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Prevalency and Risk Factors Associated with Brucellosis in Cattle in the Municipality of São Francisco do Brejão, Maranhão, Brazil

Margarida Paula Carreira de Sá PRAZERES
Postgraduate, Federal University of Maranhão,
Departament of Health Sciences, São Luiz, MA Brazil

Nancyleni Pinto CHAVES
Lecturer, Federal University of Maranhão,
Departament of Health Sciences, São Luiz, MA Brazil

Alcione Miranda dos SANTOS
Postgraduate, Federal University of Maranhão,
Departament of Health Sciences, São Luiz, MA Brazil

Alcina Vieira de CARVALHO-NETA
Lecturer, Federal University of Maranhão,
Departament of Health Sciences, São Luiz, MA Brazil

Ana Lúcia ABREU-SILVA
State University of Maranhão, Faculty of Veterinary Medicine, Departament of Patology,
Cidade Universitária Paulo VI, Cx. Postal 9, Tirical, São Luiz, MA 65055-70, Brazil.
Tel.: +55 98 3257-3676; Fax: +55 98 3257-3676; Email address: anabreu@uema.br

Abstract – **This objective study was to determine the prevalence of brucellosis-positive cattle and identify the possible associated risk factors in dairy cattle herds. The study design, serum samples and information regarding farm properties were as used within the context of the National Program for Brucellosis and Tuberculosis Control and Eradication, for studying bovine brucellosis in the state of Maranhão. In total 736 animals originating from 96 herds were enrolled in the study. Of the analysed samples 3.94% of animals tested positive for Brucella, the resulting herd prevalence was 30.43%. The variables considered to be risk factors for infection were: number of animal movements, pasture used in common with other farms, abortion residues left in the field, animals over 64 months of age and acquisition of cattle with the purpose of fattening. Brucellosis presents prevalence low and that factors relating to the characteristics of the farm properties and management of the animals are associated with the disease**.

Keywords – **Brucellosis, Cattle, Odds Ratio, Statistical Analysis.**

I. Introduction

Brucellosis is one of the most important and widespread zoonoses in the world, according to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) [1, 2, 3, 4].

The etiological agent for brucellosis is a facultative intracellular bacterium belonging to the genus *Brucella* [5]. This genus is composed of nine species (*Brucella abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis*, *B. canis*, *B. pinnipediae*, *B. cetaceae* and *B. microti*) [6]. *Brucella abortus* is a smooth and highly pathogenic species that causes severe disease, especially among cattle [7]. This species also presents clinical-epidemiological importance and is considered pathogenic to humans [8].

Bovine brucellosis causes a wide variety of losses. Among cattle herds, there is decreased milk and beef productivity, devaluation of the market price for animals and products of animal origin from infected regions, an increased interval between births, occurrence of abortions among diseased females, sterility and the recommendation that reactive animals should be sacrificed, with consequent expenditure on purchasing replacement animals for the herd [9].

Bovine brucellosis is distributed worldwide, but is especially concentrated in underdeveloped countries. This disease was eradicated in several countries in the northern and central regions of Europe, Canada, Australia, Japan and New Zealand [10], but has become a reemerging issue in many countries, such as Israel, Egypt, Kuwait, Ethiopia, Saudi Arabia, Brazil and Colombia [1, 2, 3]. In Brazil, bovine brucellosis occurs endemically throughout the country. Official data indicate that the prevalence of seropositive animals was between 4 to 5% in the period from 1988 to 1998 [11].

The objective of this study was to estimate the prevalence of *Brucella abortus* in dairy cattle and to identify possible risk factors for infection at herd level.

II. Material and Methods

A. Study Area

This study was carried out in the municipality of São Francisco do Brejão, located in the state of Maranhão in a region near the Tocantins River, at the following geographical coordinates: 47.38° latitude south and 5.12° longitude west, with a territorial area of 745.59 km^2. The study area was selected based on the existence of livestock-rearing consisting predominantly of dairy cattle, with significant milk production. This municipality has a total herd size of 74,742 animals distributed in 441 dairy farms, with a production of 50,000 L of milk/day [12].

B. Animals

The study population consisted of dairy cattle, of both sexes, with a minimum age of 8 months for non-immunized males and vaccinated females over 24 months of age. Castrated male cattle and females at the puerperium stage (i.e. fifteen days before or fifteen days after birth) were not included in this study, in accordance to the technical regulations of the National Program for

Brucellosis and Tuberculosis Control and Eradication (PNCEBT) [11].

C. Sample Delineation

This cross-sectional study was carried out in accordance with the sampling design that was developed for seroepidemiological studies on bovine brucellosis in the state of Maranhão, within the context of the PNCEBT, using serial serological tests and an epidemiological survey.

The representatives of the study population were selected through simple random sampling. The number of cattle herds was first determined and then the number of animals. The number of herds (primary units) was calculated using the degree of confidence of the result (95%), sampling error (10%) and a mean prevalence among the herds of 30% [11], by means of a formula for simple random samples [13].

The sample planning for the secondary units (animals) sought to estimate the minimum number of animals to be examined, within each farm property, so as to make it possible to classify as foci of brucellosis or as free from the disease. On the farms with cattle herds greater than 100 head, the samples gathered consisted of 15 animals; with herds less than or equal to 100 head, 10 animals; and with herds of up to 10 heads, all the animals, while taking into consideration the exclusion criteria [14]. The prevalence of brucellosis was taken to be of the order of 6% [11], with a sample error of 2% and confidence interval of 95%, thus obtaining a minimum sample size of 560 cattle. However, following the methodology described above, a total of 736 samples, from 69 herds, were evaluated. Out of the total number of samples, 92.8% (n = 683) were female and 7.2% (n = 53) were male.

D. Blood Sample Collection and Epidemiological Data

The fieldwork consisted of blood sample collection and filling out of epidemiological questionnaires. The blood collection was carried out by means of jugular vein puncture, using a disposable sterile needle and a previously identified vacuum tube. The serum samples thus obtained were stored in plastic microtubes and frozen at -20ºC, until the serological tests were performed.

All herds were visited and information on potential risk and general information about the farms was obtained using a questionnaire. The questionnaire was administered to farmers or managers on all farms by personal interview. All questions were related to variables derived from a literature review of potential risk factors for brucellosis in ruminants.

The questionnaire included 61 questions. To keep it simple, the most questions 45 (70.4%) were closed (answers limited to ‘‘yes’’, ‘‘no’’ and ‘‘sometimes” ‘‘maybe’’) and 16 questions were semi-closed (including information on number of animals and locations). There wasn’t open questions. All such questions were pre-coded.

Information collected in the survey was classified into four sections: variables concerning herds characteristics, such as size of herd, breed, type of production (milk or meat), infrastructure (type of buildings, presence of piped water), location (proximity to other farms), premises cleaning (manure removal and disinfection frequency); farming and management such as animals introduction (females, males and from non-free or unknown status), serologic tests and quarantine, attitude with positive animals; farmers knowledge about brucellosis variables concerning the farmer, such as age, educational level, major occupation.

E. Serological Tests

The buffered acidified antigen (BAA) test was carried out as a screening test to detect anti-*Brucella* antibodies, using the antigen produced by the Technology Institute of Paraná (TECPAR).

The samples that reacted to the BAA were then subjected simultaneously to the 2-mercaptoethanol (2-ME) test (MERCK) and slow serum agglutination in tubes (SAL) test (TECPAR), at dilutions of 1:25, 1:50, 1:100 and 1:200.

The serological analyses were carried out at the Infectious Disease Diagnostic Laboratory of the Veterinary Medicine Course of the State University of Maranhão (UEMA).

F. Data analysis

The information from the questionnaires and the results from the serological tests were stored in a database (Stata 9.0). Farm properties were considered to be positive (foci) for the presence of *Brucella abortus* if they presented at least one reactive animal. Considering that the sample of primary units was random, the prevalence of foci was calculated using the number of foci and number of sampled properties as the parameters [15].

Logistic regression model was used [16] to identify factors associated with occurrences of brucellosis foci among the herds. This model included explanatory variables relating to possible the risk factors associated the occurrence of brucellosis bovine in the herd.

To identify factors relating to occurrences of brucellosis among the animals, multilevel logistic model with complex sampling was used [17]. The model described here consists of two stages. In the first stage, the herd was sampled (primary sampling units (PSU)) and the final stage, the animals within of each herd were sampled (elementary sampling units (ESU)).

At the initial stage, herd j is sampled with probability p_j, j=1, . . . , 69, and, at the subsequent stage, bovine$i$ is sampled with conditional probability $p_{i|j}$, given that herd j was sampled in the first stage. Therefore, the weight $w_{ij}$for bovine i in herd j was obtained by $w_{ij} = w_{1j}. w_{2ij,}$ wherew_{1j}=1/ p_j is the herd weight, given as the reciprocal of the probability of inclusion of herd j in the sample and w_{2ij} =1/$p_{i|j}$ is the within-herd base weight, given as the reciprocal of the probability of selection of bovine i from within the selected herd j.

We let y_{ij} be the occurrence of brucellosis in the bovine$i$ from the herd j. Specifically, this is defined as follows

$$\log \frac{\pi_{ij}}{1-\pi_i} = \beta_0 + \beta_{ij}x_{ij} + \ldots + \beta_p x_p$$

The multilevel logistic model assume that the probability π_{ij} of the bovine i from the j herd to be soropositive for brucellosis is related to the observed independent variables by thefollowing structural equation:

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$$log\left\{\frac{\pi_{ij}}{1-\pi_i}\right\} = x'_{ij}B+u_j+E_{ij}$$

where Xij is a vector of all the covariates (including health and the rural livestock-rearing characteristics), B is the parameter vector,$u_j$are multivariable normal random effects varying over herdsand E_{ij} the error term. The *gllamm* command of the STATA 9.0 *software* was used.

In both models considered in this study, backward stepwise method was used to assess multivariable effects risk factors, after first removing all those variables not univariately significant at 20% level. Independent variables that presented a p-value <0.05 in the multivariable analysis were kept in this model. Hosmer and Lemeshow goodness-of-fit test for the final selected model was calculated.

In addition, odds ratios (OR) were estimated, corresponding 95% CI and to check for potential multicolinearity, the *variance inflation factor* (VIF) was calculated.

III. Results

A. Seroprevalence of Bovine Brucellosis

Out of the 736 bovine samples examined, 34 (4.61%) were reactive in the screening test (BAA). Of these, 29 (3.94%) were confirmed by means of the SAL and 2-ME tests, all of which with 1:200 titration. Thus, the seroprevalence of brucellosis among cattle in the municipality of São Francisco do Brejão was 3.94% (95% CI: 2.65% - 5.61%).

Among the 69 farms evaluated, 21 were positive, thus showing that 30.43% (95% CI: 19.30% - 41.57%) of the herds were foci for the disease.

B. Univariate and Multivariable Logistic Regression for Brucellosis Foci

The univariate analysis on the risk factors associated with occurrences of brucellosis foci among the herds in the municipality of São Francisco do Brejão are detailed in Table I.

Table 1: Univariate logistic regression model for the risk factors most associated with occurrences of bovine brucellosis foci on the farm properties sampled in São Francisco do Brejão, Maranhão.

Variable	Herd/farm				
	Diseased/ exposed	%	OR	CI	P-value
Size of the cattle herd					
Up to 611 head	15/59	25.42			
611 to 1222	3/5	60	4.44	0.66 - 28.91	0.123
>1222	3/5	60	4.44	0.66 - 28.91	0.123
MILk production/day					
≤ 150 L	11/45	24.44	2.20	0.76 - 6.36	0.143
>150 L	10/24	41.66			
Presence of sheep					
Yes	18/51	35.29	0.36	0.09 - 1.43	0.150
No	3/18	16.66			
PIG herds					
Up to 14 head	19/54	35.18	0.28	0.05 - 1.39	0.120
>14 head	2/15	13.33			
Abortion residues left in the fields					
Yes	3/21	14.28	3.60	0.92 - 13.95	0.064
No	18/48	37.5			
ANImals purchased from breeders in other regions					
Yes	16/62	25.80	7.18	1.26 - 40.77	0.026
No	5/7	71.42			
Animal replacement according to purpose					
Does not replace	6/23	26.08			
Reproduction	8/32	25	0.94	0.28 - 3.22	0.927
Breeding	1/2	50	2.83	0.15 - 52.74	0.485
Fattening	6/12	50	2.83	0.65 - 12.26	0.164
Fields used in common with other farm properties					
Yes	18/50	36	0.33	0.08 - 1.30	0.114
No	3/19	15.79			
Certification demanded when buying animals					
Yes	3/5	60	3.65	0.53 - 24.51	0.187
No	12/41	29.27			
Number of registered cattle movements					
Up to 13 animal traffic forms issued	12/56	21.43	8.25	2.16 - 31.49	0.002
More than 13 animal traffic forms issued	9/13	69.23			

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The following variables that were considered to be risk factors in the univariate analysis were kept in the multivariable logistic regression model for brucellosis foci (Table II): number of animal movements (OR = 9.50; CI 95%= 2.16 - 41.8) and abortion residues left in the fields (OR = 5.54; CI 95% = 1.14 - 26.13).

Table II: Multivariable logistic regression model for the risk factors associated with occurrences of bovine brucellosis foci on the farm properties sampled in São Francisco do Brejão, Maranhão.

Variable	Farm		
	OR	IC 95%	P-value
Number of registered cattle movements	9.50	2.16 – 41.80	0.003
Fields used in common with other farm properties	0.20	0.04 – 0.99	0.049
Abortion residues left in the fields	5.54	1.14 – 26.13	0.034

C. Univariate and multivariable multilevel model for brucellosis among animals

Table III presents the results from the univariate analysis on the risk factors associated with occurrences of brucellosis among animals.

Table III: Univariate analysis on the multilevel model for risk factors associated with bovine brucellosis in São Francisco do Brejão, Maranhão.

Variable	Animals				
	Diseased/exposed	%	OR	CI	P-value
Age of the animals					
Up to 63 months	8/298	2.68	5.47	0.38 - 57.78	0.005
> 64 months	19/411	4.62			
Size of the cattle herd					
Up to 611 head	19/600	3.16			
611 to 1222	4/58	6.89	7.98	0.86 - 73.92	0.067
> 1222	6/49	12.24	9.60	0.57 - 160.21	0.115
Presence of goats					
No	28/686	4.08	9.72	0.99 - 94.72	0.050
Yes	1/21	4.76			
Presence of buffalos					
No	27/674	4.00			
Yes	2/33	6.06	22.97	3.64 - 145.10	0.001
Presence of pigs					
No	13/290	4.48	0.13	0.02 - 1.11	0.063
Yes	16/417	3.83			
Animals acquired for fattening					
No	20/561	3.56	1.94	1.17 - 3.24	0.011
Yes	9/146	6.16			
Brucellosis certification demanded when buying cattle					
No	26/289	8.99	0.98	0.96 - 1.00	0.075
Yes	3/47	6.38			
Pasture rented on farm property					
No	18/597	3.01	17.33	1.72 - 174.99	0.016
Yes	9/112	8.03			
Milk production/day					
≤ 150 L	14/412	3.39	7.75	1.09 - 54.71	0.040
>150 L	15/265	5.66			
Animals purchased from breeders in other regions					
No	21/628	3.34	18.44	2.27 - 149.46	0.006
Yes	8/79	10.12			
Number of registered cattle movements					
Up to 13 animal traffic forms issued	16/555	2.88	22.25	4.12 - 120.23	<0.001
More than 13 animal traffic forms issued	13/152	8.55			

In the multivariable multilevel model, the age of the cattle (OR = 5.28, 95% CI = 1, 59 – 17,55) and pasture rented on farm property (OR = 9.59, 95% CI = 1,34 – 68,60) were the risk factors for the disease among the animals (Table IV).

Table IV: Multivariable analysis on the multilevel model for risk factors associated with bovine brucellosis in São Francisco do Brejão, Maranhão

Variável	Animais		
	OR	IC 95%	P-valor
Age of the animals	5.28	1.59 – 17.55	0.007
Pasture rented on farm property	9.59	1.34 – 68.60	0.024

IV. Discussion

This study deals with the prevalence of bovine brucellosis in the municipality of São Francisco do Brejão, where there was no kind of epidemiological data on the situation of this disease. The general prevalency of brucellosis observed in this municipality was 3.94%. This figure was equivalent to official data, which indicate that the prevalence of animals seropositive for brucellosis is between 4 and 5% [11], as well as previous surveys carried out in Maranhão [18].

The low prevalency identified in the present study is due to the effects from a series of official sanitary actions carried out over recent decades in practically all regions of Brazil.

Studying the prevalence of brucellosis at the farm, the result was 30.43% and was higher than the results for the states of São Paulo (9.70%), Espírito Santo (9.00%), Rio Grande do Sul (2.00%), Paraná (4.15%), Minas Gerais (6.04%), Rio de Janeiro (15.48%), Bahia (4.30%), Sergipe (11.24%), Goiás (16.20%) and Tocantins (20.99%) and lower than found in Mato Grosso do Sul (37.3%), Mato Grosso (41.19%) and Rondônia (34.57%) [19].

Based on the results from the studies mentioned above, it can be seen that the epidemiological behavior of the disease does not differ from that found nationally and internationally, in that brucellosis has greater relevance in relation to the epidemiological unit of the herd than it does in relation to individual animals, because of the low dissemination potential of the disease [20].

All the samples that were reactive were from females. No positive males were observed, although this finding was not a statistically significant variable ($P > 0.20$) regarding occurrences of brucellosis among the animals or for identifying the disease foci on the farm properties.

The univariate logistic regression analysis showed that the variables of cattle herd size, daily milk production, presence of sheep, pig herd size, habit of leaving aborted placenta residues in the fields, animal purchases from breeders in other regions, cattle replacement according to purpose, pastures used in common with other farm properties, certification demanded when buying animals and cattle movement were all associated with occurrences of brucellosis on the farms ($P < 0.20$). In the multivariable logistic regression model for brucellosis foci, the variables maintained were the number of animal movements and the habit of leaving abortion residues in the fields ($P < 0.05$).

The number of movements registered on animal traffic forms presented a statistically significant relationship with the risk of occurrence of the disease, such that on the farm properties for which more than 13 animal traffic forms were issued during the studied period, the chance that at least one of the animals in the herd would be reactive was 9.5 times higher. On these farms, there was no requirement for brucellosis examinations at the time of commercialization of the animals, which could have made it easier for infected animals to be introduced to the farm properties.

Appropriate disposal of placental residues is one of the ways of decreasing the dissemination of brucellosis. Lage et al. [21] reported that aborted fetuses and placental residues should be incinerated or buried. This variable was statistically significant, thus demonstrating that farm properties on which this occurred presented a chance of being brucellosis foci that was 5.54 times higher.

The univariate multilevel model demonstrated that the mean age of the cattle, herd size, presence of goats, buffalos and pigs, acquisition of animals for fattening, certification against brucellosis demanded when buying cattle, renting fields on the property, daily milk production, cattle purchases from breeders in other regions and the number of registered cattle movements presented statistically significant relationships with animals infected with brucellosis ($P < 0.20$). However, only the variables of buying animals for fattening and age of the cattle remained in the multivariable multilevel model.

According to the responses to the epidemiological questionnaire, 69.57% of the interviewees purchased cattle for fattening. The farms that did so were at a risk of presenting reactive animals that was 13 times higher when these animals were introduced into herd with other cattle for fattening.

In zootechnical terms, purchases of cattle for re-breeding or fattening are characterized by animals with low genetic potential, with less commercial value, which will remain on pasture until they reach the necessary weight to then be sent for slaughter. In addition, some of these animals may reproduce in the breeding stock, thereby increasing the risk of occurrence of the disease.

The average age of the cattle used in this study was high (> 64 months). The cattle in this age group presented a chance of being reactive that was approximately five times higher than shown by other age groups. In the state of Goiás, a tendency for the prevalence of brucellosis foci to be greater on farm properties with greater numbers of adult females was observed [19].

V. Conclusion

Based on the results obtained, it can be concluded that the frequency of brucellosis in the municipality of São Francisco do Brejão, Maranhão, did not vary significantly from the findings of previously surveys conducted in the state or from the averages found nationally. The risk factors that were associated with the disease in the multivariable analysis were: number of animal movements, existence of pasture used in common with other farm properties, abortion residues left in the field, age over 64 months and acquisition of cattle for fattening.

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REFERENCES

[1] H. Samaha, T. R. Mohamedb, R. M. Khoudair and H. M. Ashour. (2009). Serodiagnosis of brucellosis in cattle and humans in Egypt. Immunobiol. *[Online]*. 214(3). pp. 223-226. Available: http://www.researchgate.net/publication/24006800_Serodiagnosis_of_brucellosis_in_cattle_and_humans_in_Egypt

[2] A. M. Al-Majali, A. Q. Talafha, M. M. Ababneh and M.M. Ababneh. (2009, Mar). Seroprevalence and risk factors for bovine brucellosis in Jordan. J. Vet. Sci. *[Online]*. 10(1). pp. 61-65. Available: http://pdf.medrang.co.kr/JVS/010/Jvs010-01-09.pdf

[3] H. Mekonnen, S. Kalayou and M. Kyule. (2010. Apr). Serological survey of bovine brucellosis in barka and arado breeds (*Bos indicus*) of Western Tigray, Ethiopia. Prev. Vet. Med. *[Online]*. 94(1-2). pp. 28–35. Available: http://www.sciencedirect.com/science/article/pii/S0167587709003663

[4] D. A. Abernethy, J. Moscard-Costello, E. Dickson, R. Harwood, K. Burns, E. McKillop, S. McDowell and D. U. Pfeiffer. (2011, Mar). Epidemiology and management of a bovine brucellosis cluster in Northern Ireland. Prev. Vet. Med. *[Online]*. 98(4). pp. 223-229. Available: http://www.ncbi.nlm.nih.gov/pubmed/21144605

[5] W. Probert, K. Schrader, N. Khuong, S. Bystrom and M, Graves (2004, Mar). Real-Time Multiplex Assay for detection of *Brucella spp., B. abortus* and *B. melitensis*. J. Clin. Microbiol. *[Online]*. 42(3). pp.1290-1293. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC356861/

[6] H. C. Scholz, Z. Hubalek, I. Sedlácek, G. Vergnaud, H. A. L. Tomaso, S. Dahouk, F. Mlzer, P. Kämpfer, H. Neubauer, A. Cloeckaert, M. Maquart, S. Zygmunt, A. M. Whatmore, E. Falsen, P. Bahn, C. Gollner, M. Pfeffer, B. Huber, H. J. Busse and K. Noclker. (2008, Feb). *Brucella microt*i sp. nov., isolated from the common vole *Microtis arvalis*. Int. J. Syst. Evol. Microbiol. *[Online]*. 58(2). pp. 375-382. Available: http://www.ncbi.nlm.nih.gov/pubmed/18218934

[7] M. J. Corbel, S. S. Elberg and O. Cosivi. (Ed.). Brucellosis in humans and animals. Geneva: WHO Press, 2006, pp. 89

[8] M. Maurin, M. (2005, Jan). La brucellose à l'aube du 21e siècle. Méd. Mal. Infect. *[Online]*. 35(1). pp. 6-16. Available: http://www.sciencedirect.com/science/article/pii/S0399077X04002574

[9] G. Berhe, K. Belihu and Y. Asfaw. (2007). Seroprevalence of *Brucella abortus* infection in the crossbred dairy cattle in Tigray Region, Northern Ethiopia. Bulletin of Animal Health and Production in Africa. *[Online]*. 55(3). pp. 195-198. Available: http://dx.doi.org/10.4314/bahpa.v55i3.32808

[10] OIE. Office InternationaL des Epizootias. Manual of Standards for Diagnostic Tests and Vaccines. 5.ed. Paris: Office International des Epizzotias, 2008.

[11] Brasil. Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal (PNCEBT). Manual Técnico. Brasília: MAPA/SDA/DAS. 2006.

[12] IBGE. Instituto Brasileiro de Geografia e Estatística, 2013. Available: http://www.ibge.gov.br

[13] M. Thrusfield M. Veterinary Epeidemiology. 2nd. Ed. Blackwell Science, Cambridge, 1995, pp. 479.

[14] Brasil. Inquérito Soroepidemiológico da Brucelose: Manual de Procedimentos. Brasília: MAPA/SDA/DDA. 2001.

[15] A. G. Dean, J. A. Dean and D. Colombier. Epi-Info, version 6: A word processing database and statistics pro-gram for epidemiology on microcomputers. Atlanta: CDC, 1994

[16] D. W. Hosmer and S. Lemeshow S. Applied Logistic Regression. New York: Wiley-interscience Publication, 1989.

[17] S. Rabe-Hesketh, A. Skrondal and A. Pickles. (2004, June). Generalized multilevel structural equation modelling. Psychometrika. *[Online]*. 69(2). pp. 167-190. Available: http://www.gllamm.org/PMmsem_04.pdf

[18] M. R. Borba, M. A. Stevenson, V. S. Gonçalves, J. S. Neto, F. Ferreira, M. Amaku, E. O. Telles, S. S. Santana, V. Ferreira, J. R. Lôbo, V. C. Figueiredo and R. A. Dias. (2013). Prevalence and risk-mapping of bovine brucellosis in Maranhão State, Brazil. Prev. Vet. Med. *[Online]*. 110(2). pp. 169-176. Available: http://www.sciencedirect.com/science/article/pii/S

[19] USP. Primeiro relatório parcial: Situação epidemiológica da brucelose bovina e bubalina no Brasil. São Paulo: USP/FMVZ/VPS, 2006.

[20] B. Toma, B. Dufor, M. Sanaa, J. Benet, A. Shaw, F. Moutou and A. Louzã. Epidemiologia aplicada à luta coletiva contra as principais doenças animais transmissíveis. Lisboa: Fundação Calouste Gulbenkian, 2004.

[21] A. P. Lage, F. P. Poester and V. S. P. Gonçalves. (2005). Controle da Brucelose bovina. Cad. Técnico Vet. Zoot. 47. pp. 30-41.

AUTHOR'S PROFILE

Margarida Paula Carreira de Sá PRAZERES
Postgraduate, Federal University of Maranhão, Departament of Health Sciences, São Luiz, MA Brazil