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## A random sample survey of bovine Brucellosis in the State of Paraíba, Brazil

### Prevalência de Brucelose bovina no Estado da Paraíba

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#### Abstract

A serosurvey was conducted to estimate the prevalence of bovine brucellosis in the State of Paraíba, Brazil. A random sample of six municipalities was selected from each of three ecoregions of the State: the Agreste/Litoral region (1), the Cariri/Curimatau region (2) and the Sertão region (3). These 18 municipalities were divided in quadrants and one herd was selected at random from each quadrant. The sampling scheme included herds with more than 50 bovines, which excludes small-holder subsistence livestock production. At least 32 animals were sampled within each herd, stratified in four age categories, as follows: (1) at least 8 animals under 6 months of age, (2) at least 8 animals of 7 to 18 months of age, (3) at least 8 animals of 19 to 30 months of age and (4) at least 8 animals over 30 months of age. Antibodies to *Brucella abortus* were observed in 8 out of 18 municipalities (44.44%), in 8 out of 72 herds included in this study (11.11%) and in 8 animals out of 2343 samples collected (0.34%). The prevalence of test positive cattle by ecoregion/cattle production regions in the state showed no significant differences of risk among them. Likewise, there was no significant difference of risk among the different age categories studied. It was observed that brucellosis is widely spread across the state albeit with low animal prevalence. These results may be used in the design of a strategy for controlling bovine brucellosis in the State of Paraíba.

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#### Introduction

Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Animal Health Organisation (OIE) as one of the most important and widespread zoonosis in the world. It is an important cause of abortion in several species of domestic animals, causing

sometimes temporary or permanent infertility and great economical losses.<sup>1</sup> Bovine brucellosis due to *Brucella abortus* is endemic and the most prevalent *Brucella* infection in Brazil.<sup>2,3</sup>

The Brazilian State of Paraíba is the most eastern point of the Americas. Paraíba has a population of 3,305,616 inhabitants, covers an area of 56,584.6 km<sup>2</sup>, contains 223 municipalities and is set in a tropical climate.

Cattle production is one of the major agricultural activities in the state and stands out as one of the most important enterprises in large farms, with an estimated total herd size of 1,327,826 animals.<sup>4</sup> Cattle production is mostly extensive, on semi arid soils, with poor vegetation and low rainfall. According to the Committee for Agricultural Planning of the State of Paraíba, there are three types of cattle production regions in the State, with regard to the ecological traits: the Agreste/Litoral region, the Cariri/Curimatau region and the Sertão region.

The objective of the present study was to estimate the prevalence of bovine brucellosis in Paraíba and gain insights into the association of age categories and ecological regions with the presence of the disease.

## Materials and Methods

### Herds

A sample frame was developed containing a list of potential participants from both dairy and beef herds. To be eligible for inclusion in the study the herd would have to comprise more than 50 bovines. Such restriction tends to exclude small-holder subsistence livestock production and, therefore, targets the survey at commercially orientated livestock owners. These livestock farms had information on health status, reproduction and management, and farm managers agreed to participate. A random sample of six municipalities was selected from each of three ecoregions: the Agreste/Litoral region (1), the Cariri/Curimatau region (2) and the Sertão region (3) (Figure 1). Six municipalities were randomly selected from each ecoregion: Alagoinha, Bananeiras, Fagundes, João Pessoa, Tacima and Umbuzeiro from ecoregion 1; Boqueirão, Campina Grande, Gurjão, Monteiro, Serra Branca and Soledade from ecoregion 2; Conceição, Patos, Piancó, Pombal, Riacho dos Cavalos and Souza from ecoregion 3. These 18 municipalities were divided in quadrants and one herd was selected at random from each quadrant.

### Animals

Blood samples were randomly collected from at least eight animals of the following age categories within each herd, totaling at least 32 animals per herd: The age categories were (1) 0 to 6 months of age, (2) 7 to 18 months of age, (3) 19 to 30 months of age and (4) more than 30 months of age. Samples were collected between March and September 2000. Sera were then stored at minus 20°C until tested.

### Sampling

In order to determine the spatial distribution of bovine brucellosis in Paraíba, where there is incomplete information on the demographics of the cattle population, multilevel modeling was used. In this model, the most precise estimate of prevalence can be achieved if the number of sample units, at each level, is chosen to be proportional to the standard deviations (SD) at each of the levels.<sup>5</sup> Thirty-two animals were chosen per herd; four herds per municipality; six municipalities per region and three regions. The most precise estimate would be reached if SD of individuals (within property) / SD of property (within municipality) / SD municipality (within region) / SD region was on the same 32:4:6:3 ratio. These variance estimates were not available but the blood samples were also tested against IBR and BVD and for these diseases strong herd effects and weak (but variable) municipality and region effects were expected.

### Testing

The serological diagnosis of brucellosis was carried out using the Rose Bengal Plate Agglutination Test (RBPAT) for screening purposes. The test positive sera were then submitted to the 2-mercaptoethanol tube agglutination test (2ME), used as confirmatory test.<sup>6,7</sup> Negative and positive sera were run in each assay as controls. The interpretation of the following the Standard Norms set by the Federal Animal Health Authority (Regulamento Técnico do Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal).<sup>8</sup>

**Statistics**

To estimate the overall prevalence of brucellosis, the disease frequency was calculated for each age group. Standard errors were adjusted for over-dispersion, by farm, using an over-dispersion factor calculated by the deviance divided by the degrees of freedom for deviance. The age groups were compared for prevalence using within herd comparisons estimated by a Generalized Estimating Equations (GEE) approach.

**Results**

Antibodies to *B. abortus* were observed in 8 out of 18 municipalities (44.44%) and also in 8 out of 72 farms included in this study (11.11%). Out of 2343 samples included in the survey, 15 (0.64%) tested positive for brucellosis in the RBPAT of which 8 (0.34%) were further confirmed by the 2ME. Of the test positive samples to the RBPAT, three were discarded because they were from vaccinated animals aged under 24 months<sup>8</sup> reducing the number of test positive animals from 11 to 8 (0.34%) (Table 1). The titers obtained in the

2ME test are showed in table 1.

The results for brucellosis by cattle production regions revealed that there are no significant differences of risk among them. In spite of the low prevalence found, all three ecoregions presented test positive results, showing that brucellosis is widespread throughout Paraíba (Table 1).

The age category results for brucellosis also showed no significant difference of risk among the different categories studied (p=0.05). Age categories 1 and 2 did not present test positive results at the 2ME (Table 1).

Because the estimated prevalence was very low, higher order variances such as region and municipality could not be calculated.

**Discussion**

Based on probability sampling, there was an attempt to estimate the spatial and age risks for bovine brucellosis in the State of Paraíba but because of the low prevalence found this was not possible. It was observed that brucellosis is widely spread throughout Paraíba albeit with low prevalence.

**Table 1**

Frequency of seropositive animals in the Rose Bengal Plate Agglutination Test and 2-Mercaptoethanol Tube Agglutination Test for Brucellosis in cattle from the State of Paraíba, Brazil

Region <sup>1</sup>	Animal	Municipality	Herd <sup>2</sup>	Age <sup>3</sup>	Vaccinated <sup>4</sup>	Tube <sup>5,6</sup>	2 ME <sup>5,7</sup>	Status	
Region 1	1122*	1	1	2	Y	25	25	-	
	1134*	1	1	3	Y	100	50	-	
	1135*	1	1	3	Y	100	50	-	
	1246	1	2	4	N	50	50	+	
	9146	9	1	4	N	100	100	+	
	18327	18	3	2	N	25	0	-	
	18433	18	4	3	N	0	0	-	
	19346	19	3	4	N	100	50	+	
	Sub-Total								3
	4149(M)	4	1	4	N	50	50	+	
Region 2	5236	5	2	3	N	50	50	+	
	8215	8	2	1	N	0	0	-	
	10241	10	2	4	N	50	50	+	
	16146	16	1	4	N	25	25	+	
	Sub-Total								4
	6247	6	2	4	N	0	0	-	
Region 3	17143	17	1	4	N	200	200	+	
	Sub-Total								1
Total								8	

<sup>1</sup>Region 1 – Agreste/Litoral; Region 2 – Cariri/Curimatau; Region 3 – Sertão;

<sup>2</sup>There were 4 herds sampled from each municipality;

<sup>3</sup>Age categories: 1) 0 - 6 months; 2) 7 - 18 months; 3) 19 - 30 months; 4) more than 30 months old;

<sup>4</sup>Vaccinated animals (Y = yes, N = no).

<sup>5</sup>Titer

<sup>6</sup>Tube – Tube agglutination test done in conjunction with the 2ME test;

<sup>7</sup>2ME - 2-mercaptoethanol tube agglutination test;

<sup>8</sup>vaccinated animals with less than 24 months of age not considered for computing the number of *Brucella*-positive animals (4).

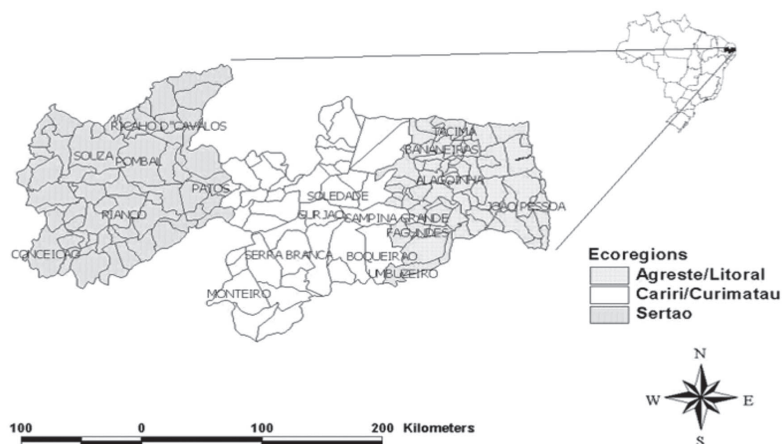


Figure 1  
Ecoregions and Municipalities sampled for the study in the state of Paraíba, Brazil.

Previous studies revealed that bovine brucellosis was widespread all over the country.<sup>2,3</sup> In 1975, a national survey showed the following animal prevalence for each region: north – 4.1%, northeast – 2.5%, midwest – 6.8%, southeast – 7.5% and south – 4.0%.<sup>9</sup> Official reporting and more recent survey results show a fairly stable endemic situation and a higher prevalence of the disease in the regions with higher cattle density.

In this survey, 544 herds were sampled in Paraíba, 15 being positive (2.7%). Moreover, 36 (6%) of the herds sampled were vaccinated. The present survey showed that out of the 72 herds sampled 8 were positive (11.11%), each positive animal belonged to a different herd, so from the 2343 samples tested only 8 were positive (0.34%) and of the 72 herds tested only 5 were vaccinated (7.24%). Comparing the results of the 1975 survey with the present survey, the number of vaccinated herds increased from 6.0% to 7.24% and the prevalence of positive herds increased from 2.7% to 11.11%. This might be due to changes in the cattle production since 1975 and also because of the sample strategy used in the present study, which targeted the sample to herds with at least 50 animals, considered large for the state of Paraíba.

The State of Paraíba presented a low prevalence of brucellosis when compared to other surveys carried out in states of different regions. The State of Rio Grande do Sul, in the extreme south of Brazil, presented 0.3% prevalence<sup>10</sup>, as a result of a successful vaccination program. The State of Santa Catarina (south region) presented 0.6%<sup>3</sup>, the State of Mato Grosso do Sul (midwest region) presented 6.3% (1998) and the State of Minas Gerais (southeast region), the largest dairy state in Brazil, presented 6.7%.<sup>11</sup>

The results by age category only showed positive animals aged over 18 months. Animals in the two younger age categories did not present positive results. Such results are consistent with the fact that susceptibility appears to be associated with sexual maturity rather than age and, therefore, sexually immature cattle do not become infected following exposure or recover quite rapidly.<sup>12</sup>

Previous research has already demonstrated the presence of Brucellosis in cattle herds of the State of Paraíba, Brazil. Results obtained in the present study demonstrate that the disease has low animal prevalence, higher herd prevalence, low vaccination coverage and is widely distributed throughout the state. The information presented in this study may help designing a more efficient

control program against in the State of Paraíba.

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## Resumo

Foi realizado um estudo para a determinação da prevalência sorológica de brucelose bovina no Estado da Paraíba. Seis municípios foram selecionados aleatoriamente em cada uma das três ecorregiões do Estado: a região do Agreste/Litoral (1), a região do Cariri/Curimataú (2) e a região do Sertão (3). Estes 18 municípios foram divididos em quadrantes e uma propriedade foi selecionada em cada quadrante. Foram amostradas propriedades com mais de 50 animais, o que exclui as pequenas propriedades de subsistência. Pelo menos 32 animais foram amostrados de cada rebanho, estratificados nas quatro faixas etárias seguintes: (1) no mínimo 8 animais até 6 meses de idade, (2) no mínimo 8 animais com 7 a 18 meses de idade, (3) no mínimo 8 animais com 19 a 30 meses de idade e (4) no mínimo 8 animais com mais de 30 meses de idade. Anticorpos anti-*B. abortus* foram observados em 8 de 18 municípios (44,44%), em 8 de 72 rebanhos (11,11%) e em 8 animais dos 2343 amostrados (0,34%). A análise dos resultados de prevalência por ecorregião do Estado não revelou diferença significativa de risco entre elas. A análise dos resultados por categorias de faixa etária também não evidenciaram diferença significativa de risco entre as mesmas. Foi observado que a brucelose está amplamente disseminada no estado embora com uma baixa prevalência animal. Estes resultados podem ser úteis no delineamento de estratégias de controle da brucelose bovina no estado da Paraíba.

## Palavras-chave:

Brucelose.  
Epidemiologia.  
Bovinos.  
Paraíba.  
Brasil.

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