccination and control of animal movement are also needed. State programs to fight brucellosis, respecting regional differences, should be encouraged.

KEY WORDS: Brucellosis. Control. Vaccination. Cattle. Zoonosis.

RESUMO

Prevalência e Fatores de Risco para Brucelose no Tocantins e o Programa Nacional Brasileiro de combate à doença.

Em 2010 foram analisados 1.818 soros de vacas com 24 ou mais meses de idade. Estes animais estavam distribuídos por 102 rebanhos em 16 dos 17 municípios da microrregião de Araguaína, Estado do Tocantins. Foram pesquisadas imunoglobulinas contra *Brucella abortus*. Os donos ou responsáveis pelas propriedades responderam a um questionário sobre fatores de risco para a brucelose. Os soros positivos no teste do antígeno acidificado e tamponado (AAT) foram submetidos

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à prova do 2-Mercaptoetanol (2-ME). As prevalências de rebanho e de animal foram de 43,5% (42,3-44,8%) e de 6,2% (6,1-6,2%), respectivamente. Excetuando uma pequena região, ao Norte da área estudada, a prevalência de rebanho deste trabalho é maior (p<0,05) do que aquela encontrada sete anos antes em todas as outras regiões do Estado. Não se verificou diferença na prevalência da doença em vacas com e sem vacinação (OR=1,52; [0,73 a 3,54]). Fazem-se necessários estímulos para remoção das fontes de infecção dos rebanhos e para boas práticas de manejo sanitário. São também necessárias normas mais consistentes para realização e monitoramento da vacinação e para controle do trânsito animal. Programas estaduais próprios, para combate à brucelose, devem ser incentivados em respeito às diferenças regionais.

DESCRITORES: Brucelose. Bovino. Controle. Vacinação. Zoonose.

INTRODUCTION

Cattle raising is the main economic activity of the State of Tocantins. The sanitary quality of the products of this sector is extremely important for its growth and for meeting the demands of consumer markets. Brucellosis is an important zoonosis, and compromises the quality of meat, milk and dairy products (Poester et al., 2002). Economic losses associated with this disease result from the condemnation of animals, abortions, stillbirths, weak calves, increased calving interval, decreased milk production, weight loss, infertility, loss of high genetic value animals, and disposal of carcasses (Lage et al., 2008).

Among the eight micro-regions of the State of Tocantins, Araguaína is the most important in livestock production, occupies an area of 26,493.499 km² at the Northern of the State, and the population, according to the last census, is 150,484 inhabitants (IBGE, 2010).

According to data from the Agency of Agricultural Protection of Tocantins State (Adapec), in May 2010, the micro-region of Araguaína had 416,035 cows aged 24 months or more. These cows were distributed in 6,477 properties located in 17 municipalities. Of these properties, 4,564 (70.46%) had 10 to 6,000 cows, with an average of 94 cows per property.

In Brazil, bovine brucellosis has a herd prevalence ranging from 0.3% to 41.5% (Chate et al. 2009; Negreiros et al., 2009, Ogata et al., 2009, Rocha et al. 2009; Sikusawa et al., 2009).

The implementation of the Brazilian National Program of Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) in 2001, created the need for systematic quantification of the disease and its determinants in populations (Brasil, 2006). However, the achievement of the objectives envisaged in the National Program requires the adequacy of measures and procedures at States' own programs. This requirement results from the socio-economic diversity of a continental country like Brazil. One objective of this study was to determine the herd and animal prevalence of brucellosis in cows aged 24 months or more from the micro-region of Araguaína. Another aim of this study was to identify and quantify risk factors for the disease and to provide subsidies for better deployment and

management of the PNCEBT, by proposing adequate measures grounded in the reality of Tocantins State or other regions with similar characteristics.

MATERIAL AND METHODS

Using the computer program WinPepi (Abramson, 2004), and considering the following parameters: confidence level of 95%; acceptable difference of 10%; prevalence herd of 21% (Ogata et al., 2009), and 4,564 properties with 10 or more cows aged 24 months, a sample of 90 properties was defined. However, as this study is part of a larger project, the sample was adjusted for the entire project. Thus, a sample of 102 properties was determined. The allocation of these properties was done by systematic sampling. To this end, a list of farms, ordered by total number of cows (low to high) and by municipality, was used. The information for this list was provided by the Tocantins Agency of Agricultural Defense (Adapec) in May 2010.

When any of the owners of the farms in the sample would not participate in the survey or when there was any other impediment, the property was replaced by another nearby with similar characteristics.

The number of cows to be allocated randomly (simple random or systematic depending on the number of animals and other conditions of the herds) was determined using the Computer Program Herdacc 3.0. For serological diagnosis of brucellosis, considering 100% specificity and more than 86% herd sensitivity, the number of animals to allocate randomly in herds was established as 20 cows. The following parameters were considered: cut-point of 1 and an expected prevalence of 8.5% (Ogata et al. 2009); sensitivity of 96% and specificity of 97.7% for the Rose Bengal Test (RBT) and sensitivity of 99.8% and specificity of 100% for the 2-mercaptoethanol test (2-ME) (Samartino et al.,2010); sensitivity of 95.8% and specificity of 100% for serial application of the tests RBT and 2-ME. The specificity and sensitivity of serial diagnosis were adjusted according to Dohoo et al. (2003).

The Field veterinary service of ADAPEC collected 1,818 blood samples from October 2010 to March 2011. These samples were collected from cows with 24 or more months of age, belonging to 102 herds distributed over 16 of the 17 municipalities of the micro-region studied. The sera were taken under refrigeration to the Laboratory of Hygiene and Public Health, at Federal University of Tocantins (UFT), where they were kept at -20° C until laboratorial examination. Sera were examined by the Rose Bengal Test (RBT) followed by application of 2-Mercaptoethanol (2-ME) (Brasil, 2006). The survey of factors related to the occurrence, maintenance and spread of brucellosis in cattle was done through a questionnaire, responded by owners or managers of properties sampled.

Prevalence rates of herds and animals positive for brucellosis in Araguaína micro-region were weighted as described by Dohoo et al. (2003). The weight of each property to estimate the prevalence of outbreaks in the micro-region was given by P1 = properties in the micro-region/properties sampled in the micro-region.

The weight of each animal to calculate the prevalence of cows with 24 months of age or older and reagents in the micro-region, was given by the expression below, where the first term refers to the weight of each animal to calculate the prevalence of positive cows in the municipalities allocated in the sample. P2=(cows on the property/cows sampled on the property).(cows in micro-region/cows sampled in the properties in the micro-region).

Two databases into EpiInfo version 3.5.1 were created: one for the variables of an epidemiological survey and other for recording the results of diagnostic tests. This same program was used for the extraction of frequencies. With the program WinPepi (Abramson, 2004) comparisons were made and the 95% confidence intervals were determined. Thematic maps were generated by the computer program TerraView 3.3.1.

RESULTS

The weighted prevalence of positive cows to serological diagnosis of brucellosis in Araguaína micro-region by confirmatory test (2-ME) was 6.2% [6.09 to 6.24%] and frequencies weighted by county, ranging from zero to 34.99% (Table 1). Of the 102 herds surveyed, 44 (43.1%) [33.37 to 53.32%] had at least one cow reagent in RBT and 2-ME, respectively, in serial use. The weighted prevalence of outbreaks in the micro-region was 43.5% (42.33 to 44.77%).

Table 1. Prevalence of cows in Araguaína micro-region, State of Tocantins, reagents for serological diagnosis of brucellosis by Rose Bengal Test (RBT) and by confirmatory test of 2-Mercaptoethanol (2-ME) - Brazil, 2011

Country	Cows	RBT	%	2-ME	%	IC 95%	% adjusted	IC 95%
Aragominas	56	5	8.93	1	1.79	0.05-9.55	1.78	1.48-2.13
Araguaína	223	37	16.59	9	4.04	1.86-7.52	3.63	3.48-3.80
Araguanã	57	16	28.07	5	8.77	2.91-19.30	9.82	9.35-10.30
Arapoema	118	3	2.54	1	0.85	0.02-4.63	2.42	2.25-2.59
Babaçulândia	213	9	4.23	7	3.29	1.33-6.65	1.99	1.82-2.17
Bandeirantes do Tocantins	80	13	16.25	9	11.25	5.28-20.28	19.98	19.62-20.36
Colinas do Tocantins	134	10	7.46	10	7.46	3.64-13.30	6.20	5.97-6.43
Filadélfia	223	36	16.14	11	4.93	2.49-8.65	6.68	6.43-6.94
Muricilândia	20	8	40.00	7	35.00	15.39-59.22	34.99	33.82-36.18
Nova Olinda	176	6	3.41	3	1.70	0.35-4.90	0.91	0.81-1.03
Palmeirante	151	4	2.65	3	1.99	0.41-5.70	1.36	1.22-1.52
Pau D'Arco	57	0	0.00	0	0.00	0.00-6.27	0.00	0.0-0.02
Piraquê	21	1	4.76	1	4.76	0.12-23.82	4.76	4.37-5.17
Santa Fé do Araguaia	96	11	11.46	4	4,17	1.15-10.33	4.87	4.65-5.09
Wanderlândia	107	5	4.67	5	4.67	1.53-10.57	5.76	5.32-6.22
Xambioá	86	4	4.65	1	1.16	0.03-6.31	3.78	3.47-4.11
Total	1,818	168	9.24	77	4.24	3.36-5.27	6.17	6.09-6.24

Large part of the properties (39.21%) had all their cows included in the sample and 50.0% to 95.23% of the cows of 22.5% of the herds investigated were also studied. Thus, in 61.7% of the herds sampled, 50.0% or more of their cows were analyzed. The seroprevalence of brucellosis in the properties sampled is presented in Figure 1.
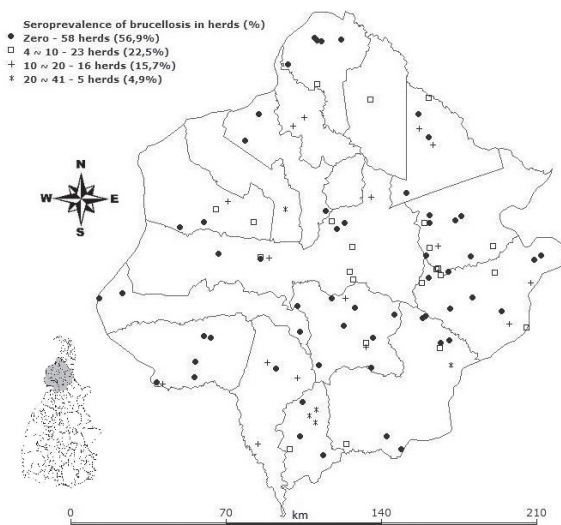

Figure 1. Seroprevalence of brucellosis in herds of cows with 24 or more months of age, in Araguaína micro-region, State of Tocantins - Brazil, 2011

The environmental management of livestock in the micro-region of Araguaína is shown in Table 2.

Table 2. Responses of owners or managers in the epidemiological survey about brucellosis in the micro-region of Araguaína, State of Tocantins - Brazil, 2011

Variable	N° of. responses	Yes	%	CI 95%
Acquisition of cows or bulls from other farms	100	91	91.00	83.6 a 95.8
Health certificate requirement	99	21	21.2	13.6 a 30.6
Quarantine	87	9	10.3	4.8 a 18.7
Picket maternity	99	40	40.4	30.7 a 50.7
Burial or burning of abortion products	96	25	26.0	17.6 a 36.0
Artificial insemination	100	5	5.0	1.6 a 11.2
Use of veterinary services	100	14	14.0	7.9 a 22.4

The seropositivity for brucellosis in vaccinated and unvaccinated cows is presented in Tables 3 and 4.

Table 3   Unweighted prevalence of positive cows to serodiagnosis of brucellosis by Rose Bengal Test (RBT) and by 2-mercaptoethanol (2-ME) confirmatory test according to their vaccination status, in the micro-region of Araguaína, State of Tocantins - Brazil, 2011

Vaccination status	No. % of cows	%	Animals reagents					
			AAT	%	IC 95%	2-ME	%	IC 95 %
Vaccinated	1,145	62.98	125	10.92	9.21-12.82	53	4.63	3.52-5.97
Not vaccinated	290	15.95	14	4.83	2.77-7.78	9	3.1	1.52-5.62
Unknown	383	21.06	29	7.57	5.23-10.56	15	3.92	2.29-6.24
Total	1,818	100.00	168	9.24	7.97-10.64	77	4.72	3.36-5.27

Table 4.   Comparison of the prevalence of positive cows to the brucellosis by the confirmatory test of 2-mercaptoethanol, according to history of vaccination, in the micro-region of Araguaína, State of Tocantins - Brazil, 2011

Vaccination status declared	Odds ratio	CI 95%
Vaccinated x Not vaccinated	1.52	0.73 a 3.54
Vaccinated x Unknown	1.19	0.65 a 2.30
Unknown x Not vaccinated	1.27	0.51 a 3.35

Forty-four percent of herds had less than 60% of their cows vaccinated, and 9.8% of properties had no vaccinated cows (Figure 2).
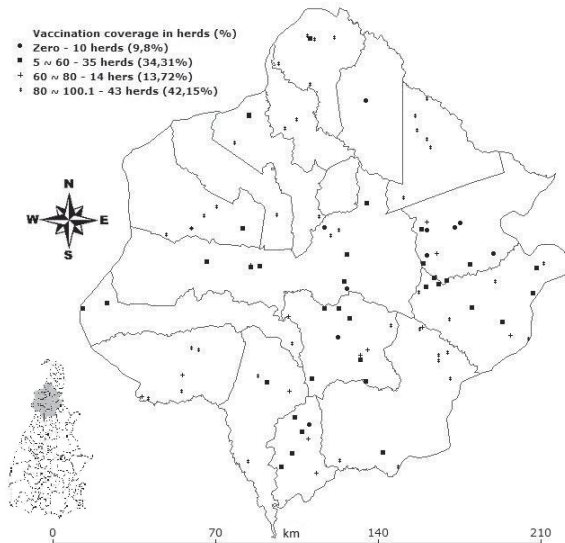

Figure 2.   Brucellosis vaccination coverage in herds of cows with 24 or more months of age, in Araguaína micro-region, State of Tocantins - Brazil, 2011

DISCUSSION AND CONCLUSIONS

The weighted animal prevalence of 6.2% [6.09 to 6.24%] found in this work is greater than that found by Ogata et al. (2009) for the State of Tocantins, which was 4.4% [3.6 to 5.3%], but within the range of variation of 2.0 to 8.5% found by those authors in the territorial divisions of the State, named by them as economic circuits.

The adjusted prevalence of animal brucellosis in this study is higher than that found for the North region of Brazil in 1977 (4.1%) and for this country in the period from 1989 to 1998 that was 4% (Poester et al., 2002).

Except for a small region in the Northern area of this study, the weighted herd prevalence of 43.5% in the micro-region [42.33 to 44.77%] is higher (p <0.05) than those found in all other regions of the State, in a survey done by Ogata et al. (2009). Although the consideration of different territorial divisions can harm comparisons between the two studies, it can be thought about a possible spread of outbreaks in the micro-region of Araguaína in the seven years that separate the two surveys.

Further diagnostic facilities and lack of voluntary adherence to the National Program of Control and Eradication of Brucellosis and Tuberculosis (PNCEBT), established in the country in 2001, may explain these findings. This adhesion is fundamental to reducing the herd and animal prevalence of brucellosis, to facilitate the removal of sources of infection and hence reduce the introduction of these infected animals into herds free from infection.

Presented results show how much the gross regional prevalence may mask the true magnitude of the problem in livestock. In this case, the prevalence of infected animals in the studied region was 6.2% [6.09 to 6.24%], however in 20.6% of the herds the prevalence varied between 10 and 41%.

Unfortunately, the data and comparisons made here do not allow us to celebrate advances in fighting the disease, despite efforts by authorities and the meat and milk industry.

For some Southern regions of the country, the reduction of prevalence to levels consistent with the eradication is referred. The success achieved in these regions and throughout the State of Santa Catarina can be attributed to a relatively low initial prevalence, small farms with fewer animals, a small movement of animals between properties and, in some cases, elimination of sources of infection (Dias et al ., 2009; Marvulo et al., 2009; Sikusawa et al., 2009).

Simple transplantation of the successful experiences of some regions in Brazil should be done cautiously, not only due to the vastness of its territory, but also by the great economic and cultural diversity in the country. Thus, the herd and animal prevalence of this study suggest the need for evaluation of the National Program not only nationally, but particularly, in States, macro and micro-regions, municipalities, and finally in properties in order to have a more realistic and useful

system constructed. The large range in the frequency of the disease in cattle shows the fragility of the average (crude prevalence) in the exposure of the severity of infection and its real potential spread (new outbreaks). The concern should not only focus on increasing the immunological capability of animals to the challenges, but also on those factors that may contribute to the survival and spread of the specific agent of the disease. In this respect, the interest of the farmers in the herd sanitation is crucial. This interest should include not only the elimination of infected animals, but also other measures of health management with emphasis on reproduction, veterinary support and animal movement. In places like the one of this study, which does not meet the sanitary control of these factors, there is a positive association between this deficiency and the increase of the disease prevalence (Chate et al., 2009, Dias et al., 2009, Gonçalves et al., 2009; Nicoletti, 1980).

With regard to animal movement, the measure of the national program, that requires the presentation of negative test for brucellosis only for interstate transit of animals for breeding, must be modified. The movement of animals is a risk factor for brucellosis that does not depend on territorial boundaries. On the other hand, the allocation set by the producers may change. By current regulations the cattle can be moved for fattening without tests for brucellosis, however they can be used later for breeding. Thus, the requirement of a negative test should involve the movement of all cattle fit to reproduction, unless the animals are sent to slaughter.

Data from this study also point to the need for an education system for animal health and inspection, based on valid criteria, in order to be able to pass on to cattle ranchers, industry and population an image of efficiency and reliability.

We believe that the environmental management of livestock in the micro-region of Araguaína can be found in others regions of the country and that it cannot contribute positively to the effective control of brucellosis. The group protection afforded by vaccination may be compromised, considering the possibility of the disease agent to be introduced and remain in the herd.

At the confidence level of 95%, the prevalence of seropositivity for brucellosis was the same in cows with and without history of vaccination ($p < 0.05$). Thus, according to the vaccination status of the cows, declared by their owners or managers, we cannot say that vaccination in the micro-region of Araguaína is a protecting factor against brucellosis. This finding differs from that found by Ogata et al. (2009). Knowing that the vaccine strain B19 was used in countries that have eradicated brucellosis, the difference in programs to combat the disease must be sought in vaccination as a process that involves multiple factors. The credit given to vaccination by producers is the first of these factors. This credit includes the recognition of the importance of vaccination as part of an appropriate health management, with emphasis on introduction of new animals in the properties, careful identification of vaccinated and infected and removal of infected animals from the herds. In parallel, a control based on consistent rules is essential. Vaccination coverage in the micro-region of Araguaína, shown in Figure 2, mirrors what has been said before.

The protection afforded by *Brucella abortus* strain 19 of about 70% (Brasil, 2006), a vaccination without consistent criteria for monitoring and lack of incentives to producers, may explain the persistence and even increase in the prevalence of brucellosis found in this work. The lack of material incentives for voluntary compliance of the creators is a problem of implementation of measures in the Programme throughout the country (Brasil, 2006). Godfroid et al. (2005) acknowledge that the programs of control and eventually eradication of brucellosis with vaccination should include the test and slaughter, and even in the later stages, the depopulation of the outbreaks. As vaccination with *Brucella abortus* strain 19 is a compulsory measure in the Brazilian program to fight brucellosis. Remember that the Brazilian Program reserves the use of RB51 vaccine for special situations such as lack of immunity in cattle. Thus, the poor vaccination coverage and lack of difference between the vaccinated and unvaccinated cows in brucellosis seroprevalence (Table 4), shows the need for evaluation of control measures recommended by PNCEBT. Own State programs to fight animal diseases should be encouraged in respect to regional differences. Should continue to be the concern of the Federation to strengthen local structures, calling them not only to participate in data collection but also in the analysis of its animal health problems, including the definition of own health programs, in line with national guidelines.

The micro-region studied is the most important in cattle raising in the State of Tocantins. However, it is necessary that this study be improved and extended to other micro-regions of the State.

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