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Detection of brucellosis in cattle and evaluation of risk factors associated with the disease in workers slaughterhouse

Detecção de brucelose em bovinos e avaliação de fatores de risco associados a doença em trabalhadores de frigorífico

Detección de brucelosis en ganado y evaluación de factores de riesgo asociados a enfermedad en trabajadores de refrigerador

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Thaise Marques Alves
ORCID: https://orcid.org/0000-0002-0934-530X
Universidade Estadual de Santa Cruz, Brazil
E-mail: thaisemarquesalves@gmail.com
Poliana de Castro Melo
ORCID: https://orcid.org/0000-0003-0774-6211
Universidade Estadual de Santa Cruz, Brazil
E-mail: policamello@yahoo.com.br
Lilia Marcia Paulin Silva
ORCID: https://orcid.org/0000-0002-0835-8723
Instituto Biológico de São Paulo, Brazil
E-mail: paulin@biologico.sp.gov.br
Nathana Kyolla Santos de Carvalho
ORCID: https://orcid.org/0000-0003-1332-1711
Universidade Estadual de Santa Cruz, Brazil
E-mail: nathannaemanuelly@hotmail.com
Amora Ferreira Menezes Rios
ORCID: https://orcid.org/0000-0003-3557-2781
Universidade Estadual de Santa Cruz, Brazil
E-mail: amoramenezes@hotmail.com
Raphaella Barbosa Meirelles Bartoli
ORCID: https://orcid.org/0000-0002-7147-5711
Universidade Federal de Jataí, Brazil
E-mail: raphaella@ufg.br
Carlos Augusto de Oliveira Júnior
ORCID: https://orcid.org/0000-0002-9609-0746
Universidade Federal do Oeste da Bahia, Brazil
E-mail: carlos.dirgel@hotmail.com
Henrrique Fernando Santos
ORCID: https://orcid.org/0000-0003-3555-0603
Universidade Estadual de Santa Cruz, Brazil
E-mail: henriqueveterinario@hotmail.com

Abstract
Brucellosis is anthropozoonosis caused by *Brucella spp.* Among the zoonotic species, *B. abortus* is the main species affecting cattle and can easily be transmitted to humans. The purpose of this study was to investigate, through epidemiological inquiry and serological analysis, animal and human health as related to smooth strain *Brucella spp.* in a slaughterhouse located in the southern region of the state of Bahia. For this purpose, blood samples were collected from workers and animals at a slaughterhouse together with the State Inspection Service. Then, the Buffered Acidified Plate Antigen test was performed for animals and humans, the Slow Agglutination Test was performed for humans only; and the Complement Fixation Test and the 2-Mercaptoethanol *Brucella* Agglutination test (2ME) were performed for animals only. In addition, an epidemiological inquiry was applied to workers in order to assess risk factors for the disease. After data analysis, it was concluded that infection by smooth strains of *Brucella spp.* was detected in 14.0% of the cattle. Additionally, one worker out 41 tested reactive to the disease.
Keywords: Cattle slaughter; *Brucella spp.*; Butcher; Zoonosis.

Resumo
A brucelose é uma antropozoonose causada por *Brucella spp.* Entre as espécies zoonóticas, *B. abortus* é a mais importante que afeta bovinos e pode ser facilmente transmitida aos seres humanos. O objetivo deste estudo foi

investigar, a partir de levantamento epidemiológico e análise sorológica, a saúde animal e humana em relação a cepas lisas de *Brucella spp.* em um matadouro localizado na região sul do estado da Bahia. Para esse fim, foram coletadas amostras de sangue de trabalhadores e animais de um matadouro que possui Serviço de Inspeção Estadual. Em seguida, o teste de antígeno tamponado acidificado foi realizado para animais e humanos; o teste de aglutinação em tubo, foi realizado apenas para humanos; e o teste de fixação do complemento e 2-mercaptoetanol foram realizados apenas em animais. Em adição, foi realizada, pesquisa epidemiológica a partir da aplicação de questionários aos trabalhadores para avaliar os fatores de risco para a doença. Após análise dos dados, concluiu-se que a infecção por cepas lisas de *Brucella spp*. foi detectada em 14,0% dos bovinos. Além disso, um trabalhador de 41 funcionários avaliados foi reativo à doença.
Palavras-chave: Abate bovino; *Brucella spp.*, Magarefe; Zoonose.

Resumen
La brucelosis es una antropozoonosis causada por *Brucella spp.* Entre las especies zoonóticas, *B. abortus* es la más importante que afecta al ganado y puede transmitirse fácilmente a los humanos. El objetivo de este estudio fue investigar, a partir de una encuesta epidemiológica y un análisis serológico, la salud animal y humana en relación con cepas lisas de *Brucella spp.* en un matadero ubicado en la región sur del estado de Bahía. Para ello, se recolectaron muestras de sangre de trabajadores y animales de un matadero que cuenta con Servicio de Inspección del Estado. Luego, se realizó la prueba del antígeno tamponado acidificado para animales y humanos; la prueba de aglutinación en tubo se realizó solo para humanos; y la prueba de fijación del complemento y 2-mercaptoetanol se realizó solo en animales. Además, se realizó una encuesta epidemiológica basada en la aplicación de cuestionarios a los trabajadores para evaluar los factores de riesgo de la enfermedad. Después del análisis de los datos, se concluyó que la infección por cepas lisas de *Brucella spp.* se detectó en el 14.0% de los bovinos. Además, un trabajador con 41 empleados evaluados fue reactivo a la enfermedad.
Palabras clave: Sacrificio de ganado; *Brucella spp.*; Carnicero; Zoonosis.

1. Introduction

Bacteria of the genus *Brucella spp.* are Gram negative coccobacilli, of which an important characteristic for pathogenicity is the fact that they are facultative intracellular (Tortora, 2012) any pathogenic species were described as composing this genus. B. melitensis, B. ovis and *B. abortus*, are found in ruminants, the latter being more common in cattle (Osterman & Moriyon, 2006). Swine are affected mainly by B. suis (Emy, 2018) and B. canis is responsible for infection in dogs (Petry, 2019). Other species, such as B. neotomae (desert rat) (Kang, 2019), B. ceti (cetaceans), B. pinnipedialis (pinnipeds) (Jahans, 1997; Scholz, 2008), B. microti (common vole, red fox and wild boar) (Scholz, 2008; Rónai, 2015; Scholz, 2009), B. papionis (baboons) (Whatmore, 2014), afect wild animals. Humans can be infected by B. inopinata (Scholz, 2010) (preferential species) and many species mentioned previously.

The composition of a polysaccharide chain (LPS) of *Brucella spp.* determines two different antigenic groups. B. melitensis, *B. abortus*, *B. suis*, *B. neotomae*, *B. ceti* and *B. pinnipedialis* belong to smooth strains, while *B. canis*, and *B. ovis* are in the rough strains group (Osterman & Moriyon, 2006; Emy, 2018; Petry, 2019; Kang, 2019) *Brucella inopinata* is informally classified by some researchers as atypical, since its phenotypical characteristics differ from the classic groups of *Brucella spp* (Zygmunt, 2012).

Smooth strains of *Brucella spp.* can affect non-specific species. Therefore, the contaminated biological material in pastures and in animal facilities can act as transmission routes for other animals, such as equines, dogs, swine, sheep, goats, and including humans (Osterman & Moriyon, 2006; Emy, 2018; Petry, 2019; Kang, 2019).

In animals, the clinical signs are mainly related to the reproductive system, such as abortion, infertility, retained placenta and the birth of weak animals. These signs are explained because the bacteria have a preference for these tissues and because the presence of erythritol in testis and pregnant uterus increases bacterial multiplication (Antoniassi, 2017).

Transmission of brucellosis in humans occurs by oral route, through consumption of contaminated products of animal origin, such as milk, dairy products and raw or undercooked meat. Another important route is inhalation, through direct contact with infected animals or secretions and the products of abortion. Transmission can also occur by accidental inoculation of vaccines (Schneider, 2013). As regards occupational health, veterinarians, butchers and livestock professionals, who are in

constant contact with potentially infected biological material, are at risk for brucellosis (Zigmunt, 2012; Skendros, 2013).

The incubation period for brucellosis in humans varies from 5 to 60 days, but it could even last for up to two years. The clinical signs are nonspecific, they include fever, malaise, sweating (nightly and profusely), chills, lethargy, anorexia, headache, muscle pain, abdominal pain, and, when chronic, the disease most frequently causes joint pain affecting the individual's quality of life (Corbel, 2006).

In Brazil, the serological diagnosis of brucellosis in cattle and buffalo herds is recommended by the National Program of Control and Eradication of Brucellosis and Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal – PNCEBT) (Brazil, 2017a). The official tests are the Buffered Acidified Antigen test (RBT), the 2-Mercaptoethanol Brucella Agglutination test simultaneously with the Slow Agglutination Test (2ME), the Fluorescence Polarization Assay (FPA), the Complement Fixation Test (CFT), and the Milk Ring Test (MRT), this last one for monitoring dairy herds (Brazil, 2017b).

The laboratorial diagnosis for humans includes the direct test (bacterial culture and PCR) and an indirect test, such as the 2ME, the SAT, the FPA and the Enzyme-Linked Immunosorbent Assay (ELISA) (Brazil,2019). Individuals exposed to the RB51 vaccine strain are not reagent in serological tests, and direct tests (Corbel, 2006; Brasil, 2019; Hyeda 2011) are recommended.

In view of its threat to public health and the economic loss in livestock, epidemiological research of this disease is essential. Therefore, the purpose of this study was to investigate, though epidemiological inquiry and serological analysis, animal and human health related to smooth strains of *Brucella spp.* in a slaughterhouse situated in the Southern region of the state of Bahia, Brazil.se the paragraph as a template.

2. Methodology

Animal procedures were approved by the Ethical Committee for Animal Experimentation of the Universidade Estadual de Santa Cruz (UESC), under protocol 018/18. Together with the State Inspection Service (Serviço de Inspeção Estadual - SIE) five visits were carried out in order to collect blood samples from bovine females aged over 24 months, slaughtered in an exclusive beef slaughterhouse. During the time the survey was being carried out (February 14 to June 31, 2019), around 2000 animals were slaughtered per month. At each visit, an average of 40 blood samples was collected in a tube without anticoagulant, immediately after bleeding.

A total of 179 blood samples were collected, these were cooled and transported to the Microbiology Laboratory of UESC within 6 hours. Then, the samples were centrifuged and the serum was recovered and kept in microtubes at 0 °C. At the Instituto Biológico de São Paulo, RBT, 2ME and CFT tests were performed following instructions from the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA) (Brazil, 2017b).

The procedures involving humans were approved by the Research Ethics Committee of UESC under protocol 2.836.725. Approximately 20 days after the last collection of animal samples, all slaughterhouse workers were asked to take part in a serum- epidemiological inquiry for brucellosis. They were oriented as to all procedures that would be performed, enlightened about the risks and benefits of their participation in this research and informed about the occupational effects and the significance of brucellosis in public health. An Informed Consent Form was signed by 41 workers who agreed to take part in the survey. The blood collection on these workers was done by a nurse, following all ethical norms, and the epidemiological inquiry was carried out by a veterinarian and a veterinary student.

All human samples were identified and sent to the Special Public Health Service (Serviço Especial de Saúde Pública – SESP) of Itabuna, where the serum collection was performed in duplicate. One sample of serum (1 mL) from each participant was sent to a private clinical analysis laboratory (Hermes Pardini®) for performing standard tube (SAT), since the official

results needed to be laid out by a biomedical specialist. The remaining aliquots were transported to the UESC, frozen and subsequently tested through RBT, in accordance to MAPA instructions (Brazil, 2017b).

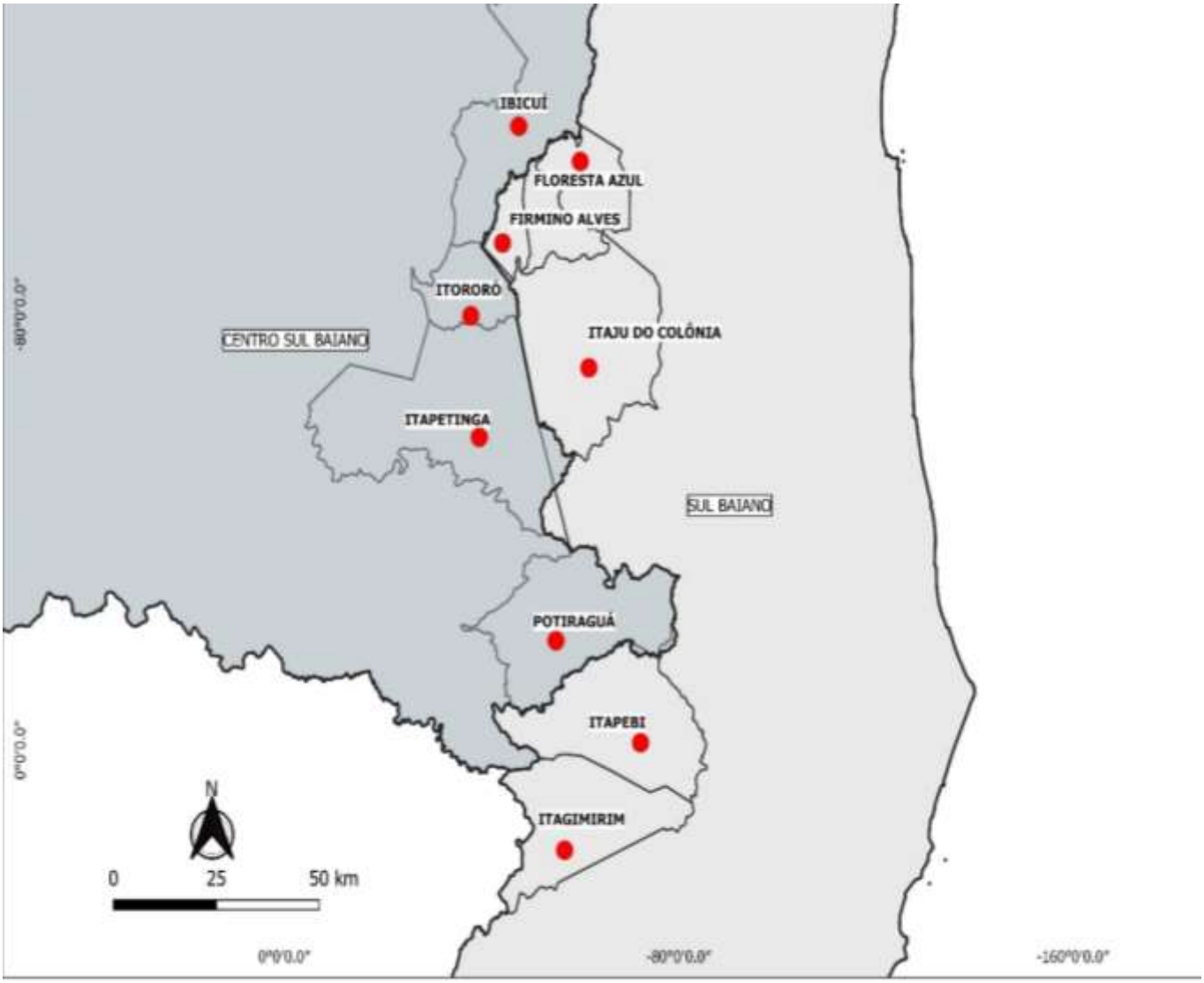
The epidemiological data and serological results from humans and cattle were initially stored in the Excel spreadsheet program. To perform epidemiological analysis both confirmatory tests (2ME and CFT) were considered gold standard. Then, both (the screening test and each confirmatory test individually) were compared to the gold standard. True and false positives and true and false negatives were calculated. Subsequently, the Open Epi version 3.01 was used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's Kappa coefficient, the latter to verify agreement between tests. The data related to risk factors for slaughterhouse workers was analyzed by *Odds ratio* and Fisher exact test with a 0.05 level of significance, both using the Open Epi program.

3. Results

A total of 179 samples of cattle serum belonging to females, aged over 24 months and unvaccinated against brucellosis were evaluated. The sampled animals were from Floresta Azul (25), Itororó (15), Itapetinga (15), Firmino Alves (10), Ibicuí (27), Itapebí (10), Itajú do Colônia (21), Itagimirim (46) and Potiraguá (10). All of these municipalities are located in the state of Bahia, Brazil (Figure 1), more precisely in the South-Central mesoregion, which contributed 67 samples, and the South mesoregion, which housed 112 animals (Table 1).

All samples were submitted to RBT and 2ME and CFT confirmatory tests. Of the tested animals, 39/179 (21.8%) were reagent in the screening test and 25/179 (14.0%) tested positive in both confirmatory tests (Table 2). The South-Central mesoregion showed 10/67 (14.9%) animals testing positive, while in the South mesoregion the number of animals testing positive was 15/112 (13.4%) after confirmatory tests (Table 1).

Figure 1: Geographical location of origin of the cattle investigated according to municipalities of origin and respective mesoregions in the state of Bahia, Brazil. Source: prepared by the author using Qgis software version 3.4.



Source: Authors.

Table 1: Number and percentage of cattle positive for brucellosis in 2ME and CFT by mesoregion and city of origin.

Mesoregion	City	Positive (%)	Total
South-Central	Ibicuí	3 (11.1%)	27
	Itororó	1 (6.6%)	15
	Itapetinga	3 (20.0%)	15
	Potiraguá	3 (30.0%)	10
		10 (14.9%)	67
South	Itagimirim	1 (2.2%)	46
	Itapebí	1 (10.0%)	10
	Itajú do Colônia	4 (19.0%)	21
	Firmino Alves	1 (10.0%)	10
	Floresta Azul	8 (32.0%)	25
		15 (13.4%)	112
Total		25 (14.0%)	179

Source: Authors.

The confirmatory tests, namely 2ME and CFT, showed maximum Kappa coefficient (1,0), indicating total agreement and, consequently, 100% sensitivity and specificity when compared. The comparison of RBT with both confirmatory tests as gold standards demonstrated 100% sensitivity, 90.91% specificity, 64.1% positive predictive value and 100% negative predictive value (Table 2).

Table 2: Serum diagnosis of brucellosis of cattle slaughtered in Southern Region of state of Bahia, Brazil.

Diagnostic test	Result	Samples	Frequency %	Sensitivity	Specifity	PPV	NPV	*Kappa*
RBT	Reagent	39	21.8%	100%	90,91%	64%	100%	0,7364
	Non reagent	140	78.2%					
2ME	Positive	25	14.0%	100%	100%	100%	100%	1
	Negative	154	86.0%					
CFT	Positive	25	14.0%	100%	100%	100%	100%	1
	Negative	154	86.0%					
Total		179	100%	-	-	-	-	-

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Kappa coefficient of diagnostic tests of brucellosis calculated using OpenEpi software version 3.0. Frequency data based on sampling of 179 as 100%. Source: Authors.

Regarding the epidemiological survey and serological results of the 41 workers from the slaughterhouse, one of them was reagent in RBT and positive for brucellosis in SAT, and ELISA detected IgG. Based on answers obtained in the epidemiological inquiry, the Odds ratio was calculated, but it was not statistically significant in any category ($p > 0.05$). As regards the importance of answers referring to the disease transmission path, they have been described in Table 3.

Table 3: Evaluation of risk factors related to brucellosis in workers of a slaughterhouse.

Category	Exposed	Affected	Frequency of exposed (%)	*Odds ratio*	P
Performs function of butcher and farmer	8	0	19.5	NA	-
Performs administrative function in the area or works in external area of slaughterhouse	5	0	12.2	NA	-
Always worked as butcher	26	1	63.4	1,23	0,6837
Already had contact with birth remains or abortion of domestic animals	18	0	43.9	NA	-
Has direct contact with blood of domestic animals	35	1	85.4	0,4	0,4524
Has indirect contact with blood of domestic animals	5	0	12.2	NA	-
Have already performed vaccination against brucellosis	7	0	17.1	NA	-
Have already consumed unboiled or unpasteurized milk	20	1	48.8	2,2	0,4829
Have already consumed dairy products made with unboiled or unpasteurized milk	31	1	75.6	0,7	0,6155
Have already consumed undercooked beef	30	0	73.2	NA	-

Odds ratio calculated in software Open Epi version 3.0, verified through Fisher exact test. *NA: not applicable. Source: Authors.

During the epidemiological inquiry and before the blood sample collection, the individual found positive for brucellosis reported chronic joint pain, but dismissed fever episodes, sweating and reproductive dysfunction.

4. Discussion

The PNCEBT was created in 2001 and since then has gone adapting according to evolution of the disease in the country. In spatial and temporal studies of brucellosis carried out in Brazil between 2014 and 2018, 19,631 cattle tested positive, with incidence among 0.03 and 33.93/100,000 animals (Ribeiro, 2020). This research shows the occurrence of the disease in Brazil and indicates the need for improvement in controlling it.

In this study, 14.0% of sampled animals tested positive for brucellosis. These animals were selected from several different farms in the Southern Region of the state of Bahia and this number was found in only one slaughterhouse in the region. It is noteworthy that the products from this slaughterhouse qualify for the State Inspection Service (SIE) label, which allows the product to be marketed throughout the state, thus posing a threat to the population through contaminated animals.

The prevalence of brucellosis in the Southern region of Bahia was 0.86%, represented by 36 out of 3,565 females that tested positive (Alves, 2009). The higher proportion of animals testing positive during this study may be a consequence of the number of discarded animals in the slaughterhouse. It is widely known that reproductive problems are one of the main reasons for discarding dairy cattle (Ribeiro, 2003). Although the purpose of this study was not the identification of the prevalence of brucellosis, the high proportion of animals testing positive, especially in some cities, points to the need for further studies on this subject.

According to the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal – RIISPOA) (Brazil, 2017c) animals bred for milk and meat production must be monitored for brucellosis and, in the case of animals testing positive, they must be euthanized or slaughtered in an authorized slaughterhouse. However, it can be assumed that diagnosis frequency is insufficient, since the animals that tested positive were routed and received by the slaughterhouse without any indication of positive reaction to brucellosis.

The RIISPOA provides for the release of carcasses for consumption of animals that are positive for brucellosis in the absence of indicative lesions. The regulation also provides, that if this is the case, the udder, genital tract and blood should be condemned (Brazil, 2017c). However, a previous study performed by De Macedo (2019) demonstrated *Brucella spp.* in bursitis lesions in some animals. It should be noted that bursitis is not a typical sign of brucellosis and is not included in the RIISPOA. Therefore, these animals are not condemned in the slaughter line and may pose a risk of contamination to the population.

The first paragraph of the aforementioned RIISPOA (Brazil, 2017c) article states that animals with brucellosis must be slaughtered separately. Reagent and non-reagent animals investigated in this study passed through the slaughter line in a concomitant period. This is a non-conformity to regulation, which probably occurred due to lack of awareness of the serological condition for brucellosis in these animals.

According to the PNCEBT, all cows should be vaccinated against brucellosis (Brazil, 2017a) this was not done on the 179 animals assessed in this study. This revealed a double flaw: in the inspection of farms, where vaccination is not carried out, and in the inspection of slaughterhouses, where unvaccinated animals are accepted. These inspection failures increase the risk of contamination for the population resident in Bahia and favor the continuity of bacteria in the environment.

The 2ME and CFT confirmatory tests showed maximum agreement in this study, detecting the same animals as positive. In a previous study, Meirelles-Bartoli & Mathias (2010) found a *Kappa* coefficient of 0,86 for these tests. The

efficacy of both confirmatory tests has already been proven in vaccinated and unvaccinated animals (Paulin, 2002). Beyond that, the reliability of results is higher when serology is performed by two confirmatory tests concomitantly (Meirelles-Bartoli & Mathias, 2010). On the other hand, RBT showed 100% sensitivity and negative predictive value in this study, which are desirable characteristics for a screening test.

Regarding risk factors, 48.8% of workers claim to have consumed raw milk, 75.6% have already consumed products made from unpasteurized milk and 73.2% have already eaten undercooked meat. This behavior is worrying because it increases the risk of transmission of brucellosis and other zoonoses. In a previous study, DNA from *Brucella spp.* was identified by PCR in 10 samples of unpasteurized milk out of 80 tested, confirming the risk factor (Paula, 2015).

As to contact with infectious sources, 85.4% are in direct contact with bovine blood, while 43.9% have already handled birth or abortion remains of domestic animals. Fetal remains, placenta and aborted fetuses of infected animals can contain the bacteria and be an important reservoir (Antoniassi 2017). As regards to inhalation as a route of transmission (Corbel, 2006) individuals exposed to this factor are more likely to be infected (Skendros, 2013), especially in slaughterhouses which are closed locations with reduced air circulation.

In addition, it was found that 63.4% of workers act professionally only at slaughterhouses, while 19.5% also perform some livestock activity and 17.1% have already vaccinated animals against brucellosis. According to MAPA standards, vaccination against brucellosis is to be performed by veterinarians holding Official Veterinary Service authorization and assistant vaccinators are to be previously trained and registered (Brazil, 2017d). In this study, the workers acting as vaccinators did not mention any training. Performing vaccination against brucellosis without procedural knowledge increases the accident risk factor. It is a well-documented fact that these accidents can occur even with authorized professional workers and may result in infection (Brazil, 2019).

Despite the importance of risk factors, no association was found between exposure and the occurrence of human brucellosis, and the Odds ratio was also not significant, $p > 0,05$. It is worth mentioning that the number of volunteers in this study was not high, which may explain the absence of association. However, the biological character of the data reinforces the relevance of discussing the values found. Note that one volunteer (2.4%) submitted to at least four risk factors tested positive for brucellosis in this study. Of these risk factors, some, such as direct contact with animals and consumption of raw milk and its derivatives, are often related in infected individuals (Soares, 2015). This data stresses the importance of educational measures for the population at risk.

5. Conclusion

Infection from smooth strain *Brucella spp.* was found in cattle slaughtered in the slaughterhouse studied. The seropositivity of one individual for brucellosis, associated to the epidemiological data suggests that the disease is present and may be explained through exposure of workers to *Brucella spp.* in slaughterhouses, of occupational origin, and because of educational and cultural habits, due to lack of information or consumption of potentially contaminated food. Therefore, the data alert as to the occurrence of brucellosis among both animals and humans, reinforces the need for measures to be taken towards prevention, education, and control in order for the country to achieve eradication and promote public health development as proposed by the PNCBET.

The exposed study reinforces the debate on preventive health and the discussion on neglected zoonoses; as well as stimulating research for deeper epidemiological and microbiological research.

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