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## **Anti-*Brucella abortus* antibodies in free-ranging equids from Mossoró, Rio Grande do Norte, Brazil**

### **Anticorpos anti-*Brucella abortus* em equídeos errantes do município de Mossoró, Rio Grande do Norte**

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#### **Abstract**

The aims of the present study were (i) to determine the occurrence and (ii) to evaluate possible factors associated with infection by *Brucella abortus* in free-ranging equids from Mossoró, Rio Grande do Norte, Brazil. Sera from 227 free-ranging equids (178 donkeys, 43 horses and 6 mules), captured by the highway police and the prefecture agents, were screened by the rose bengal test (RBT) and confirmed for *B. abortus*-antibodies by the standard tube agglutination (STAT) and the 2-mercaptoethanol (2ME) tests. Of the 227 equids tested, four (1.76%) were positive for *B. abortus* antibodies. All were horses, which resulted in an observed frequency of infection for this species of 9.30% (4/43). No association was found among seropositivity for *B. abortus* and the age and sex. Thus, data from the present study showed that infection by *B. abortus* is present among horses in Mossoró, Rio Grande do Norte, Brazil.

**Key words:** Brucellosis, *B. abortus*, free-ranging equids, Mossoró

#### **Resumo**

Os objetivos deste trabalho foram (i) determinar a ocorrência da infecção por *Brucella abortus* em equídeos de vida livre no município de Mossoró, Rio Grande do Norte e (ii) avaliar possíveis fatores associados a esta infecção. Soros de 227 equídeos (178 asininos, 43 equinos e 6 muare), capturados pela polícia rodoviária e funcionários da prefeitura, foram coletados por punção venosa. A pesquisa de anticorpos anti-*Brucella abortus* foi realizada empregando-se, como triagem, o teste do antígeno acidificado tamponado (AAT) e como confirmatório o teste de redução pelo 2-Mercaptoetanol (2ME). Dos animais testados, quatro (1,76%) foram positivos para anticorpos anti- *B. abortus*. Todos os

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positivos eram equinos (9,30%, 4/43). A análise das variáveis levantadas (sexo e idade) como possíveis fatores associados à infecção por *B. abortus* não revelou a existência de associação entre estas e a soropositividade. Assim, o presente estudo permite concluir que a infecção por *B. abortus* está presente em equinos do município de Mossoró, Rio Grande do Norte.

**Palavras-chave:** Brucelose, *B. abortus*, equídeos errantes, Mossoró

*Brucella abortus* infection in horses was a well-studied subject during the 1970s and 1980s but recently, only a few reports could be found in the literature. Brucellosis in equids is important due to its clinical manifestations, which could result in the reduction of working capacity. Moreover, infected equids could be a potential source of infection to other species, including man (DENNY 1973). The frequencies of *Brucella* spp. infection in equids observed in studies throughout the world vary from 0.0% to 73.1% in horses, 0.0% to 7.4% in donkeys and 0.0% to 0.95% in mules (HIPÓLITO; SOUZA; GIÓVINE, 1943; HUTCHINS; LEPHERD, 1968; VIANA; REIS; SANTOS, 1981; LANGONI; SILVA, 1997; OMER et al., 2000; SILVA et al., 2001; REFAI, 2002; RIBEIRO et al., 2003; MUSA, 2004; OCHOLI et al., 2004; CVETNIC et al., 2005; ACOSTA-GONZÁLEZ; GONZÁLES-REYES; FLORES-GUTIÉRREZ, 2006; AGUIAR et al., 2008; ABO-SHEHADA, 2009; ARAÚJO et al., 2009; JUNQUEIRA JUNIOR, 2012). The most frequent clinical manifestations of brucellosis in equids are lesions of articular surfaces and synovial membranes, as bursitis, synovitis, osteoarthritis and osteomyelitis (LANGENEGGER; SZECHY, 1961; COLLINS et al., 1971; DENNY 1973). Reproductive problems are less frequent, although abortion has already been reported in mares and donkeys (CROSSMAN; BONSON, 1968; ROBERTSON et al., 1973; HINTON; BARKER; MORGAN, 1977).

In Brazil, the epidemiological situation of brucellosis in horses, and especially in donkeys and mules is poorly known, due to the scarcity of research focusing this disease (LANGENEGGER; SZECHY, 1961; GODOY; BARG, 1976; VIANA; REIS; SANTOS, 1981; LANGONI; SILVA, 1997;

RIBEIRO et al., 2003; JUNQUEIRA JUNIOR, 2012). *B. abortus* is the most commonly agent associated with brucellosis in equines, and horses usually get infected when raised in close contact with infected cattle (MCNUTT; MURRAY, 1924; CARPENTER; BOAK, 1937; STONE, 1938; FITCH; DODGE, 1939; MACMILLAN, 1985). The isolation and identification of *Brucella* spp. provide the conclusive diagnosis of brucellosis in horses, however, due to biosafety and technical issues associated with the culture of *Brucella* spp., serological tests, which shows good correlation with the infection status, were widely used for the identification of infected animals (DENNY, 1972).

Considering the zoonotic aspects of brucellosis, losses from the infection for breeders and the lack of information on the epidemiology of brucellosis in equids, the aims of this study were (i) to determine the occurrence and (ii) to evaluate possible factors associated with *B. abortus* infection in free-ranging equids from the municipality of Mossoró, Rio Grande do Norte, Brazil.

The survey was conducted between August 2009 and March 2010, in the municipality of Mossoró, Rio Grande do Norte, Brazil. All free-ranging equids captured by the highway police and the agents of the prefecture of Mossoró in the period were included in the study. Blood of 227 equids – 178 donkeys, 43 horses and 6 mules-, were collected by venipuncture and serum was separated and stored at  $-20^{\circ}\text{C}$  until the time of analysis. The gender and age of each animal were recorded. The age of animals was estimated by modification in the occlusal face, the eruption of the teeth and by the continued erosion of its constituents (SILVA et al., 2003).

Sera were screened by the rose bengal test (RBT) (Instituto de Tecnologia do Paraná – TECPAR,

Brazil) and confirmed for *B. abortus*-antibodies by the standard tube agglutination (STAT) and the 2-mercaptoethanol (2ME) tests (TECPAR, Brazil) (ALTON et al., 1988; BRASIL, 2006). Animals presenting serological titers greater than or equal to 50 in STAT were considered positive (DENNY, 1973). Prevalence and confidence intervals were calculated by the function binCI of the package binGroup (ZHANG et al., 2011) using the R software, version 2.14.1 (R DEVELOPMENT CORE TEAM, 2011). Association of seropositivity and gender or age was studied by the chi-square and Fisher exact test, using an  $\alpha$  error of 0.05 (SAMPALIO, 2002). Factors associated to infection were analysed using the software Epi-Info 6.4 (DEAN et al., 1995). This experimental protocol was approved by the Ethics

Committee in Animal Experimentation of the Universidade Federal de Minas Gerais (CETEA-UFMG, Protocol 182/10).

Of the 227 equids tested, four (1.76%, 95% Confidence Interval (CI): 0.48% to 4.45%) were positive for antibodies anti-*B. abortus*. No donkeys (0%, 95% CI: 0%; 2.05%) nor mules (0%, 95% CI: 0%; 45.93%) showed positive results. All four positive animals were horses; the frequency of positive animals among horses was 9.30% (95% CI: 2.59% ; 22.14%) (4/43). The titers shown by positive horses for STAT and 2ME were, respectively, 100 and 50, 100 and negative, 100 and negative, and 50 and negative. No association was found between gender or age and seropositivity for *B. abortus*. The results are summarized in Table 1.

**Table 1.** Number of equids positive and negative to *B. abortus* infection according to the possible factors associated with brucellosis.

Factors associated		Positive	Negative	P <sup>1</sup>
Sex				0.339
	Male	1/21	20/21	
	Female	3/22	19/22	
Age				0.825
	Up to 2 years	0/1	1/1	
	3 to 5 years	0/5	5/5	
	6 to 8 years	3/27	25/27	
	9 to 11 years	1/6	5/6	
	Above 12 years	0/4	4/4	

<sup>1</sup>probability of random occurrence (P) in the univariate analysis.

Source: Elaboration of the authors

The presence of seropositive horses in the equine population of the Mossoró suggests that these animals were in contact with *B. abortus*, since the diagnostic tests employed have good correlation with the infection (MACMILLAN et al., 1982). Cattle are the main host of *B. abortus* (CORBEL; ELBERG; COSIVI, 2006), and although the mechanism of transmission of brucellosis to horses is unclear, it is considered to be favored by cohabitation between these two domestic species (DENNY, 1972). In most cases, horses infected with *B. abortus* have great

contact with cattle (DENNY, 1973). It is also worth considering that, after being captured, the animals were confined, transferred to another location, and, then released again. Thus, animals with different health status are grouped together for a short period and then released, enlarging the risk of transmission of *B. abortus* among the animals.

Bovine brucellosis is endemic throughout Brazil (POESTER; GONÇALVES; LAGE, 2002) and serological surveys conducted in cattle in some

States of the Northeastern Region have confirmed the presence of seropositive herds and animals in the region (ALVES et al., 2009; SILVA et al., 2009). Although data on the situation of brucellosis in the State of Rio Grande do Norte is scarce, the occurrence of positive horses observed in this study may reflect the presence of infection in cattle population of the state.

A direct comparison among the frequencies of the positive animals (horses, mules and donkeys) found in serological surveys conducted in Brazil and in different countries and the results obtained in the present study is difficult, mainly due to differences in experimental designs, serological assays, diagnostic cutoff points, and size and representativeness of the sampling. Although donkeys and mules show a similar infection course as that observed on horses (LANGENEGGER; SZECHY, 1961; CROSSMAN, BONSON, 1968; PORTUGAL et al., 1971; ENRIGHT, 1990), it is hard to assess the real impact of *Brucella* spp. infection on these species or their importance in the epidemiology of brucellosis. The assessment of the situation of brucellosis in donkeys and mules is of particular importance in the Northeastern region, since this is the Brazilian region which concentrates the largest population of these two species (IBGE, 2006). The absence of seropositive animals among donkeys and mules in the present study suggests that the infection is not present or the prevalence is too low in their population in Mossoró. Moreover, in the case of mules, the number of animals captured was probably too small to assess the prevalence, as suggests the large confidence interval of the prevalence.

In conclusion, the present study indicates that infection with *B. abortus* is present in free-range horses of the municipality of Mossoró, Rio Grande do Norte, Brazil.

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