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Original Article

Epidemiological study of swine brucellosis in household farms in Federal District of Brazil

Estudo epidemiológico de brucelose em suínos em criatórios de subsistência do Distrito Federal, Brasil

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A B S T R A C T

Brucellosis is caused by 10 species of *Brucella* sp. and is responsible for huge economic losses. There are no specific prophylactic measures present for household pigs, which can be also reservoirs, in the brazilian National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis. Therefore, we conducted a cross-sectional study of blood serum samples from 1793 household pigs from 823 farms in Federal District of Brazil. Serum samples were collected between August and October in 2011, 2012, and 2014 by the veterinarians under the Secretary of Agriculture. The Rose Bengal plate test (RBPT) was used for screening for antibodies against smooth *Brucella* sp. Positive results were confirmed by the slow agglutination test (SAT) and 2-mercaptoethanol test (2-ME). Among the 1793 samples screened with RBPT, 125 were positive for antibodies against smooth *Brucella* sp.; 46 (32%) of these samples showed agglutination in SAT, but only one (0.8%) sample was considered positive by 2-ME. The animal with the positive blood serum was traced back in a household farm whose owner had supplemented feeds with cow milk whey. For preventing swine brucellosis, it is necessary to prevent the cross-infection either by the consumption of contaminated cow milk or by direct contact with infected animals. The government policy should enhance the sanitary education for encouraging household farmers to employ prophylactic measures and ensure biosecurity. The present study results indicate that swine brucellosis risk in household farms occurs in a low prevalence rate in pigs from the Federal District of Brazil.

Palavras-chave:

Brucella sp.

Zoonose

R E S U M O

A brucelose, enfermidade causada por 10 espécies de *Brucella sp*, pode afetar várias espécies de animais. A doença é responsável por perdas econômicas em diversos setores, inclusive na suinocultura. Não existem medidas profiláticas específicas para suínos no Programa Nacional de Controle e Erradicação da Brucelose Animal e Tuberculose -

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Vigilância
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PNCEBT. Foi realizado um estudo transversal durante o período de agosto a outubro em 2011, 2012 e 2014 para o estudo de soroprevalência. Os analistas veterinários da Secretaria de Agricultura coletaram sangue de 1793 suínos de 823 criatórios no Distrito Federal. O teste Rosa de Bengala (RBPT) foi utilizado como triagem para a pesquisa de anticorpos contra *Brucella sp*. Os resultados positivos foram confirmados pelo teste de aglutinação lenta (SAT) e teste de 2-mercaptoetanol (2-ME) em série. Das 1793 amostras testadas pelo RBPT, 125 foram reagentes; sendo que 32% (46) destas amostras apresentaram aglutinação no SAT, mas apenas uma (0,8%) amostra foi confirmada positiva pelo teste 2-ME. O animal positivo foi rastreado em uma chácara de subsistência cujo dono tinha hábito de fornecer soro de leite de vaca aos suínos. Para prevenir a brucelose suína, é necessário evitar a infecção cruzada pelo consumo de leite de vaca contaminado ou pelo contato direto com animais infectados. A política do governo deve melhorar a educação sanitária para incentivar a agricultura familiar a empregar medidas profiláticas e garantir a biossegurança. Os resultados do presente estudo indicam que o risco de brucelose suína é baixo entre os criatórios de suínos do Distrito Federal.

INTRODUCTION

Brucellosis is one of the most important bacterial zoonotic diseases (RAGAN; VROEGINDEWEY; BABCOCK, 2013) worldwide (VALLAT, 2013). It causes economic losses because of reproductive failures in farm animals (CZIBENER et al., 2016) and exerts an economic impact in international trade (OIE, 2016).

Brucella infection has been documented in both domestic and wild animals (NYMO et al., 2013). Although *B. melitensis, B. abortus, B. suis, B. neotomae, B. ovis, B. canis, B. microti, B. pinnipedialis,* and *B. ceti* cause infection in specific hosts, cross-infections may occur (FOSTER et al, 2007).

In Brazil, *B. suis* has been identified in some regions (LEITE et al., 2014). *B. abortus* is known to circulate nationwide (LEITE et al., 2003) and has been reported in cattle (NING et al., 2013) and pigs (AMARAL et al., 2005).

Brucellosis prevalence in swine herds is reported to be 0.34% (BRASIL, 2000). Epidemiological studies can contribute in deciding whether specific actions should be taken. Surveillance is focused on the sanitary conditions in a specific region. The aim of this study was to evaluate swine brucellosis incidence in household pig farms in Federal District of Brazil

MATERIALS AND METHODS

Blood Serum Samples

Blood serum samples were collected in duplicates for the epidemiological survey of the Minister of Agriculture. Sample collection was conducted between August to October in 2011, 2012, and 2014 by the veterinary centers under the Secretary of Agriculture. Approximately 10 mL of blood were collected from the jugular vein and the samples were stored at −18°C until the time of analysis. Each sample was collected from domestic pigs born at their respective farms.

The collection of samples for this study was approved by the Committee for Ethics in Animal Use of the University of Brasilia (UNBDOC 66698/2016). Sample numbers varied according to the number of household farms registered (Table 1).

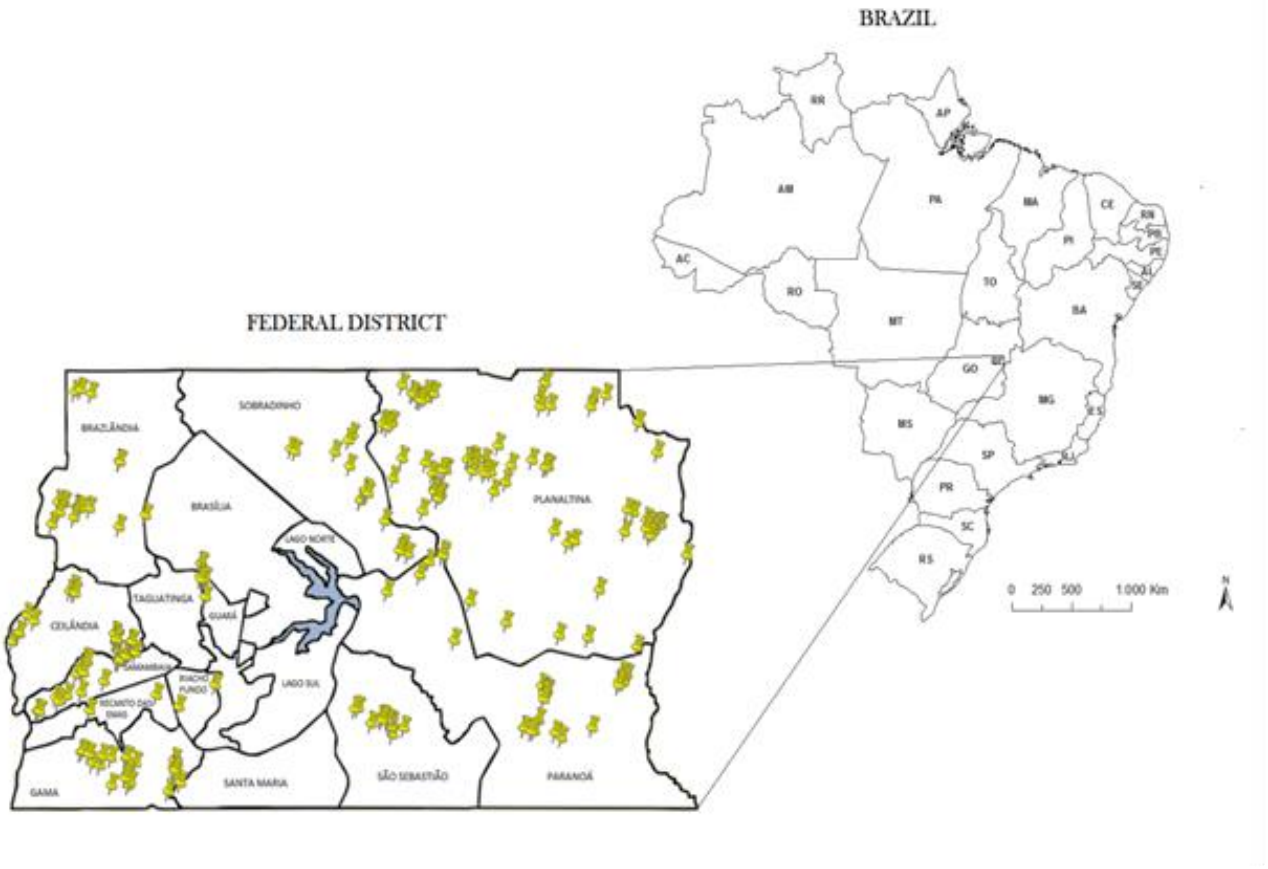
Table 1 – Number of household farms and adult pigs sampled.

Year	household farms registered	household farms sampled	adult pigs sampled
2011	565	210	542
2012	865	293	581
2014	1665	320	670
Total	3095	823	1793

The sample sizes calculation to estimate the true prevalence considered an assumed prevalence of ≥1% in household farms and ≥10% in adult pigs, a confidence level of 0,95 and a desired precision of 0,5. The number of household farms (primary unit) and adult pigs (elementary unit) sampled was calculated by Epitools, sample size calculations to estimate true prevalence. The selection of the household farms (primary unit sample) and the pigs (elementary unit samples) was randomized.

The household farms are situated in 16 localities: Brazlândia, Ceilândia, Cidade Estrutural, Fercal, Gama, Itapoã, Núcleo Bandeirante, Paranoá, Planaltina, Recanto das Emas, Riacho Fundo, Samambaia, Santa Maria, São Sebastião, Sobradinho, and Taguatinga (Figure 1).

Figure 1 - Approximate location of the household farms from which the samples were collected in Federal District of Brazil.



Detection of anti-Brucella immunoglobulin G (IgG)

For detecting antibodies against smooth *Brucella* sp., the Rose Bengal plate test (RBPT; TecPar®) was used as a screening method. Samples that were positive after RBPT underwent confirmation by agglutination test (SAT) and 2-mercaptoethanol test (2ME). Samples that were positive after RBPT were also incubated in a culture medium at 37°C for bacterial culture.

Statistical analysis

Brucellosis prevalence in household farms was calculated by the software STATA®[1]. A p value of < 0.05 was considered statistically significant. Odds ratio with a 95% confidence interval was used to find possible risk factors.

RESULTS

The screening test applied with higher frequency in routine – RBPT – showed more positive results than the other serological tests also considered for screening – SAT. Of 1793 sample tested only one was confirmed by 2-ME (Table II).

Table 2 – Serum samples positives among the serological tests

Sorological Test	Number of serum sampled positive	Percentual of serum sampled positive
RBPT	125	7%
SAT	45	2,5%
2-ME	1	0,05%

This pig with the positive blood serum screened in 2011 was found in a household farm whose owner had supplemented feeds with cow milk whey. The owner does not raise cattle anymore, and the pig had been slaughtered. He stated that all the pigs were born at the farm and he had not purchased pigs since then. Currently, the farm has five pigs, all of which were tested negative.

In 2012 and 2014, there were no positive results. However, assuming that disease was prevalent in pig

herds, disease prevalence had to be less than 1.1% in 2012 and less than 1.2% in 2014, considering the sample size of this study (Table III).

Table 3 – Serum samples positives among the serological tests.

Year	Positive	Prevalence	Confidence interval
2011	1	0,47%	95 % (0 -2,6%)
2012	0	0	97,5% (0 -1,251%)
2014	0	0	97,5% (0 -1,146%)

DISCUSSION

The results of this study are in concordance with those of Braga et al. (2013), who reported 1.04% *Brucella* sp. prevalence in a swine intensive care system in Piauí, and those of Aguiar et al. (2006), who reported 0.9% *Brucella* sp. prevalence in a household farm in Rondônia, respectively. These authors followed the same protocol that we followed—i.e., RBPT as the screening test and SAT and 2-ME as confirmatory tests.

RBPT is recommended for brucellosis screening in pigs (MUÑOZ et al., 2012). RBPT showed a higher sensitivity than SAT, similar to the findings of Dieste-Pérez et al. (2015), who compared RBPT to other serological tests. Although SAT is suitable for detecting recent infections, it is not efficient during the incubation period or in acute stages (KUNDA et al., 2007), because the infected animal could not have sufficient antibodies to be detected by serological tests (PRAUD et a., 2012). SAT is not also recommended immediately after calving or abortion (KUNDA et al., 2007).

The 2-ME test involves laboratory procedures similar to SAT, but it includes the addition of 2-mercaptoethanol in the tube, which breaks the disulfate chains and neutralizes the immunoglobulins IgM, reducing the false positive results, only once the IgG agglutinations are able to resist (NIELSEN, 2002).

Although RBPT inhibits IgM agglutination in an acidic medium (pH 3.65 ± 0.05), nonspecific reactions have been reported (NIELSEN, 2002). The explanation of high levels of sera agglutination on RBPT could be particularly because of the cross-reactions with *Yersinia enterocolitica* O:3 and *Y. enterocolitica* O:9 (NIELSEN, 2006). However, other bacteria, such as *Escherichia hermanni, Salmonella* O:30, *Stenotrophomonas maltophilia, Vibrio cholera* O:1, *E. coli* O:157, *Francisella tularensis, E. coli* O:116, *S. maltophilia*, and *V. cholera*, all of which have similar liposaccharide (LPS-O), are also relevant (NYMO et al., 2013).

Approximately 38% positive samples showed growth in culture medium after incubation at 37°C, but the bacteriological exam did not reveal the bacteria involved in cross-reactions. It was identified strains of *Escherichia coli*, *Streptococcus* sp. and *Salmonella* spp. It is believed that antibodies against LPS-O were the cause for the nonspecific reactions (GANESH et al., 2014).

Disease control must be the focus in the case of animal reservoirs (GODFROID et al., 2013), and prophylactic measures are recommended so that the domestic animals will not be affected (PAPPAS, 2010). In Europe (KREIZINGER et al., 2014) and in USA (LEISER et al., 2013), feral hogs have been monitored via epidemiological surveys. In Brazil, the official serological surveys on household pigs are conducted biennially, with aim for diagnosing classical swine fever. However, technical visits by the veterinarians from the government service improve swine surveillance and contribute to the control of other diseases, because they allow for the verification of the sanitary conditions and guide the farmers to improve these conditions.

The Bovine Brucellosis National Program was launched with the aim of decreasing brucellosis prevalence, and vaccination with Brucella Strain 19 vaccine (SB 19) in 3–8-month-old bovine female (BRASIL, 2006). Prophylactic measures, such as preventing farming of multiple species and avoiding grazed grass calving, play an important role in brucellosis control (NING et al., 2013). Despite this, no specific measures exist for swine herds. Indeed, the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis, Brazil (PNCEBT) goes a long way to not spread the pathogenic agent to pigs, because it contributes to keep the *Brucella* circulation at low levels.

Even with the PNCEBT, compulsory measures are established by Swine Health National Program for reproductive and commercial pig herds to prevent *Brucella* infection, because commercial pig farms employ biosecurity measures. Reproductive swine are tested in every 6 months for several diseases, including brucellosis. These pigs are monitored at slaughterhouses via *antemortem* and *postmortem* veterinary examinations as well as by periodic laboratory tests. Therefore, these programs could have contributed to the low brucellosis incidence in household pig farms.

However, the risk of swine brucellosis infection exists when the pigs are fed the milk of cows infected by *B. abortus*. This is considered a common practice in dairy farms that produce cheese and offer the milk whey for the pigs. Although the actual frequency of this practice has never been analyzed, the government policy should enhance education regarding sanitary practices for encouraging small farm-owners to employ prophylactic measures and ensure biosecurity.

Although this study revealed low circulation rates of swine brucellosis in Federal District of Brazil, the practice of providing bovine milk whey in feeds is a potential risk for the spread of *B. abortus* to swine. An important point to increase vigilance in swine farms would be to include a question regarding the supplementation with bovine milk whey to these animals in the Classical Swine Pest Form. If this is ensured, the Classical Swine Pest survey itself may be useful for the control of brucellosis, because prophylactic measures can be applied by the farmers after veterinarians provide them with animal guidelines.

CONCLUSION

The findings of this study revealed that Brucella infection occurs in a low prevalence rate in pigs from the Federal District of Brazil.

MANUFACTURER

[1]STATACORP. 2011. Stata: Release 12. Statistical Software. College Station, TX: StataCorp LP.

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