

# Analysis of the risk factors for bovine brucellosis in dairy herds of the Rio Branco microregion, Acre, Brazil

## *Análise dos fatores de risco para brucelose bovina em rebanhos leiteiros da microrregião de Rio Branco, Acre, Brasil*

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**ABSTRACT:** Bovine brucellosis (BB) is a compulsory notifiable bacterial disease caused by *Brucella abortus*, which is responsible for severe reproductive disorders in cattle, considerable damages to trade and public health damage. The objectives of this study were to identify the frequency of BB and to analyze the risk factors in the dairy herds of the Rio Branco microregion, in the state of Acre, Brazil. Thus, 527-bovine-serological samples were submitted to the buffered acidified antigen test and to 2-mercaptoethanol. Risk factors were characterized by the administration of epidemiological questionnaires. The logistic regression test was used to evaluate the existence of a significant association between the variables. The frequency of BB in the Rio Branco microregion was 10.6% (56/527). The inappropriate destination of the aborted fetus and placental remnants (*odds ratio* — OR = 13.6), the slaughter of cattle within the property (OR = 3.4) and the age range above 4 years old (OR = 2.9) were the most significant risk factors identified by the logistic regression model. According to the results of this study, it is recommended to intensify BB control and prophylaxis measures in the microregion of Rio Branco, especially those related to sanitary and reproductive management in the livestock breeding.

**KEYWORDS:** *Brucella abortus*; ruminant; logistic regression; Western Amazon.

**RESUMO:** A brucelose bovina (BB) é uma bacteriose de notificação obrigatória, causada pela *Brucella abortus*, responsável por distúrbios reprodutivos graves em bovinos, prejuízos consideráveis ao comércio e agravos em saúde pública. Objetivaram-se com este estudo identificar a frequência de BB e analisar os fatores de risco nos rebanhos leiteiros da microrregião de Rio Branco, Acre. Assim, 527 amostras sorológicas de bovinos foram submetidas ao teste do antígeno acidificado tamponado e ao 2-mercaptoetanol. Os fatores de risco foram caracterizados por aplicação de questionários epidemiológicos. Utilizou-se o teste de regressão logística para avaliar a existência de associação significativa entre as variáveis. A frequência da BB na microrregião de Rio Branco foi de 10,6% (56/527). O destino inadequado do feto abortado e restos placentários (*odds ratio* — OR = 13,6), o abate de bovinos na propriedade (OR = 3,4) e a faixa etária acima de 4 anos (OR = 2,9) foram os fatores de risco mais significativos apontados pelo modelo de regressão logística. Conforme os resultados deste estudo, recomenda-se a intensificação nas medidas de controle e profilaxia da BB na microrregião de Rio Branco, sobretudo relacionadas ao manejo sanitário e reprodutivo nas criações.

**PALAVRAS-CHAVE:** *Brucella abortus*; ruminante; regressão logística; Amazônia Ocidental.

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## INTRODUCTION

Bovine brucellosis (BB) is a compulsory notifiable bacterial disease caused by *Brucella abortus*, a highly pathogenic agent that is responsible for severe reproductive disorders in cattle. Direct contact, inhalation and ingestion of contaminated food are the main forms of transmission, with great zoonotic potential (WHO, 2009). This disease is considered re-emergent and occurs mainly in tropical countries, showing high prevalence rates (WARETH et al., 2017).

Brazil's cattle breeding industry has been standing out for its significant participation in the financial scenario, contributing considerably to the gross domestic product. However, infectious bovine diseases have negative influence on the national economy (HIRSH; ZEE, 2003). Being a disease with chronic evolution and low mortality, BB propagates throughout properties in a silent way, but it interferes economically with breeding, due to the fall in animal productivity, reproductive alterations, sacrifice of reagents, partial or total condemnations of carcasses in refrigerators and trade barriers (LUCAS, 2006).

The National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e da Tuberculose Animal — PNCEBT), created by the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento — MAPA) in 2001, establishes guidelines to track and to reduce the occurrence of this disease in the country. The last estimate of national prevalence was 3.4% (BRASIL, 2006). In Acre, the epidemiological situation of this disease is not outlined. The only report in the state, restricted to the municipality of Rio Branco, establishes incidence of 11.7% (COSTA; ALENCAR, 1979).

Thus, considering the negative economic impact, the risks for public health and the absence of current health data in the state, the objective of this study was to identify the frequency of BB and to analyze the risk factors that are potentially relevant for dairy herds in the microregion of Rio Branco, Acre, Brazil.

## MATERIAL AND METHODS

### Bioethics and Biossecurity Committee Approval

This research was approved by the Committee on Ethics in the Use of Animals of the Universidade Federal do Acre, Rio Branco *Campus*, under permit number 01/2016.

### Characterization of the study area

Acre, located in the western Amazon region of Brazil, has area of 164,221.32 km<sup>2</sup>, having interstate borders with Amazonas

and Rondônia and international borders with Bolivia and Peru. The climate is equatorial and the vegetation is composed by the Amazon forest. The state is geographically organized by the mesoregion of the Juruá Valley, divided by the microregions of Cruzeiro do Sul and Tarauacá, and the mesoregion of the Acre Valley, divided by the microregions of Rio Branco, Sena Madureira and Brasileia (GOVERNO DO ESTADO DO ACRE, 2016).

It is estimated that there are 2,916,207 head of cattle in the state, out of which 82,070 are appropriate for milk production. The microregion of Rio Branco (Fig. 1) corresponds to 58.4% of all dairy basin, and it is the selected area for the study. This territory is constituted by the capital, Rio Branco, and the municipalities of Acrelândia, Bujari, Capixaba, Plácido de Castro, Porto Acre and Senador Guiomard (IBGE, 2016).

### Sampling

The sample population consisted of dairy cows aged over 24 months old, bred under intensive and semi-intensive systems. The sample size was determined by the calculation for descriptive epidemiological surveys, assuming 12% of value as the estimated prevalence (COSTA; ALENCAR, 1979), in a confidence interval of 95% (95%CI) and statistical error of 5%. At least 361 animals were necessary (THRUSFIELD, 2004). However, it was possible to analyze 527 serological samples, 513 from females and 14 from males, from 20 herds, which were selected considering their non-probabilistic convenience.

### Serological diagnosis

Blood samples were collected by jugular venipuncture, and the serum was extracted by centrifugation at 5,000 G for 10 minutes. According to the technical regulation of PNCEBT/MAPA (BRASIL, 2006), the buffered acidified plate antigen test was used, and positive samples were submitted to the confirmatory diagnosis by the 2-mercaptoethanol test. The used antigens were commercially manufactured by the Paraná Institute of Technology (Instituto de Tecnologia do Paraná — Tecpar) and licensed by MAPA under no. 3,362/1990 and 3,359/1990, respectively.

### Characterization of the risk factors

In order to obtain information about the structural conditions of the facilities and the used sanitary and reproductive management practices, epidemiological questionnaires were administered to producers, totaling 30 objective questions (OLIVEIRA et al., 2013; CAMPE et al., 2016).

### Statistical design

The dependent (serological test positivity) and independent variables (risk factors) were submitted to a bivariate exploratory

analysis using Fisher's exact method. As a working hypothesis (H1), the positive association between these variables was considered at the significance level of 5%. The values were described as p value, *odds ratio* — OR, 95%CI and standard error of the estimate (DOHOO et al., 2009).

For a more in-depth interpretation, the multiple logistic regression test was administered, aiming at the elaboration of statistical models. The final model was based on the alteration of the Akaike information criterion (AIC) of each model; it was obtained with the minimum AIC and  $p < 0.05$ , according to the formula expressed in Equation 1:

$$\text{Logit}(p) = \alpha + \beta_0 + \beta_1X_1 + \beta_2X_2 + \dots + \beta_nX_n + e \quad (1)$$

In which:

p = the dependent variable;

$\alpha$  = the intercept;

$\beta_0$  = the constant;

$\beta_i$  = the regression coefficient;

$X_i$  = the independent variables;

e = the error (CAMPE et al., 2016).

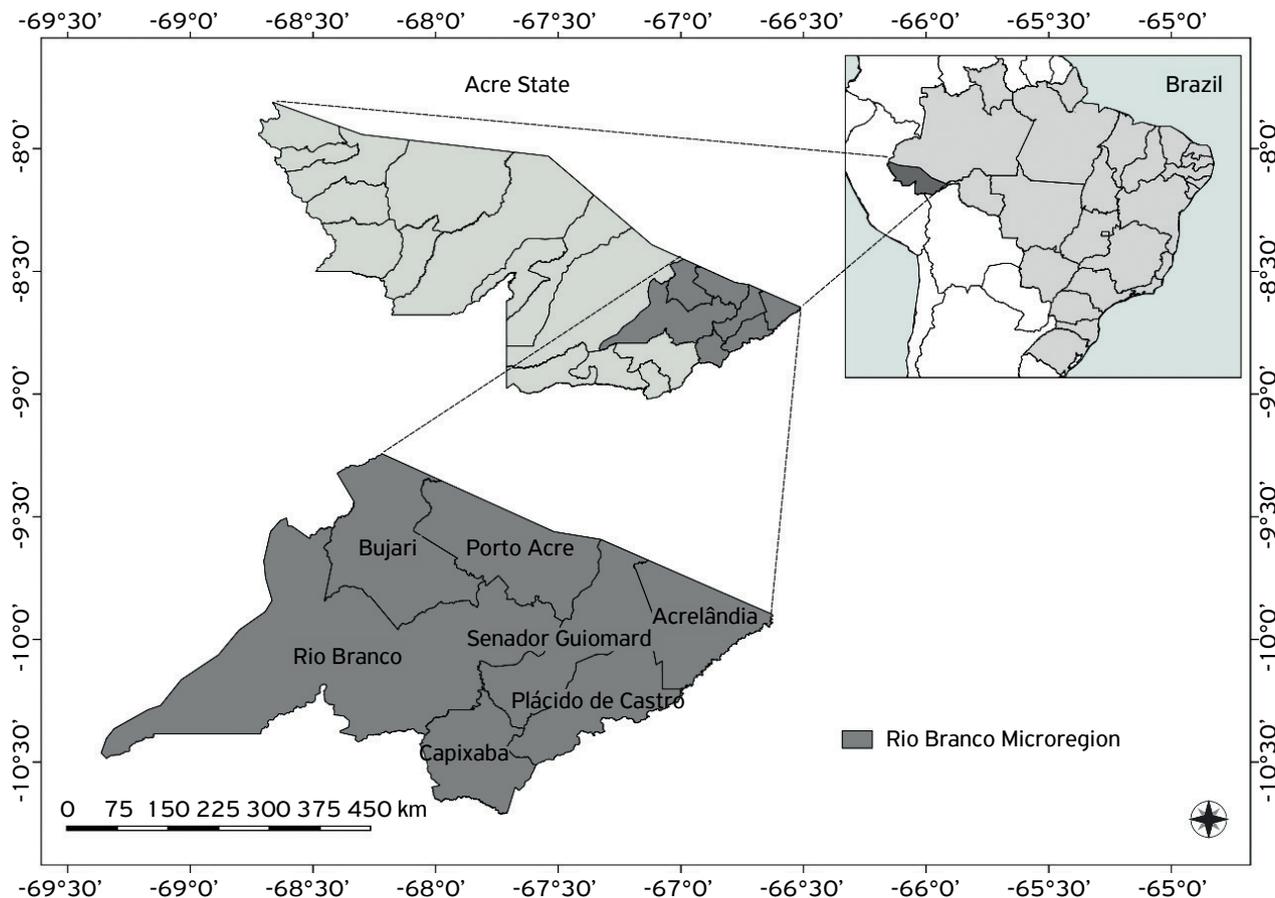
The statistical analyses were performed by the R software (The R Foundation for Statistical Computing, New Zealand).

## RESULTS

The frequency of BB in the Rio Branco microregion was 10.6% (56/527). Seroreagent cattle were detected in five (71.4% — 5/7) cities of the study area (Table 1). As for the number of outbreaks, 60% (12/20) of the properties had at least one positive animal to the serological test. The infection rate in the municipalities and in the herds ranged from 2.2 to 21.4% and from 1.9 to 26.1%, respectively. All positive animals were females.

**Table 1.** Distribution of the frequency of bovine brucellosis in the cities of the Rio Branco microregion, Acre, Brazil.

Cities	Outbreaks / Properties	Seroreagents / Population
Acrelândia	4/4 (100%)	16/94 (17%)
Bujari	2/2 (100%)	9/42 (21.4%)
Capixaba	0/1 (0%)	0/30 (0%)
Plácido de Castro	1/2 (50%)	1/46 (2.2%)
Porto Acre	0/2 (0%)	0/48 (0%)
Rio Branco	3/4 (75%)	8/105 (7.6%)
Senador Guimard	2/5 (40%)	22/162 (13.6%)
Total	12/20 (60%)	56/527



**Figure 1.** Geographical situation of the Rio Branco microregion, Acre, Brazil.

In the exploratory bivariate analysis of risk factors, there was significant association ( $p < 0.05$ ) for several variables associated with hygiene-sanitary and reproductive management failures, in addition to the age range above 4 years old (Table 2). The inappropriate destination of aborted fetuses and placental remnants was highlighted by the high *odds ratio* (OR = 13.6;  $p > 0.01$ ). The practice of quarantine for newly purchased animals or the performance of serological tests to diagnose BB were found in none of the sampled properties. However, the vaccination of females aged between 3 and 8 months old was observed in all farms.

The final statistical model that best elucidated the occurrence of BB throughout the herds, representing the lowest AIC value and the maximum significant association ( $p < 0.01$ ), was composed by the following variables: inappropriate destination of aborted fetuses and placental remnants, age range between 4 and 6 years old, and slaughter of cattle within the property (Table 3).

## DISCUSSION

The frequency of BB in the Rio Branco microregion (10.6%) was well above the prevalence rate in the country (3.4%), but similar to the one reported in Pará (10.9%) (MINERVINO et al., 2011). The rates for the other northern states were 4.1, 4.4 and 6.2% in Roraima, Tocantins and Rondônia, respectively (OGATA et al., 2009; VILLAR et al., 2009; SOUZA et al., 2012).

The number of outbreaks (60%) demonstrates concern about the spread of the disease over the herds. The percentage of outbreaks in Brazil varies between 0.32 and 41.5% (CHATE et al., 2009; SIKUSAWA et al., 2009), and higher

values reflect the absence of sanitary barriers for the inlet of infected cattle in several farms (BRASIL, 2006).

In the state of Acre, the study by COSTA; ALENCAR (1979) was, until then, the only BB report, in which an incidence of 11.7% was determined for the municipality of Rio Branco, with 65.4% of properties considered as outbreaks. It is possible to observe that, after almost four decades, *B. abortus* infection is still intense in the state, even in low-density dairy herds (POESTER et al., 2002; SOUZA et al., 2012).

In the northern states, the rapid and remarkable growth of livestock farming over the last decades has implied the importation of animals and genetic material from other states with more consolidated cattle breeding, but without proper sanitary control (VILLAR et al., 2009). Moreover, in the Rio Branco microregion, several failures in hygienic-sanitary and reproductive management had significant association with the occurrence of BB.

Abortion, which is usually observed in the final third of the pregnancy and which is a result of *Brucella* infections and cell death in the gravid uterus, is an important sign of the disease, due to the intense tropism of the bacteria by reproductive

**Table 3.** Final multiple logistic regression model for the risk factors associated with bovine brucellosis in the Rio Branco microregion, Acre, Brazil.

Variables	p*	z	SE
Intercept	3.6	-6.3	1.6
Inappropriate destination of aborted fetuses	0.004	2.8	0.6
Age range (4 to 6 years old)	0.008	2.6	0.4
Slaughter of cattle within the property	0.01	2.5	0.4

\*Significant association at the 0.01 level; SE: standard error of estimate.

**Table 2.** Risk factors associated with bovine brucellosis in the Rio Branco microregion, Acre, Brazil.

Variables	Cases / Exposed	p	OR	95%IC	SE
Inappropriate destination of aborted fetuses	53/266 (19.9%)	0.000**	13.6	4.4 – 44.1	8.2
External purchase of cattle	49/271 (18.1%)	0.000**	5.2	2.3 – 11.6	2.1
Absence of disinfection	43/197 (21.8%)	0.000**	4.6	2.4 – 8.8	1.5
Shared pasture	47/250 (18.8%)	0.000**	4.6	2.2 – 9.6	1.7
Slaughter of bovines within the property	15/46 (32.6%)	0.000**	3.4	1.7 – 6.6	1.1
Age range (4 to 6 years old)	36/177 (20.3%)	0.000**	2.9	1.6 – 5.1	0.8
Absence of maternity pens	56/354 (15.8%)	0.000**	1	0.1 – 0.2	0.1
Contact with wild animals	39/250 (15.6%)	0.011**	1.9	1.2 – 3.1	0.58
Use of reproductive biotechnology	9/145 (6.2%)	0.026*	0.4	0.2 – 0.9	0.2
Intensive breeding system	24/176 (13.6%)	0.771	1.1	0.6 – 1.9	0.3
Semi-intensive breeding system	32/295 (10.8%)	0.815	0.9	0.5 – 1.8	0.5

\*Significant association at the 0.05 level; \*\*significant association at the 0.01 level; OR: *odds ratio*; 95%CI: confidence interval of 95%; SE: standard error of the estimate.

organs. The aborted fetus and placental remains are potentially infective, due to the high bacterial load found in these tissues, which contaminate pastures (PATHAK et al., 2016). In this study, high *odds ratio* (OR = 13.6;  $p < 0.01$ ) were observed (VILLAR et al., 2009; AHASAN et al., 2017; CAMPE et al., 2016). It represents a threat of imminent risk to farms and lack of knowledge about the proper destination of aborted tissues.

Another relevant aspect is the cattle age range, which is poorly evaluated as a risk factor in Brazil, but has recently been described as a positive association with seroprevalence in other Asian and European countries (AHASAN et al., 2017; CAMPE et al., 2016), especially in animals that are older than 4. It is known that the advancing age is directly proportional to the time of exposure to risk factors, as well as the intensification in the management (BRASIL, 2006).

The slaughtering of cattle within the farm itself, in turn, being them breeding animals or females at the end of their productive life, also constitutes a risk activity. Carcasses and innards can contaminate pastures, drinking troughs, soil, and water sources, and they induce the formation of aerosols containing bacterial particles, which remain viable for long periods, compromising the health of animals and humans themselves (OGATA et al., 2009; MÉNDEZ-LOZANO et al., 2015).

All the females of the study were vaccinated with sample B19 and were aged between 3 and 8 months old, as recommended by the PNCEBT (BRASIL, 2006). However, they showed considerable seropositivity, which was justified by the expectation of the 80% protection that the vaccine gives to herds. The RB51 vaccine sample has been alternatively used in several countries in animals aged over 8 months old or as a booster of B19, demonstrating greater resistance to infection

and reducing abortion rates (YAZDI et al., 2009; BASTOS et al., 2012). However, it is still not used in the state.

In order to reduce the incidence and prevalence of BB in Acre, to certify properties as monitored or free, and to contribute to the maintenance of bovine health, minimizing economic barriers and risks to public health (BRASIL, 2006), the State Program for the Control and Eradication of Brucellosis and Tuberculosis (Programa Estadual de Controle e Erradicação da Brucelose e Tuberculose — PECEBT) was implemented in 2012 in the state (IDAF, 2016), but with preliminary actions. The reduced number of independent veterinarians and agricultural inspectors authorized for the diagnosis, control and prophylaxis of these diseases has been a challenge to the program's progress.

According to the results of this study, it is recommended to intensify BB control and prophylaxis measures in the Rio Branco microregion, avoiding greater economic losses in cattle breeding, as well as risks for the public health. The strategic organization of the program in the state of Acre will allow significant reduction in the occurrence of this disease, consolidating the official animal health service and strengthening the local productive chain. Greater attention is needed to be paid to sanitary and reproductive management, especially in terms of discarding aborted tissues, as well as discouraging the slaughter of cattle within farms and creating strategies for a more efficient vaccination campaign.

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