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Seroprevalence of antibodies against smooth *Brucella* in small ruminants in the states of Sergipe, Bahia, Ceará and Paraíba

[*Soroprevalência de anticorpos contra Brucella lisa em pequenos ruminantes dos estados de Sergipe, Bahia, Ceará e Paraíba*]

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ABSTRACT

Brucellosis is a zoonotic disease widespread almost all over the world, representing a significant economic and public health problem. *Brucella melitensis*, *B. suis* and *B. abortus* are considered the most pathogenic species for humans. The most virulent species, *B. melitensis* is endemic in many parts of the world, particularly the biovar 3 in the Mediterranean and Eastern Europe. Some Latin American countries are seriously affected by biovar 1, especially Mexico, Peru and northern Argentina. Furthermore, while Brazil is considered free of this etiologic agent, one recurrent question is whether this infection really does not occur in Brazil or there is a lack of research/data on the subject. To answer this question, this study aims to investigate the occurrence of antibodies against smooth Brucella in goats and sheep in the states of Sergipe, Paraíba, Ceará and Paraíba. All samples were screened by the Rose Bengal test (RBT). The complement fixation (CFT) and the fluorescence polarization (FPT) tests were used as confirmatory tests. There were no positive samples in the confirmatory tests (both CFT and FPT). We, therefore, conclude that this result reinforces the previous knowledge regarding the exotic status of *B. melitensis* infection in Brazil.

Keywords: brucellosis, sheep, goats, *Brucella melitensis*, serology

RESUMO

A brucelose é uma das doenças de caráter zoonótico mais difundidas no mundo, representando um grande problema econômico e de saúde pública. A Brucella melitensis, *a* B. suis *e a* B. abortus *são consideradas as mais patogênicas espécies para humanos. A espécie apontada como a mais virulenta é a* B. melitensis, *endêmica em várias partes do mundo, particularmente o biovar 3 na região do Mediterrâneo e na Europa Oriental. Alguns países da América Latina são seriamente afetados pelo biovar 1, especialmente México, Peru e norte da Argentina. O Brasil é considerado livre desse agente etiológico, porém sempre há o questionamento se a infecção não ocorre ou se falta pesquisa. Diante dessa questão, o objetivo deste trabalho foi investigar a ocorrência de anticorpos contra amostras lisas de* Brucella *em caprinos e ovinos dos estados de Sergipe, Bahia, Ceará e Paraíba. Todas as amostras foram submetidas à triagem pelo teste do antígeno acidificado tamponado (AAT). Como testes confirmatórios, utilizou-se a reação de fixação de complemento (RFC) e também o teste de polarização fluorescente (TPF). Nenhuma amostra foi positiva nos testes confirmatórios (RFC e TPF). Esse resultado comprova que a infecção por* B. melitensis *é exótica no Brasil.*

Palavras-chave: brucelose, ovinos, caprinos, Brucella melitensis, *sorologia*

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INTRODUCTION

Human and animal brucellosis is caused by bacteria of the Brucella genus, which comprises twelve species: *Brucella melitensis*, *B. abortus*, *B. suis*, *B. neotomae*, *B. ovis*, *B. canis*, *B. ceti*, *B. pinnipedialis*, *B microti*, *B. inopinata* (Banai and Corbel, 2010), *B. vulpis* (Scholz *et al.*, 2016), *B. papionis* (Whatmore *et al*., 2014). Each *Brucella* species or biovar has a preferred host (Banai and Corbel, 2010). While *B. melitensis*, *B. suis* and *B. abortus* are considered the most pathogenic species for humans; *B. melitensis* is also considered the most virulent (Xavier *et al*., 2010).

According to the World Health Organization (WHO), brucellosis is one of the seven most neglected and underreported diseases (Donev, 2010). The incidence of brucellosis caused by *Brucella melitensis* is very high in sheep and goats, especially in Southeast European and Mediterranean countries.

Rigorous and efficient surveillance of brucellosis not only has economic value but also shows efforts to reduce the impact of the disease on the human population (Mamisashvili *et al*., 2013). The control of human infections depends almost entirely on disease control in animals (Rubach *et al*., 2013; Nicoletti, 2010).

In Latin America, the true incidence of human brucellosis is still unknown. However, *B. melitensis* remains the main cause of infection in humans, and *Brucella* isolated from humans reflects its presence in the animal population (Lucero *et al.*, 2008).

Moreno (2002) reported that in Central America, sheep and goat brucellosis caused by *Brucella melitensis* was identified in Guatemala and there is suspicion in Panama. In South America, in a retrospective study involving 1,933 human and animal samples, *B. melitensis* was the most isolated species from humans (Lopes *et al*., 2010).

According to Samartino (2002), studies on the prevalence of brucellosis in goats reported variable prevalences in Argentina. In the provinces of Tucumán, Catamarca and La Rioja, a prevalence of 0.5 and 0.8% was found in goats. Other studies conducted in the northwestern region of the country showed a prevalence rate of 20-25%. A study in the Province of Salta found 14.9% of reactive animals, and a survey carried out in San Luis showed a prevalence rate of 4.5%. Also, the *Brucella melitensis* biovar 1 has been isolated from infected goats in this country.

Furthermore, *Brucella melitensis* biovars 1 and 2 have been isolated, identified and reported in goat herds in Paraguay (Baumgarten, 2002).

Despite having the largest population of commercial cattle in the world, the lack of official data available for Brazil prevents the assessment of the true incidence of *Brucella melitensis* (Pappas *et al*., 2006). Brazil is considered free from *Brucella melitensis* (Seleem *et al.*, 2010; Poester *et al.*, 2002), but the question remains: the infection does not occur, or is there a lack of research to detect it? Given the importance of this question, this study aims to investigate the occurrence of antibodies against smooth Brucella in blood samples from small ruminant herds in the states of Sergipe, Bahia, Ceará and Paraíba.

MATERIAL AND METHODS

The sample size was calculated considering an expected prevalence of 1%, sampling error of 1% and confidence level of 95% (z = 1.96), using the following equation (Thrusfield, 2010):

$$n = \frac{p\,(1-p)Z^2}{d^2}$$

n: sample size;
p: expected prevalence;
z/Z: confidence level; and,
d: sampling error (prevalence error)

The minimum number of animals to be examined was calculated at 374 per state and per species. The study was conducted in the states of Paraíba, Sergipe, Bahia and Ceará (Fig.1). A total of 3,215 blood samples of apparently healthy sheep were collected between 2010 and 2011, of which 931 samples in Ceará, 923 in Sergipe, 918 in Bahia, and 443 in Paraíba.

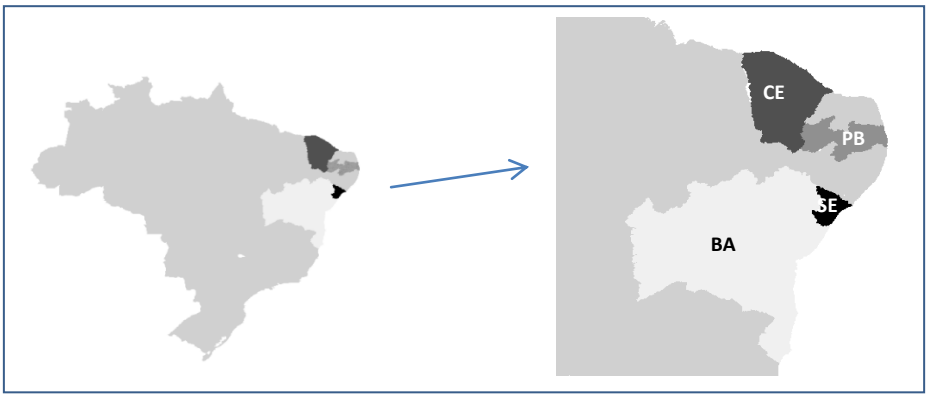

Figure 1. States sampled for the investigation of antibodies against *Brucella* spp in goats and sheep, Brazil, 2010-2011.

The number of sheep herds analyzed was 178, distributed in 34 municipalities: Monteiro, Sumé, São João do Cariri, Quixaba, Pombal, Cacimba de Areia, Passagem and Prata, in the State of Paraíba. The towns of Poço Verde, Simão Dias, Lagarto, Nossa Senhora da Glória, Poço Redondo, Canindé de São Francisco, Gararu and Tobias Barreto located in the State of Sergipe. The towns of Remanso, Juazeiro, Casa Nova, Curaçá, Campo Alegre de Lourdes, Pilão Arcado, Sento Sé and Sobradinho located in the State of Bahia. And, finally, Tauá, Independência, Quixeramobim, Parambu, Pacajus, Quixadá, Granja, Santa Quitéria, Canindé and Sobral located in the State of Ceará (Fig. 2).

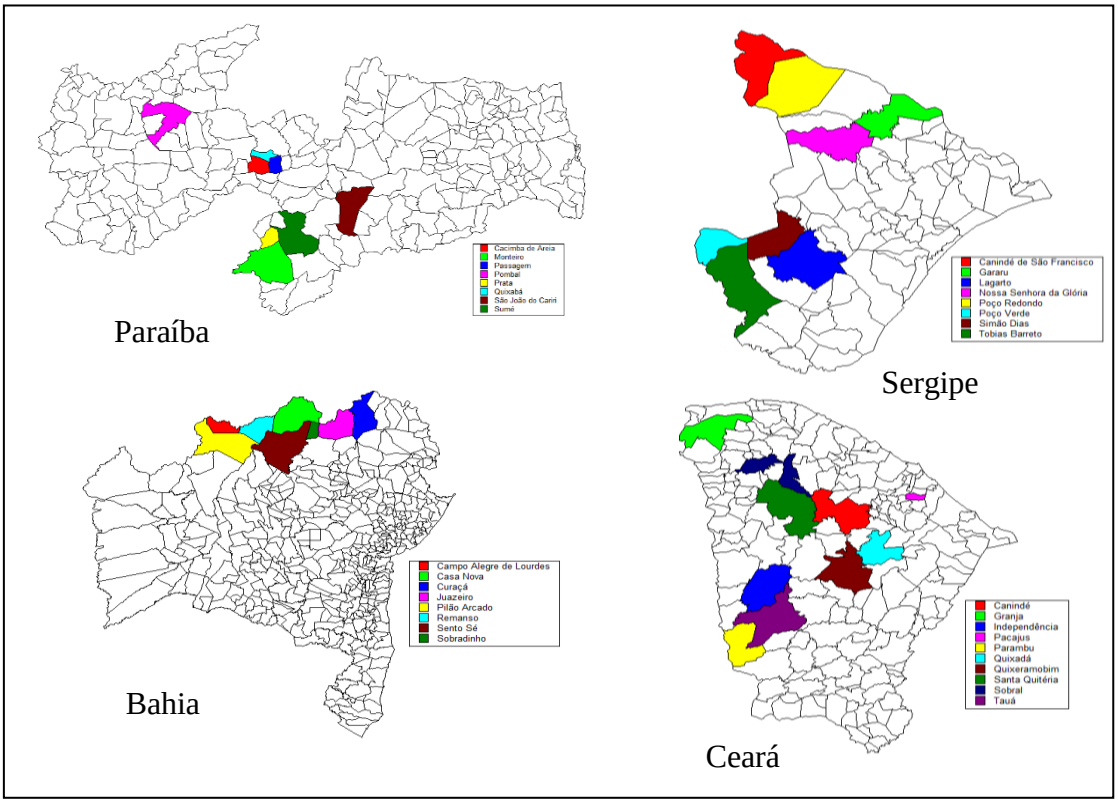

Figure 2. Municipalities sampled for the investigation of antibodies against *Brucella* spp in sheep from the State of Paraíba, Sergipe, Bahia and Ceará, Brazil, 2010-2011.

A total of 3,216 blood samples of apparently healthy goats were collected between 2010 and 2011, of which 1215 in Paraíba, 817 in Ceará, 685 in Bahia, and 499 in Sergipe. The number of herds analyzed was 178, distributed in 39 municipalities: Monteiro, Sumé, São João do Cariri, Quixaba, Pombal, Cacimba de Areia, Passagem, Prata and Camalaú located in Paraíba. The towns of Poço Verde, Simão Dias, Lagarto, Nossa Senhora da Glória, Poço Redondo, Canindé de São Francisco and Gararu located in Sergipe. Remanso, Juazeiro, Casa Nova, Curaçá, Campo Alegre de Lourdes, Pilão Arcado, Sento Sé and Sobradinho located in Bahia. And, finally, Aquiraz, Caucaia, Horizonte, Beberibe, Eusébio, São Gonçalo do Amarante, Independence, Parambu, Tauá, Quixadá, Banabuiu, Santa Quitéria, Granja, Sobral, and Canindé located in Ceará (Fig. 3).

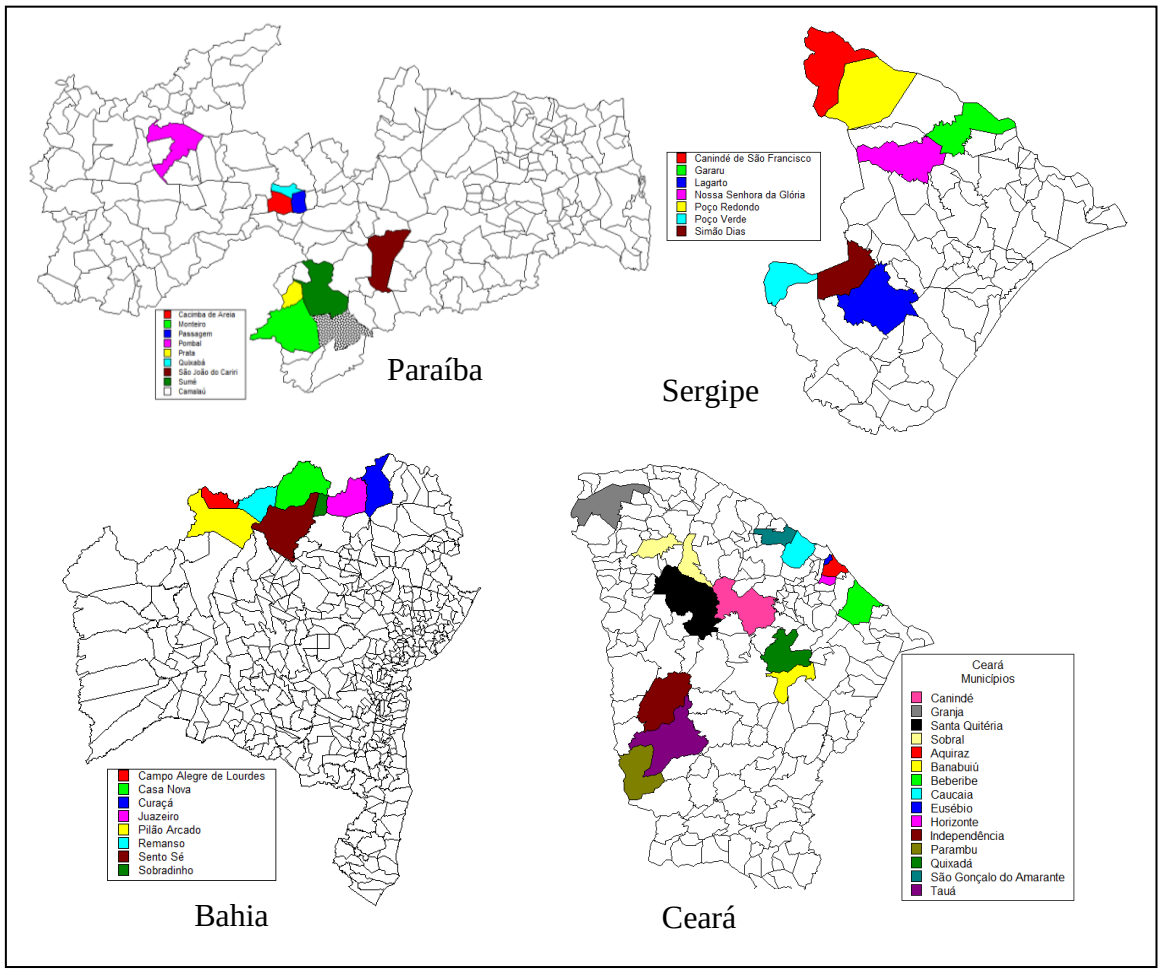

Figure 3. Municipalities sampled for the investigation of antibodies against *Brucella* in goats from the States of Paraíba, Sergipe, Bahia and Ceará, Brazil, 2010-2011.

Non-probabilistic sampling was performed to select the participating farmers, as participation in the study was always voluntary. The considered sampling universe consisted of all properties listed by breeder associations, municipal departments of agriculture, agricultural defense agency and technicians from extension companies. The two mesoregions with the highest number of sheep and goat in the state were selected.

In each mesoregion, the cities with the largest sheep and goat herds were chosen. In the farms, the animals were selected based on a non-probabilistic way, respecting the following ratio: 60% adult females (aged 6 months), 30% young

(up to 6 months old), and 10% adult males (aged 6 months or older), a maximum of 20 animals total.

The screening test chosen to diagnose brucellosis was the buffered, acidified plate antigen (BPA) test, using *Brucella abortus* as antigen at 8% concentration, buffered in acidic pH (3.65) and stained with Rose Bengal, following the rules of the technical manual of the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal, PNCEBT) (Brasil, 2006).

The samples reactive to the BPA were submitted to the complement fixation (CFT/RFC) and fluorescence polarization (FPT/TPF) confirmatory tests. The positive animal was only confirmed as infected when reactive/positive after the confirmatory tests while the uninfected animal was negative in the screening test and/or in the confirmatory tests.

In the complement fixation reaction, the microtechnique with incubation at 37ºC in the two phases of the reaction was used as recommended by Alton *et al.* (1988), with antigen prepared with *Brucella abortus* sample 1119/3 to test for serum-agglutination in tubes. All reagents used were standardized following the technique mentioned above. The final test result was expressed as a titer, that is, the highest serum dilution that showed at least 25% of complement fixation, with samples with a titer greater than or equal to four being considered positive.

For the fluorescent polarization test (FPT/TPF) the "*Brucella abortus* Antibody Test Kit", produced by Diachemix, USA. The kit consists of four components: Positive Control, Negative Control, 25x Concentrated Buffer and Conjugated Antigen. The reading was done on a Sentry 100 model fluorescent polarization analyzer (Diachemix, USA). The test was carried out in borosilicate tubes (10 x 75mm) in good condition. The test results were interpreted following the manufacturer's recommendations, positive samples had a value greater than 20 mP above the mean of the values obtained for the negative control while the negative samples had a reading less than 10 mP above the mean of the negative control.

RESULTS AND DISCUSSION

Samples were reactive in the screening test, however, none were reactive in the confirmatory tests. No positive results were detected among the samples tested, thus strengthening the information regarding no evidence of the occurrence of *Brucella melitensis* in the studied region in Brazil. According to Papas *et al.* (2006), the lack of available official data prevents the assessment of the true prevalence of the pathogen in Brazil. The result of this study confirms the conclusions of Seleem *et al.* (2010) and Poester *et al.* (2002) stating that *Brucella melitensis* is not present in Brazil. Salaberry *et al.* (2011) also did not find animals reactive to *Brucella abortus* when studying 334 sheep in the municipality of Uberlândia, MG.

Taking into account the high number of samples and the breadth of this work carried out in 42 municipalities from four different states highly representative of the goat and sheep herds in the country, we believe that the present result can be considered as a reflection of the reality in Brazil, because, according to IBGE (Pesquisa…, 2018), the Northeast region concentrates 94% and 67% of the Brazilian goat and sheep herds, respectively.

Brucellosis has great economic importance due to the impact on animal production caused by the losses arising from reproductive problems, as well as a decrease in animal productivity, and because it represents a public health problem especially since brucellosis caused by *Brucella melitensis* is the most serious form of the disease as it is the most pathogenic and the most virulent for human beings. Therefore, the result found in this work is of paramount importance for animal production and public health, as it reflects the sanitary aspect of goat and sheep herds regarding a serious disease that can create commercial barriers, and especially the aspect related to the protection of human health. Furthermore, the study conclusion is compatible with the knowledge that the infection by *B. melitensis* is rare in Brazil.

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