DOI: 10.1590/1808-1657v74p3012007

RISK FACTORS AND SEROPREVALENCE OF *BRUCELLA* SPP. IN CATTLE FROM WESTERN AMAZON, BRAZIL

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ABSTRACT

The seroprevalence of *Brucella* spp. and the possible associated risk factors were estimated for 2,109 adult cows in Monte Negro county, State of Rondônia, Brazil. A questionnaire was completed for each farm where cattle were sampled. Laboratory tests were Rose-Bengal Agglutination, Standard Tube agglutination, and Mercaptoethanol. The adjusted overall prevalence of *Brucella* spp. seropositive cows for Monte Negro county was 15% and at least 54 herds (63%) were positive. A logistic regression analysis suggested that the herd size of more than 25 cows and the presence of pigs were significant factors associated with the seropositivity (herd size: OD = 2.8; *P*=0.02; presence of pigs: OD = 2.5; *P* = 0.04). Other significant variables associated to the infection, analysed by Chi-Square were the presence of seropositivity cows with the herd that were repeat breeders and birth of weak calves ($P < 0.05$).

KEY WORDS: *Brucella* spp., prevalence, epidemiology, Amazon, Brazil.

RESUMO

SOROPREVALÊNCIA E FATORES DE RISCO PARA A INFECÇÃO POR *BRUCELLA* SPP. EM VACAS DA AMAZÔNIA OCIDENTAL, BRASILEIRA. A soroprevalência e os possíveis fatores de risco para a infecção por *Brucella* spp. foram estimados em 2.109 vacas do Município de Monte Negro, Rondônia. De cada animal selecionado foi coletado soro sangüíneo e para as propriedades envolvidas foi preenchido um questionário. As amostras coletadas foram analisadas pelas provas de Antígeno Acidificado Tamponado corado com Rosa Bengala, Soroalgutinação Lenta em Tubos e 2-Mercaptoetanol. A prevalência total da infecção por *Brucella* spp. em vacas no Município de Monte Negro foi de 15%, sendo 54 (63%) fazendas com animais reagentes. A regressão logística sugeriu que rebanhos com mais de 25 vacas (OD = 2,8; *P* = 0,02) e a presença de suínos (OD = 2,5; *P* = 0,04) foram fatores associados a soropositividade dos bovinos. Outras variáveis associadas à infecção pelo teste do Qui-Quadrado foram repetição de cio e nascimento de bezerros enfermos ($P < 0,05$).

PALAVRAS-CHAVE: *Brucella* spp., prevalência, epidemiologia, Amazônia, Brasil.

INTRODUCTION

The transmissible diseases of domestic animals that affect the reproductive system, such as abortion, lowered fertility and infertility, stillbirth or birth of weak calves are important because they lower livestock productivity (Radostits*et al*., 2000). Several of these diseases are potentially zoonotic. Brucellosis, associated with *Brucella abortus* infection is one of such diseases, making it and one of the most important and widespread zoonoses in the world (Corbel, 1997).

B. abortus is the most widespread agent of Brucellosis in the world, including Brazil (Corbel, 1997). Other *Brucella* species reported in Brazil are *B. suis* in pigs, *B. ovis* in sheep, and *B. canis* in dogs (Poester et al., 2002). Recently Megid et al. (2005) reported the isolation of *B. abortus* biovars 1, 2 and 3 in cattle and provided the first confirmed evidence of *B. abortus* biovar 1 infection in water buffalo in

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Brazil. On the other hand, *B. suis* has also been isolated from cattle in Brazil (Giorgi et al., 1972). Since *B. suis* and *B. abortus* present cross-reaction in serological tests (they are both smooth *Brucella*), they can not be distinguished by serological tests.

The Brazilian Amazon region is a new geographical area where agricultural development and livestock farming have been recently introduced. The state of Rondônia is one such area in the western Amazon. Brucellosis is a common disease of cattle in Brazil, but there is little information about its occurrence in the Amazon region (Homem et al., 2000; Molnár et al., 1999). The region presents typical features of the frontiers of human occupation, where its human inhabitants, and domestic and wild animals share the same habitat (Labruna et al., 2002).

The present study investigated the seroprevalence of anti-*Brucella* spp. antibodies in cattle from Monte Negro County, Rondônia state, Western Amazon, Brazil, and possible the risk factors associated with this infection in cattle.

MATERIAL AND METHODS

Monte Negro County is located in Rondônia state, Western Amazon, Brazil (10º 15' 35"S, 63º 18' 06"W). It has a population of approximately 13,000 inhabitants, most of them rural dwellers in small, family-employed farms. The region has a high rainfall that averages of 2,000 mm annually; there is a moderate drought period, from during April to-October. Temperature ranges from 25 - 29º C and the relative humidity is 70% - 80% throughout the year (Camargo et al., 2002). In 2001, Monte Negro County had a total cattle population of 116,736, distributed amongst 722 farms (Idaron, personal communication.). The Nelore was the predominant beef breed; dairy cattle were mostly crossbred between zebu and several European breeds.

The number of farms to be studied was determined using EpiInfo 6.04, with an estimated prevalence of 50%, absolute precision desired of 10% and confidence interval of 95%. As a result, 86 farms were randomly selected.

Only cows over 24 months old were included in the present study because sera from these cows should no longer be seropositive to *Brucella* spp if vaccinated between 3 - 8 months old (Poester et al., 2002; Ribeiro et al., 1997). The minimum number of cows to be tested in each farm was estimated assuming that there was a 95% probability of detecting at least one seropositive animal per farm with a prevalence of 5% seropositive cows in each herd (Thrusfield, 1995).

A questionnaire, for each farm, was completed by the farm owner or manager to identify possible independent variables associated with the presence of seropositive cattle on the farm: farm size, number of cows, stocking rate, presence of dogs, pigs or horses, jungle contact (cattle grazing inside the jungle), history of B19 vaccination, and water source. It was also investigated reproductive disorders and seropositivity associated conditions: abortion, lowered fertility and infertility, stillbirth, repeated breeders, retained placenta, birth of weak calves (can die in the first hours of life) and neonatal mortality.

Serum samples were obtained between May - October 2002 and stored at –20ºC until analysis. In the laboratory, serum samples were examined by the Rose Bengal Agglutination Test (RBT) as a screening test for anti-smooth *Brucella* spp. antibodies (Alton et al., 1976). To confirm that specific anti-smooth *Brucella* spp antibodies were present in the sera, those RBT positive samples were retested using a Tube Agglutination Test (SAT) and the Mercaptoethanol Test (MET) (Alton et al., 1976). The antigen used was an inactive suspension of *B. abortus* 1119-3. For RTB the antigen was 8% concentrated with pH 3.65. For SAT and MET, antigen was 4.5% concentrated. The antigens were standardized according to references of the Pan American Center of Zoonosis, and were produced by the Institute of Technology of Paraná, Brazil. Reactions were considered positive when a titer of 25 was detected in MET.

Seroprevalence for *Brucella* spp. infection was calculated separately for each farm and the total sample. As farms with different herd sizes were evaluated, the overall seroprevalence was adjusted by herd sizes to estimate a prevalence value that could be applied for the entire cattle population of Monte Negro County (Thrusfield, 1995). Different farming activities were also compared for seroprevalence; dairy, beef or mixed cattle farms, using the Chi-Square test (χ^2) within EpiInfo 6.04.

A logistic regression analysis (Hosmer & Lemeshow, 1989) allowed multivariate models to be constructed to describe the dependent variable (presence of seropositive cattle) as a function of the independent variables. The independent variables identified in the questionnaire were subjected to univariate analysis and those with statistical association ($P < 0.20$ in χ^2 or Fischer's exact test) were tested in the multivariate model by the stepwise forward method. Variables were included in the multivariate model if they displayed statistical significance of $P < 0.05$. The goodness-of-fit test was performed by according to Hosmer and Lemeshow statistic (Hosmer & Lemeshow, 1989). Other analyses (χ^2) investigated associations between seropositive cattle and other reproductive problems within the herd. Analyses were performed using SPSS for Windows (1999).

Table 1 - Independent variables adopted in the questionnaire completed by 86 farms in Monte Negro County, Rondônia state, Brazilian Western Amazon, Brazil.

Independent variables	Number of farms (%)			
	Beef	Dairy	Mixed	Total
Farm size (> 110 ha)	9 (82)	20 (40)	17 (68)	46 (53)
Number of cows (> 25 cows)	9 (82)	21 (42)	15 (60)	45 (52)
Stocking rates (> 3.5 cattle per ha)	4 (36)	35 (70)	15 (60)	54 (63)
Jungle contact	0	4 (8)	2 (8)	6 (7)
Water sources (water reservoir)	6 (54)	19 (38)	8 (32)	33 (38)
Presence of dogs	8 (73)	47 (94)	24 (96)	79 (92)
Presence of pigs	5 (10)	29 (60)	15 (30)	49 (57)
Presence of horses	11 (15)	41 (55)	23 (30)	75 (87)
B19 vaccination	9 (23)	16 (41)	14 (36)	39 (45)

Table 2 - Seroprevalence to *Brucella* spp infection in cattle types among 86 farms in Monte Negro County, Rondônia state, Brazilian Western Amazon, Brazil.

Farm type	Number of Herds			Number of Animals		
	Examined	Positive	%	Examined	Positive	%
Beef	11	10	91[a]	584	95	16[a]
Dairy	50	31	62[a, b]	1,011	68	7[b]
Mixed	25	13	52[b]	514	40	8[b]
Total	86	54	63	2,109	203	10

*Different letters in the same column means significance difference ($P < 0.05$).

RESULTS

The 86 farms investigated comprised 11 beef, 50 dairy, and 25 mixed cattle farms. Serum was collected from 2,109 cows; 584 beef cattle, 1,011 dairy cows, and 514 cattle on mixed farms. The average herd size was 60 cows (range 3 – 1,250). Other variables studied in the farms are presented in Table 1.

At least one seropositive cow was detected in 54 herds, giving a herd prevalence of 63% (52 - 73%; CI: 95%). A total of 227 cows were seropositive by the RBT. However, only 208 cows reacted by the SAT and 203 by the ME. This last one was the confirmatory test adopted in the present study. The number and proportion of seropositive herds and cattle are summarized in Table 2. The adjusted overall prevalence of *Brucella* spp. seropositive cows for Monte Negro County was 15% (8 – 19%; CI: 95%).

By the univariate analysis, the presence of seropositive cows were statistically coupled ($P<0.20$) with the variables: number of cows, stocking rate, water source, presence of pig and horses. The Multivariate analysis (Table 3) showed that herd size comprising more than 25 cows (OD: 2.8; 95% CI: 1.1-7.2; $P = 0.02$) and the presence of pigs in the farm (OD: 2.5; 95% CI: 1.0-6.5; $P = 0.04$) were associated with at least one seropositive cow within it. The final model showed good performance ($\chi^2 = 0.14$; $P = 0.93$). Farms that claimed repeat breeders (47/86; 54.6%) and birth of weak calves (52/86; 60.5%) were statistically associated ($P < 0.05$) with the presence of seropositive cows.

DISCUSSION

Brucellosis is considered an important cause of productive losses in cattle. In addition, it is a zoonosis thoroughly diffused all over the world (Radostits et al., 2000). According to the Department of Animal Health of the Brazilian Ministry of Agriculture, the mean seroprevalence of cattle brucellosis in Brazil was 4 - 5% (Brasil, 2000). The present study showed the seroprevalence to be 15% for Monte Negro County similar to the Northern region of Brazil (Brasil, 2000). The farm seroprevalence in this present study was 63%, slightly higher than that found by Homem et al. (2000) in the eastern Brazilian Amazon.

Table 3 - Univariate and multivariate analysis investigating independent variables listed with cattle seropositive to *Brucella* spp.

Variable	Farm number			Analyze				
				Univariate		Multivariate		
	Examined	Positive	%	χ^2	*P*	OD	*P*	IC (95%)
Farm Size								
< 110 ha	40	23	57					
> 110 ha	46	31	67	0.70	0,34	-	-	-
Number of cows								
< 25 cows	41	21	51			1		
> 25 cows	45	33	73	4.50	0,03	2,8	0.02	1.1 – 7.2
Stocking rate								
> 3.5 bovine/ ha	54	22	41					
< 3.5 bovine/ ha	32	20	62	3.80	0.05	-	0.09	-
Jungle Contact								-
No	80	49	61					
Yes	6	5	83	0.96	0,27	-	-	-
Water sources								
River	53	30	57					
Water reservoir	33	24	73	2.26	0,13	-	0.10	-
Dog								
Presence	79	49	62					
Absence	7	5	71	0.22	0,47	-	-	-
Pig								
Absence	37	19	51					
Presence	49	35	71	3.60	0.05	2.5	0.04	1.0 – 6.5
Horse								
Absence	11	5	45					
Presence	75	49	65	1.60	0.20	-	0.7	-
B19 vaccination								
Absence	47	27	57					
Presence	39	27	71	1.26	0.26	-	-	-

Herds containing more than 25 cows were considered a significant risk factor for the seropositive cattle; the larger the herd the higher the chances of having an infected animal. Generally, beef herds are much larger than dairy or mixed herds. Higher seroprevalence rates have been reported previously among beef herds in Brazil (Poester et al., 2002). In a typical Brazilian beef herd cattle grazed constantly at pasture with different age and productive groups all intermingled. This factor may be important when devising control and eradication programs for brucellosis.

The presence of pigs was associated with seropositive cows in the farms. According to a review performed by Corbel (1997), bovine brucellosis caused by *B. suis* is emerging as an increasingly serious public health problem in South America. This statement is not confirmed in Brazil, where recently studies have demonstrated only *B. abortus* isolation only from bovine milk and foetus (Langoni et al., 2000; Megid et al., 2005). In fact, the *B. suis* isolation from cattle reported by Corbel (1997)is an old data from thedates 1970s (Giorgi et al., 1972). There is no later report of *B. suis* isolation from cattle in Brazil. On the order hand, *B. abortus* is rarely found to infecting pigs, and pigs are considered to be a dead-end hosts (Radostits et al., 2000). Recently, Aguiar et al. (2006) observed in the same area that pigs of the same areaof were not infected by *Brucella* spp. Apparently, the presence of pigs cannot contribute to the maintenance of the disease in cattle and this result may be just a confounding effect.

It was not observed association with the presence of dogs in the farms. Recently, in this region, Aguiar et al. (2005) found no positive dogs for brucellosis. Horses are mentioned as possible risk factors (Radostists et al., 2000). However, it has not been proven to be the case, hence it should be further documented, since horses are susceptible to the infection by *Brucella* spp.

The associations of repeat breeders and weak calves with seropositivity herds indicate that there may be a significant economic impact of the disease in Monte Negro County. Losses due to brucellosis can be of major importance, mainly due to decreased milk production by abortion and reproductive disorders (Radostits et al., 2000). It is emphasized the importance of cattle brucellosis in Monte Negro County, as cause of damage to the herd and animal production.

ACKNOWLEDGEMENTS

We are very grateful to Dr. F. Ferreira (Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo) for his technical assistance during statistical analysis; to T. Bronhall, and to the Agency of Animal Sanitary Defense of the State of Rondônia (IDARON) for the information provided and field support. This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). D.M. Aguiar, M.B. Labruna, S.A. Vasconcellos and S.M. Gennari are in receipt of a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, and G.T. Cavalcante from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES.

The present research agrees with the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation and was approved by the Biomedical Science Institute/USP (protocol nº 086/03).

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Received on 4/1/07
Accepted on 14/12/07