Serological prevalence of *Brucella* spp. in feral pigs and sympatric cattle in the Pantanal of Mato Grosso do Sul, Brazil

Prevalência sorológica de *Brucella* spp. em porcos ferais e bovinos em simpatria no Pantanal do Mato Grosso do Sul, Brasil

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Abstract

The aim of this study was to estimate the prevalence of anti-*Brucella* antibodies in feral pigs and sympatric cattle in the Pantanal sub-regions of Paiaguás and Nhecolândia. The study was conducted in Corumbá, State of Mato Grosso do Sul, Brazil. A total of 105 feral pigs and 256 cattle were sampled on 12 farms. Blood samples were collected from all the animals for serological diagnosis with buffered acidified antigen (BAA) for screening, confirmatory 2-Mercaptoethanol (2-ME) test, and comparative fluorescent polarization assay (FPA). The positive prevalence of feral pigs was 1% (1/105) in BAA and FPT, with no positive result confirmed of BAA in 2-ME. The prevalence of positive sampled cattle was 11.32% (29/256), 4.3% (10/256), and 7.42% (19/256) in the BAA, 2-ME, and FPT tests, respectively. The degree of agreement obtained among the serological tests in cattle was Kappa = 0.506 (p < 0.001), 95% CI (0.282-0.729). The results of serological tests showed that brucellosis is widespread in cattle herds of the studied region, but the same type of exposure to the agent did not occur in feral pigs according to the used diagnostic tests.

Key words: Brucellosis. Diagnosis. Bovine. Serology. Suidae.
obtido entre os testes sorológicos utilizados nos bovinos foi de Kappa = 0,506 (p < 0.001), 95% CI (0.282 – 0.729). Os resultados dos testes sorológicos demonstraram que a brucelose está disseminada nos rebanhos bovinos da região estudada, porém o mesmo tipo de exposição ao agente não ocorreu nos porcos ferais de acordo com os testes de diagnóstico utilizados.


### Introduction

In 2000, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) initiated the national program for the control and eradication of brucellosis and tuberculosis (PNCEBT), outlining strategies to combat brucellosis and tuberculosis at a national level (BRASIL, 2006). In addition to the strategies to combat these diseases, the epidemiological information on the participation of free-living animals should be considered since the control plan does not take into account farms where wildlife animals live with domestic animals (GODFROID et al., 2013).

Feral or wild forms of *Sus scrofa* are among the most harmful exotic species due to the impacts caused in agriculture and natural environments through damages to crops, disease transmission, and destruction and predation of nests and wild animal specimens (LOWE et al., 2000).

Feral pigs are reservoirs of several etiological agents, viral and bacterial, that cause diseases in production animals and humans (RUÍZ-FONS et al., 2006; MENG et al., 2009). Among these diseases, swine brucellosis, caused by *Brucella suis*, is widely reported in feral pigs of several countries (DREW et al., 1992; LEISER et al., 2013). *Brucella abortus* is also described in feral pigs in sympatry with cattle, which inhabited previously endemic sites (STOFFREGEN et al., 2007).

In the Pantanal, feral pigs, regionally called “Porco-Monteiro”, live in the same environment of cattle (MOURÃO et al., 2002), being this region endemic for bovine brucellosis (PELLEGRIN et al., 2006; CHATE et al., 2009; LEAL FILHO et al., 2016). Therefore, the serological survey on the frequency of brucellosis in feral pigs is essential for understanding the epizootiology of this disease in those animals (LEISER et al., 2013). The aim of this study was to determine the prevalence of anti-*Brucella* antibodies in feral pigs and sympatric cattle in the Pantanal sub-regions of Paiaguás and Nhecolândia.

### Material and Methods

The study was conducted in Corumbá, Mato Grosso do Sul State, Brazil. The capture area comprised two sub-regions of the Pantanal: Nhecolândia and Paiaguás. Both regions present a high density of feral pigs (MOURÃO et al., 2002). The points where the captures took place comprised nine private farms of beef cattle, two of them located in the sub-region of Paiaguás and the remaining in the sub-region of Nhecolândia.

The sample size was calculated using the statistical program Epi Info® version 7. From May 2013 to January 2015, 105 adult feral pigs (66 females, 28 males, and 11 castrated males) were captured and 256 females were sampled on farms of both sub-regions of the Pantanal (Figure 1), constituting an average of 30 cattle and 11 feral pigs on each of the 9 farms. Females included in this study were vaccinated with strain B19 up to eight months of age and were 24 months or older to avoid interference of vaccine antibodies in the serological test results.

Feral pigs were captured as soon as located in the field. The approach was carried out using a vehicle and the animals were manually captured or using a noose and then their limbs were tied for a complete physical containment. Subsequently, the feral pigs were anesthetized with a combination of 10% xylazine hydrochloride (Sedomin, Konig,
Blood collection was carried out by puncturing the lateral saphenous vein of feral pigs and puncturing the jugular vein of the cattle. The collected samples were stored in styrofoam with ice for transportation to the screening site. After a complete clot retraction, the tubes were centrifuged at 3000 rpm per 10 minutes to obtain the serum, which was stored in a freezer at −20 °C.

The serological tests used in cattle and feral pigs included screening with the buffered acidified antigen (BAA) test and the 2-Mercaptoethanol (2-ME) confirmatory test, being considered as seropositive the animals that resulted positive in both tests. The second diagnostic test for serological evaluation was the fluorescent polarization assay (FPA), which was used as described in the diagnostic kit manual (BRUCELLA FPA®, United States) at 1:50 dilution. For this test, samples with a result of 20 millipolarization units (mP) over the mean of the negative control were considered positive.

To measure the degree of agreement between the results of the serological tests 2-ME and FPA, the Kappa statistical test was performed using the software IBM SPSS Statistics 20. Data were calculated and interpreted according to Landis and Kock (1977): 0-0.20 = bad; 0.21-0.40 = poor; 0.41-0.60 = moderate; 0.61-0.80 = good; and 0.81-1 = excellent.
All captures and procedures in feral pigs were approved by the animal ethics committee/CEUA of the Federal University of Mato Grosso do Sul/UFMS, registered under the number 500/2013 and authorization of IBAMA with SISBIO under the number 35296-7.

**Results and Discussion**

From the 105 serum samples from feral pigs, a prevalence of 1% (1/105) was obtained for BAA and 1% (1/105) for FPA, with no positive samples in the 2-ME confirmatory test. In the sampling of cattle sera, 11.32% (29/256) of the samples were positive in BAA, but after the 2-ME confirmatory test, this percentage decreased to 4.3% (10/256), with two farms not presenting positive results in the 2-ME serological test. With the use of FPA, a prevalence of positive cattle of 7.42% (19/256) was obtained for the presence of anti-Brucella antibodies. In addition, all farms presented at least one positive animal.

The degree of agreement obtained between the serological tests 2-ME and FPA used in cattle was Kappa = 0.506 (p<0.001), 95% CI (0.282-0.729), which is considered as a median concordance (LANDIS; KOCK, 1977).

The serological prevalence of feral pigs was 1% in the FPA test, being this animal considered as positive because it was a single test, different from the other feral pig that was positive in BAA, but a confirmation was not obtained using the 2-ME confirmatory test. Therefore, it was a nonspecific reaction and led to a 0% prevalence related to the 2-ME confirmatory test. When comparing the results of the tests BAA and 2-ME, the prevalence was different from that obtained by Paes et al. (2009), who observed a positivity in 8/162 in the BAA test in feral pigs captured in the sub-region of Nhecolândia. From this total, the authors confirmed 1.2% (2/162) using the 2-ME confirmatory test.

Leiser et al. (2013) reported that the prevalence of *Brucella* spp. in feral pig populations can be influenced by several factors, such as the difference between the sampled locations, temporal variation, and used diagnostic techniques. In addition, a high variation may occur in the percentages of seropositive animals for brucellosis in feral pig populations, such as those registered in the United States, which ranged from 0.3 to 52.6%.

The frequency obtained from the 2-ME results in cattle was lower when compared to the data described by Leal Filho et al. (2013), who found a value of 8.9% for individuals in the Pantanal region of Mato Grosso do Sul. The prevalence obtained in the FPA test for both the sampled cattle and the herds was higher in comparison to the 2-ME test. This shows that brucellosis is well disseminated in the region, which was also observed by Pellegrin et al. (2006) and Monteiro et al. (2006).

The agreement of the serological tests used in cattle was median. This difference was mainly due to the sensitivity and specificity of the tests, resulting in a higher prevalence when using the FPA test because it is more sensitive and corroborating with the studies carried out by Nielsen et al. (1999) and Mathias et al. (2010).

The serological results of cattle indicate their exposure to *Brucella* spp. In locations where brucellosis is enzootic in ruminants, feral pigs can be infected by *B. abortus*, causing the disease in pigs (EFSA, 2009). However, no relationship was found in our study by means of both serological tests used in feral pigs and cattle living in the same environment. Perhaps direct techniques such as the polymerase chain reaction or bacterial isolation can assist in the response since in feral pigs the diagnoses of the serological tests are less informative, either because of the low frequency in these animals or because it is difficult for the serological tests to detect the anti-*Brucella* due to the characteristics of the agent in the feral pigs (LEISER et al., 2013).

Ray (1979) confirms that the test in individuals is not ideal and observed that in pigs, antibody titers
tend to decline faster when compared to cattle. Therefore, the use of serology does not necessarily reflect the real frequency of contact of feral pigs with Brucella spp. and epidemiological investigations in these populations should involve additional levels of complexity, such as the approaches of genomic and genetic techniques for an appropriate management of the situation (LEISER et al., 2013).

**Conclusion**

The results of the serological tests showed that brucellosis is widespread in cattle herds of the studied region. However, the same type of exposure to this agent did not occur in feral pigs according to the diagnostic tests used in this study, probably with localized cases of contact of feral pigs with Brucella spp.

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