DOI: 10.5433/1679-0359.2018v39n6p2437

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

Namor Pinheiro Zimmermann[1*]; Igor Alexandre Hany Fuzeta Schabib Peres[2]; Paulo Henrique Braz[3]; Raquel Soares Juliano[4]; Luis Antônio Mathias[5]; Aiesca Oliveira Pellegrin[4]

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.

Resumo

O objetivo deste estudo foi estimar a prevalência de anticorpos anti-*Brucella* em porcos ferais e bovinos simpátricos nas sub-regiões pantaneiras do Paiaguás e Nhecolândia. O estudo foi conduzido no município de Corumbá, Estado de Mato Grosso do Sul, Brasil. Foram amostrados 105 porcos ferais e 256 bovinos em 12 propriedades, em todos os animais foram coletadas amostras de sangue para o diagnóstico sorológico com Antígeno Acidificado Tamponado (AAT) para triagem, teste confirmatório 2-Mercaptoetanol (2-ME) e comparativo com Teste de Polarização Fluorescente (TPF). A prevalência de porcos ferais positivos foi de 1% (1/105) no AAT e TPF, não tendo sido confirmado nenhum resultado positivo do AAT no 2-ME. A prevalência de bovinos positivos amostrados foi de 11,32% (29/256), 4,3% (10/256) e de 7,42% (19/256) nos testes AAT, 2-ME e no TPF, respectivamente. O grau de concordância

[1] Prof., Centro Universitário da Grande Dourados, UNIGRAN, Dourados, MS, Brasil. E-mail: namorvet@gmail.com
[2] Analista, Empresa Brasileira de Pesquisa Agropecuária, Pantanal, Embrapa Pantanal, Corumbá, MS, Brasil. E-mail: igor.peres@embrapa.br
[3] Prof., Instituto Federal Farroupilha, IFFAR, Frederico Westphalen, RS, Brasil. E-mail: pauloh.braz@hotmail.com
[4] Pesquisadoras, Empresa Brasileira de Pesquisa Agropecuária, Pantanal, Embrapa Pantanal, Corumbá, MS, Brasil. E-mail: raquel.juliano@embrapa.br; aiesca.pellegrin@embrapa.br
[5] Prof., Faculdade de Ciências Agrárias e Veterinárias /Universidade Estadual Paulista, FCAV/UNESP, Jaboticabal, SP, Brasil. E-mail: lmathias@fcav.unesp.br
[*] Author for correspondence

Received: Nov. 08, 2017 - Approved: Sept. 13, 2018

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.
Palavras-chave: Brucelose. Diagnóstico. Bovino. Sorologia. Suídeos.

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 (RUIZ-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

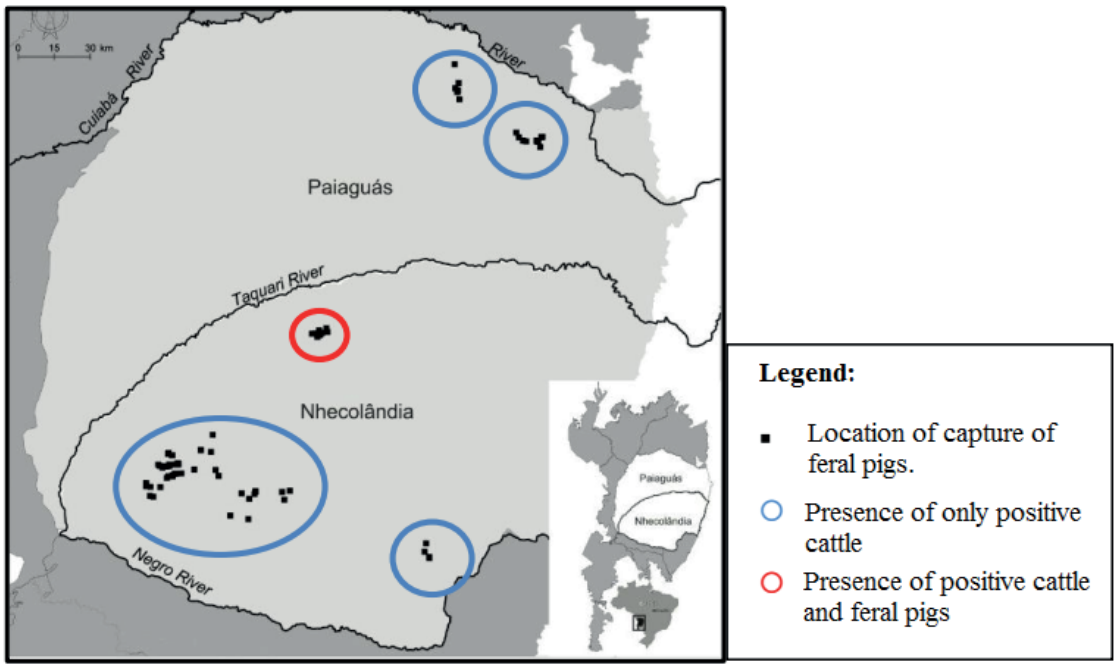
The study was conducted in Corumbá, Mato Grosso do Sul State, Brazil. The capture area comprised two sub-regions of the Pantanal: Nhecolândia and Paiaguás. Both regions present a high density of feral pigs (MOURÃO et al., 2002). The points where the captures took place comprised nine private farms of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

Brazil), 5% ketamine (Cetamin, Syntec, Brazil), and 0.5% midazolam (Dormire, Cristália, Brazil) (1 mg/kg, 5 mg/kg, and 0.25 mg/kg, respectively). During the chemical containment and collection procedures, body temperature, heart rate, respiratory rate, peripheral reflexes, percentage of peripheral oxygenation, and noninvasive blood pressure were checked every 10 minutes, in addition to a physical examination. A standardized form was used to register the capture site using a GPS (GARMIN® GPSMap 76CSx) and the information specific to each animal. All feral pigs were identified with numbered earrings to avoid recapturing the same animal.

Figure 1. Map of the Pantanal sub-regions with the indication of the sampling sites.



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.

Acknowledgments

We thank the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia of the State of Mato Grosso do Sul (FUNDECT). Part of this work was executed with funds from project TO 083/2014, call No. 011/2014.

References

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento - MAPA. Programa Nacional de Controle e Erradicação da Brucelose e da Tuberculose Animal (PNCEBT). Brasília: MAPA/SDA/DSA, 2006. 188 p.

CHATE, S. C.; DIAS, R. A.; AMAKU, M.; FERREIRA, F.; MORAES, G. M.; COSTA NETO, A. A.; MONTEIRO, L. A. R. C.; LÔBO, J. R.; FIGUEIREDO, V. C. F.; GONÇALVES, V. S. P.; FERREIRA NETO, J. S. Situação epidemiológica da brucelose bovina no Estado do Mato Grosso do Sul. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 61, n. 1, p. 46-55, 2009.

DREW, M. L.; JESSUP, D. A.; BURR, A. A.; FRANTE, C. E. Serologic survey for brucellosis in feral swine, wild ruminants, and black bear of California, 1977 to 1989. *Journal of Wildlife Diseases*, Lawrence, v. 28, n. 3, p. 355-363, 1992.

EUROPEAN FOOD SAFETY AUTHORITY - EFSA. Porcine brucellosis (*Brucella suis*) 1 scientific opinion of the panel on animal health and welfare. *EFSA Journal*, Parma, v. 7, n. 1114, p. 1-112, 2009. Available at: <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.1144/epdf>. Accessed at: 5 nov. 2016.

GODFROID, J.; GARIN-BASTIJI, B.; SAEGERMAN, C.; BLASCO, J. M. Brucellosis in terrestrial wildlife. *Scientific and Technical Review of the Office International des Epizooties,* Paris, v. 32, n. 1, p. 27-42, 2013.

LANDIS, J. R.; KOCH, G. G. The measurement of observer agreement for categorical data. *Biometrics*, Arlington, v. 33, n. 1, p. 159-174, 1977.

LEAL FILHO, J. M.; BOTTENE, I. F. N.; MONTEIRO, L. A. R. C.; PELLEGRIN, A. O.; GONÇALVES, V. S. P.; FERREIRA, F.; DIAS, R. A.; AMAKU, M.; TELLES, E. O.; GRISI-FILHO, J. H. H.; HEINEMANN, M. B.; FERREIRA NETO, J. S. Control of bovine brucellosis from 1998 to 2009 in the state of Mato Grosso do Sul, Brazil. *Semina: Ciências Agrárias,* Londrina, v. 37, n. 5, p. 3467-3478, 2016.

LEISER, O. P.; CORN, J. L.; SCHMIT, B. S.; KEIM, P. S.; FOSTER, J. T. Feral swine brucellosis in the United States and prospective genomic techniques for disease epidemiology. *Veterinary Microbiology*, Barcelona, v. 166, n. 1-2, p. 1-10, 2013.

LOWE, S.; BROWNE, M.; BOUDJELAS, S. *100 of the world's worst invasive alien* species: a selection from the global invasive species database. New Zealand: The Invasive Species Specialist Group (ISSG), 2000. Available at: <https://portals.iucn.org/library/sites/library/files/documents/2000-126.pdf>. Accessed at: 19 out. 2016.

MATHIAS, L. A.; CORBELLINI, L. G.; MAIA, L. Validação inter laboratorial do teste de polarização fluorescente para o diagnóstico sorológico da brucelose bovina. *Ciência Rural*, Santa Maria, v. 40, n. 10, p. 2135-2140, 2010.

MENG, X. J.; LINDSAY, D. S.; SRIRANGANATHAN, N. Wild boars as sources for infectious diseases in livestock and humans. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* London, v. 364, n. 1530, p. 2697-2707, 2009.

MONTEIRO, L. A. R. C.; PELLEGRIN, A. O.; ISHIKAWA, M. M.; OSÓRIO, A. L. A. R. Investigação epidemiológica da brucelose bovina em um estrato do Estado de Mato Grosso do Sul. *Pesquisa Veterinária Brasileira*, Seropédica, v. 26, n. 4, p. 217-222, 2006.

MOURÃO, G.; COUTINHO, M.; MAURO, R.; TOMÁS, W.; MAGNUSSON, W. *Levantamentos aéreos*

de espécies introduzidas no Pantanal: porcos ferais (porco monteiro), gado bovino e búfalos. Corumbá: Embrapa Pantanal, 2002. 22 p. (Boletim de Pesquisa e Desenvolvimento, n. 28).

NIELSEN, K.; GALL, D.; SMITH, P.; VIGLIOCCO, A.; PEREZ, B.; SAMARTINO, L.; NICOLETTI, P.; DAJER, A.; ELZER, P.; ENRIGHT, F. Validation of the fluorescence polarization assay as a serological test for the presumptive diagnosis of porcine brucellosis. *Veterinary Microbiology*, Barcelona, v. 68, n. 3-4, p. 245-253, 1999.

PAES, R. C. S.; RIBEIRO, O. C.; CARNEIRO MONTEIRO, L. A. R.; FIGUEIREDO, A. O.; COSTA NETO, A. A.; OLIVEIRA, J. M.; ROSA, G. O. da; KEUROGHLIAM, A.; PIOVEZAN, U.; HERRERA, H. M. Enfermidades de ocorrência no porco-monteiro (*Sus scrofa*) no Pantanal Sul-Mato-Grossense, Brasil. *Suiform Soundings*, Bogor, v. 9, n. 1, p. 29-34, 2009.

PELLEGRIN, A. O.; LEITE, R. M. H.; SERENO, J. R. B.; LAGE, A. P.; LEITE, R. C.; RAVAGLIA, E. *Brucelose bovina no Pantanal Sul-Mato-Grossense*: dados preliminares. Corumbá: EMBRAPA Pantanal, 2006. 4 p. (Comunicado técnico, n. 58).

RAY, W. C. Brucellosis (due to *Brucella abortus* and *suis*). In: STEELE, J. (Ed). *Handbook series in zoonoses.* Section a: bacterial, rickettial and mycotic diseases. Roca Raton: CRC Press, Inc, 1979. p. 99-127.

RUIZ-FONS, F.; VICENTE, J.; VIDAL, D.; HÖFLE, U.; VILLANÚA, D.; GAUSS, C.; SEGALÉS, J.; ALMERÍA, S.; MONTORO, V.; GORTÁZAR, C. Seroprevalence of six reproductive pathogens in European wild boar (*Sus scrofa*) from Spain: the effect on wild boar female reproductive performance. *Theriogenology*, New York, v. 65, n. 1, p. 731-743, 2006.

STOFFREGEN, W. C.; OLSEN, S. C.; JACK WHEELER, C.; BRICKER, B. J.; PALMER, M. V.; JENSEN, A. E.; HALLING, S. M.; ALT, D. P. Diagnostic characterization of a feral swine herd enzootically infected with Brucella. *Journal of Veterinary Diagnostic Investigation*, Guelph, v. 19, n. 3, p. 227-237, 2007.