SCIENTIFIC ARTICLE

Bovine brucellosis seroprevalence and flow network analysis in slaughterhouses in the state of Ceará

Soroprevalência da brucelose bovina e análise da rede de fluxo em abatedouros no estado do Ceará

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Abstract

The present study aimed to assess the prevalence and risk factors associated with bovine brucellosis in slaughterhouses in the state of Ceará using spatial distribution and flow network analysis. Four slaughterhouses were sampled in Ceará: two under municipal inspection and two under state inspection. Blood samples were randomly collected from bovine animals, resulting in a total of 964 samples. The collected sera were subjected to the Acidified Buffered Antigen (AAT) test, and the complement fixation test (FC) was performed for positive cases. An epidemiological questionnaire was applied to 38 producers who slaughter animals at the sampled facilities to assess the risk factors for brucellosis. An apparent prevalence of 1.55% (15) was found in the AAT test and 0.2% (n=2) in the FC test. A higher percentage of reactive animals was observed (66.6%) in properties where cattle farming is not the main activity, with OR = 4.75. The absence of contact with neighboring animals is a factor associated with protection, with a lower prevalence of seroreactive animals (23.5%) when animals were raised without contact with others (OR = 0.30). Therefore, bovine brucellosis in herds and animals can be considered low in the studied region and under all production systems. Nevertheless, despite the importance of this disease to the economic and public health aspects and the advances of the PNCEBT Program, brucellosis is still circulating in Ceará.

Keywords: bovine culture, *Brucella abortus*, risk factors, animal movement, one health.

Resumo

Objetivou-se conhecer a prevalência e fatores de risco associados a brucelose em bovinos em abatedouros no estado do Ceará, determinando sua distribuição espacial e análise da rede de fluxo. Foram amostrados quatro abatedouros no Ceará referente a três mesorregiões do estado (Centro Sul, Sertões e Sul cearense), sendo dois abatedouros sob inspeção municipal e dois sob inspeção estadual para colheita de amostras de sangue em animais bovinos de forma aleatória, gerando um total de 964 amostras. Os soros colhidos foram submetidos ao teste do Antígeno Acidificado Tamponado (AAT) e para os casos positivos foi realizado o teste da Fixação de Complemento (FC). Foi aplicado um questionário epidemiológico para avaliação dos fatores de risco para brucelose, sendo entrevistados um total de 38 produtores que abatiam seus animais nos estabelecimentos participantes da pesquisa. Foi encontrada uma prevalência aparente de 1,5% (n=15) na prova do AAT e 0,2% (n=2) na FC. Duas variáveis apresentaram-se associadas à presença de bovinos positivos na AAT, a existência de atividades econômicas paralelas à bovinocultura (OR = 4,75). A ausência de contato com animais da vizinhança (OR = 0,30), apresentando-se como fator protetor. Pode-se afirmar que a prevalência da brucelose bovina na área estudada é baixa, no entanto, ainda é observada a circulação do agente no estado do Ceará, sendo importante a manutenção das medidas de controle previstas no Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT) e inclusão de medidas adicionais, tendo em vista a relevância da enfermidade na Saúde Pública e no cenário agropecuário do país.

Palavras-chave: bovinocultura, *Brucella abortus*, fatores de risco, trânsito animal, saúde única.

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Introduction

Brucellosis is caused by microorganisms of the genus *Brucella*. It is an anthropozoonosis that affects bovines, pigs, goats, sheep, dogs, and other species (Ferreira et al., 2018; Godfroid, 2002). It is a bacterial disease with worldwide distribution. In Brazil, epidemiological surveys highlight differences in its regional prevalence (Viana et al., 2010). Brucellosis is responsible for severe losses in cattle herds worldwide. Control measures in areas with confirmed foci include mandatory sanitary barriers for the international trade of animal products, resulting in losses to the industrial sector due to the condemnation of milk and meat from infected animals, expenses to implement control and disease eradication programs, abortions, decreased reproductive rates, increased parturition intervals, death of calves, and decreased milk and meat production (Brasil, 2006; Olinto et al., 2021).

Even being an occupational disease that affects workers in direct contact with sick animals, it also has a populational character as the ingestion of contaminated animal products becomes an important means of transmission of these bacteria (Meirelles-Bartoli et al., 2014). Brucellosis is also listed by the World Health Organization (WHO) as one of the most important diseases in the world, along with tuberculosis and rabies (Roth et al., 2003). This reemerging disease, in addition to a potential source of infection, has been classified by the Center for Disease Control and Prevention (CDC) as a Category-B infectious organism, an agent with potential for bioterrorism, which, allied to the prolonged pathogenesis, highlights the need for authorities to prepare to identify and contain these potential agents (Zaki, 2010).

As it occurs with most diseases, the control and probable eradication of brucellosis require effective actions at all levels of public service as well as the commitment of the private sector (Rocha et. al., 2009). Early identification and notification, as well as information distribution across countries, are essential for a quick response, both at national and global levels (Zanella, 2016). Therefore, the flow network analysis of animal transport patterns is highly important as an exploratory tool for performing strategic actions in order to improve epidemiological surveillance, especially in highly vulnerable areas and with the potential for pathogen propagation, thus improving the effectiveness of disease control (Felipe et al., 2013). In Brazil, there are few published data on brucellosis in humans, and, in most cases, the reports correspond to seroepidemiological studies conducted with professionals at risk of occupational brucellosis and case reports. In this scenario, the Unified Health System (SUS) of Brazil informs about hospital morbidity cases by brucellosis, with 258 hospitalizations from January 2009 to December 2019 due to the disease, mostly in the South and Southeast regions (Brasil, 2019).

In the state of Ceará, the classification with regard to the degree of risk for brucellosis first requires determining its prevalence. Therefore, this study investigated the seroprevalence of bovine brucellosis and performed a flow network analysis in slaughterhouses in the state of Ceará, aiming to understand the impact of brucellosis in cattle herds in the region.

Material and methods

The study was conducted from March to December 2019 in the slaughterhouses of the municipalities of Jucás and Catarina, under the Municipal Inspection Service, and in Iguatu and Juazeiro do Norte, under state inspection by the state of Ceará. These municipalities are part of three state mesoregions: Hinterlands (Sertões), Central-South, and South. The studied population consisted of bovines, and sampling was performed randomly without discriminating between breed or sex. Furthermore, there were no known positive animals at slaughter according to the Animal Transport Certificate (GTA). The number of animals to be collected was calculated by considering a bovine population of 278,883 in the municipalities that transport animals to the four selected slaughterhouses (Agência de Defesa Agropecuária do Estado do Ceará, 2019) and an expected frequency of 50%, with a 5% sampling error and a 99% confidence interval, using the statistical software *Epi-Info* 7.0 (Center for Disease Control and Prevention, 2018). The calculation signaled the need for 660 samples, and a total of 964 bovine serum samples were collected at the end of the study.

Samples were randomly collected at each selected slaughterhouse: Iguatu (n=425), Jucás (n=217), Catarina (n=200), and Juazeiro do Norte (n=122), and animals came from different locations in the region. Blood samples were collected at slaughter after exsanguination using 10 ml sterile disposable syringes previously identified with the GTA number, sex, and collection date. The collected material was stored in BD Vacutainer Serum Plus Blood Collection® clot activator tubes. The Buffered Acidified Antigen (ATT) test was used for sample screening according to the methodology described by PNCEBT. In the present study, the serum was obtained by centrifugation at 4.000 rpm for ten minutes at a particular veterinary laboratory enabled and accredited by the Agricultural Protection Agency of the state of Ceará (ADAGRI), located in Iguatu-CE. The AAT test is performed on a glass plate using a 30 μL micropipette for serum and antigen deposition. The presence of granules indicated a positive result. Data interpretation implied that the material was reagent if granules were present. An antigen with a 001/19 range produced by Microsules® was used in the experiment. Only the reactive samples in this test were subjected to the complement fixation (FC) test, a reference test required for international animal transport (Brasil, 2004). The FC test was performed at the Federal Laboratory of Agricultural Protection of Pedro Leopoldo – MG. The interpretation of test results is the responsibility of the requester, based on the history of the animal and/or herd and the current legislation (IN 34 de 08 de setembro) (Brasil, 2017), and considering a reactive result when: titer ≥4 with a minimum 25% of complement fixation.

In all properties that resulted in positive AAT tests, a questionnaire was applied to assess the degree of risk of disease spread. Furthermore, cattle raisers whose animals did not test positive were also included in the epidemiological survey. Properties that performed the protection measure against the disease (vaccination) were removed from the risk factor analysis.

The risk factor analysis for positivity according to the ATT test in the rural properties was performed with the software SPSS 20.0 by comparing the independent variables of the epidemiological questionnaire (breed, raising system, among others) with the dependent variable of sanitary status of the herd for brucellosis. In the univariate analysis, if the chi-square test showed a p-value below 0.20, the variable was selected for multiple logistic regression analysis and odds ratio (Hosmer & Lemeshow, 1989).

The prevalence coefficient was obtained by dividing the number of positive samples in the AAT test at each slaughterhouse by the total number of samples collected at each slaughterhouse and multiplying the result by 100 (Kahn & Sempos, 1984; Organização Panamericana da Saúde, 2010). However, the AAT results refer to apparent prevalence, which is the proportion of sick individuals detected by the test in the population. This information depends on the sensitivity and specificity of the diagnostic test, considering that the confirmatory test (FC) was performed to obtain the actual prevalence, which is the proportion of truly sick individuals in the population (Youden, 1950).

With regard to the spatial distribution, theme maps were prepared to depict the geographic distribution of animals with the transport flow of bovines, in which each point of origin is a property where samples were collected from animals sent to slaughterhouses with a GTA number. The map depicting the location of properties with positive animals in the AAT test was also prepared. The open-source software *Qgis* was used to prepare the maps, whose geographic coordinates were collected in the Universal Transverse Mercator projection of each visited location with the aid of GPS equipment (Global Position System). Finally, the georeferenced data were used in the Quantum GIS software (*QGIS* 2.18.22) to prepare the figures.

Results

Fifteen (964) samples were positive in the AAT test. These samples referred to 183 different properties, of which 23 producers slaughtered animals at the Catarina slaughterhouse, 71 at the Iguatu slaughterhouse, 50 at the Juazeiro do Norte slaughterhouse, and 39 at the Jucás slaughterhouse, Ceará (Figure 1). With regard to the confirmatory test (FC), only two samples (0.21%) were positive, with antibody titers equal to 8 and 32 (Table 1).

The result of the univariate analysis to study the risk factors related to the presence of seroreactive animals in the AAT test demonstrated that, in the properties where cattle raising was not the main activity, a higher percentage of reactive animals was observed (66.6%), with OR = 4.75. On the other hand, the absence of contact with neighboring herds showed to be a protective factor, with lower seroreactivity (23.5%) when animals were raised without contact with others (OR = 0.30). These two variables showed p <0.2 and were analyzed by binary logistic regression

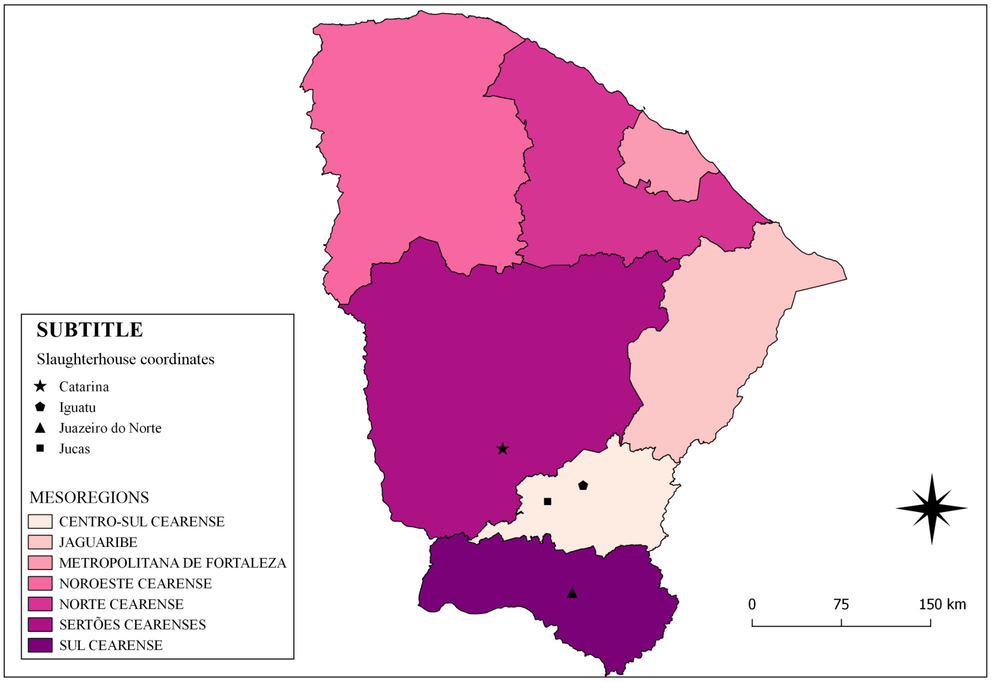

Figure 1. Map of the Mesoregions of the state of Ceará based on IBGE data, 2017.

Table 1. Data on the number of properties and samples collected in each slaughterhouse.

Slauther location	Number of samples	Number of properties	Positive reagent in AAT	Positive reagent in FC
Jucás	217	39	2	1
Catarina	200	23	4	0
Iguatu	425	71	8	1
Juazeiro do Norte	122	50	1	0
Total	964	180	15	2

(Table 2). However, no variable was confirmed in this analysis as a risk factor for positivity in the ATT test among the studied herds.

With regard to vaccination against brucellosis, only five owners (13.1%) reported vaccinating their 3 to 8-month-old females, justifying the lack of demand in veterinary clinics and the unsatisfactory vaccination rates. According to the results of the questionnaire, none of the animals that tested positive in the FC had been vaccinated.

With regard to spatial distribution, the reactive cases in the AAT test were dispersed over the entire studied territory, predominating in the Central-South region of Ceará, where 66.7% of positive cases in the screening test were located in two municipalities (Jucás and Iguatu). It is worth noting that these are the municipalities that most sent animals to slaughter in the Central-South region of the state, according to the 2019 report of the Agricultural Defense Agency of Ceará.

Figure 2 shows the spatial distribution of properties that sent animals to slaughter in the municipality of Iguatu - CE. During the analysis of the GTAs received by the slaughterhouses, it is noted that the facilities, in addition to receiving animals from their own municipalities, also receive animals from neighboring locations, such as the Iguatu slaughterhouse, which receives animals from five municipalities, suggesting the occurrence of animal trade between producers and municipalities.

Table 2. Univariate analysis with the distribution of the possible risk factors associated with positivity in the AAT test in the state of Ceará.

Variable	No. of herds	Positive (N / %)	*P*
Breed			
Purebred	1	1 (100.0)	0.364
Mixed	32	11 (34.4)	
Cleaning frequency			
Daily	2	0 (0.0)	0.543
Weekly	5	2 (40.0)	
Occasionally	26	10 (38.5)	
Veterinary Assistance			
Yes	10	3 (30.0)	0.710
No	23	9 (39.1)	
Main source of income			
Cattle farming	27	8 (29.6)	0.159*
Other activities	6	4 (66.6)	
Herd size			
Up to 80 animals	22	9 (40.9)	0.703
> 80 animals	11	3 (27.3)	
Breeding system			
Extensive	28	10 (35.7)	1.000
Semi-confinement	5	2 (40.0)	
Contact with other herds			
Yes	16	8 (50.0)	0.157*
No	17	4 (23.5)	
Purchase of new breeders			
Yes	25	9 (36%)	1.000
No	8	3 (37.5)	
Rental of pasture areas			
Yes	18	6 (33.3)	0.731
No	15	6 (40.0)	
Existence of parturition areas			
Yes	6	3 (50.0)	0.643
No	27	9 (33.3)	
Destination of carcasses			
Buried	7	1 (14.3)	0.377
Burned	11	5 (45.4)	
Open-sky	15	6 (40.0)	

* Variables selected for multivariate analysis. Five properties that vaccinated against brucellosis were removed from the risk factor analysis.

Discussion

According to the study conducted by Meirelles-Bartoli and Mathias (2009) on the use of FC to confirm AAT test results for the serological diagnosis of bovine brucellosis, the authors stated that

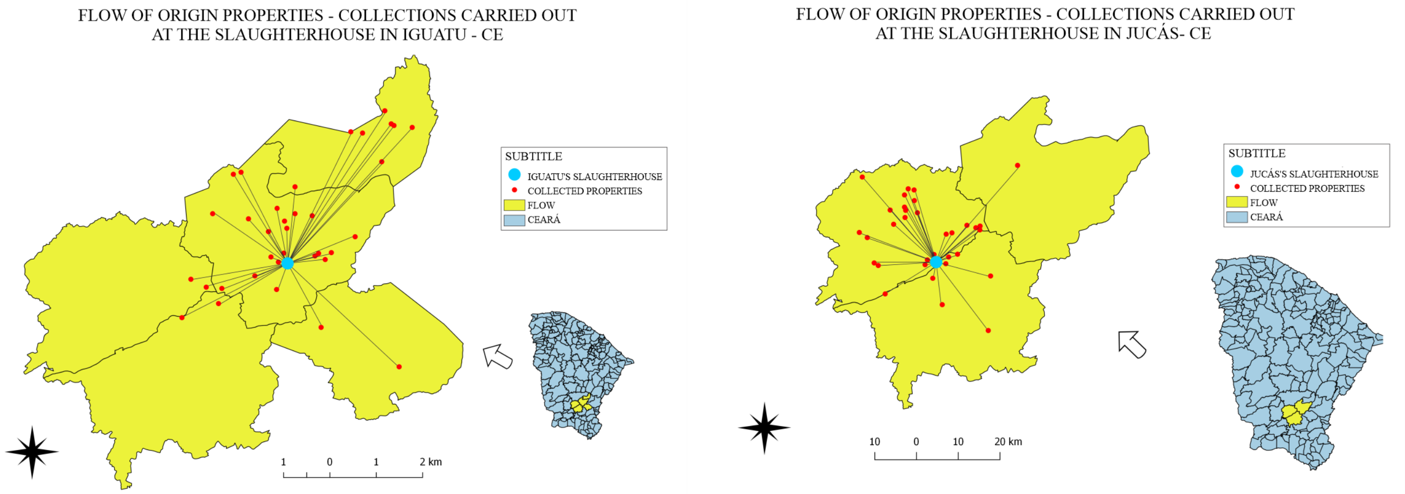

Figure 2. (A) Map of the municipality of Iguatu – CE with the network of neighboring municipalities showing the origin of the sampled animals slaughtered in Iguatu, at the Agropecuária Lavor LTDA slaughterhouse, from March to August 2019; (B) Map of Jucás – CE with the network of neighboring municipalities showing the origin of the sampled animals slaughtered in Jucás, at the Municipal Slaughterhouse of Jucás, from March to August 2019.

the possibility of a positive result in the screening test among non-vaccinated animals is higher than in animals vaccinated with B19, constituting one of the leading causes of false-negative results in the serological diagnosis of bovine brucellosis. The occurrence of false-negative results is usually due to prozone phenomena, which, in turn, can be reduced by serum dilution or retesting after some time or by performing a more specific and confirmatory test such as the FC (Organização Mundial da Saúde Animal, 2018). In a study conducted by Meirelles-Bartoli and Mathias (2010), the authors reported relative sensitivity values of 99.6%, 98.8%, and 91.1%, respectively, for AAT, 2-ME, and FC. The comparison between the three tests adopted by the program highlighted a good agreement between the Acidified Buffered Antigen (AAT) and the confirmatory tests (2-ME, with Kappa = 0.80; and FC, with Kappa= 0.73).

On the other hand, false-positive reactions in the AAT occur due to the presence of non-specific antibodies in infections caused by other bacteria, e.g., *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Actinomyces* spp., and other genera that might cause cross-reactions in serological tests, complicating the diagnosis (Costa et. al., 2001). The complement fixation test allows detecting IgG1antibodies in the serum, both in early infection and chronic cases. An advantage of this test is the low interference from antibodies present in the serum of already vaccinated animals compared to agglutination tests, allowing to differentiate between infected and vaccinated animals (Paulin & Ferreira Neto, 2008).

There was a 1.88% apparent prevalence in Iguatu, where most slaughtered animals come from dairy or mixed herds. In these cases, the prevalence is usually higher due to the extensive animal management practices, as noted by Klein-Gunnewiek et al. (2009), who observed a brucellosis prevalence of 6.25% in beef cattle, 14.52% in dairy herds, and 20.56% in mixed herds in the central-west region of Rio de Janeiro, corroborating the results of the present study, where 78.9% of sampled animals were raised under an extensive breeding system.

In the last ten years, 59 cases were reported in 2012, 34 in 2013, 12 in 2014, 10 in 2015, four in 2016, three in 2017, two in 2018, and four in 2019, according to the temporal distribution of bovine brucellosis cases in Ceará. According to the 2017 notification report, available on the website of the Agricultural Defense Agency of Ceará, there are few notifications of positive animals due to tests performed by self-employed qualified veterinarians. In August, only two animals were slaughtered in the state, in the municipality of Barro – CE, which is part of one of the mesoregions studied in this research (South).

The extensive study performed by the Ministry of Agriculture in 1975 (rapid serum agglutination test) showed a 2.5% prevalence of brucellosis foci in the Northeast region, close to the prevalence obtained in the present study. However, it is worth noting that the herd size increased over the years, and since there is no specific study for the state of Ceará, along with the whole situation mentioned before and considering that brucellosis testing is not mandatory for cattle herds in Brazil, greater participation by cattle raisers and broader program coverage could result in better results with regard to the occurrence of this disease in Brazil, especially in Ceará. In the Northeast region, Alves et al. (2009) divided the state of Bahia into producing regions where 10 to 15 bovine females were sampled per studied property and screened for antibodies, followed by a confirmatory test for bovine brucellosis, obtaining a prevalence of 0.66%. In the state of Maranhão (Carvalho et. al., 2016), a study was conducted to estimate the frequency of bovine brucellosis in dairy herds, in which 26/525 animals (4.95%) tested positive in the AAT test.

In the present study, with regard to socioeconomic variables, of the 38 cattle raisers interviewed, most (71%) have incomplete primary education, 23.6% have secondary education, and only 5.2% have higher education. This result showed similar numbers to those of the 2017 census, which reported more than 60% of incomplete primary education levels among cattle raisers in Ceará (Instituto Brasileiro de Geografia e Estatística, 2017). According to Teixeira and Costa (2011), in their study on the impact of living conditions and education on the incidence of tuberculosis in Brazil, education is a highly important variable to determine the risk of disease incidence as it correlates with the income level and the level of information with regard to disease control and preventive measures.

Mixed breed management was the main type of management practice, corresponding to 97%. According to Matope et al. (2010), mixed herds have an increased risk of infection than purebred animals. Producers can raise cattle in three types of management: confinement, semi-intensive, and extensive. The extensive system predominates in the present study, with 79%. Blasco (2004) states that there is a higher prevalence of animals in extensive systems than in other managements. The concentration of brucellosis foci in an area could be related to aspects of the livestock. Extensive cattle production usually implies a large number of animals and routine entry of reproductive animals into the herd, thus resulting in a wider spread of the disease due to poor sanitary control and sanitation, given the large dimensions of the properties (Braga, 2010).

Cattle transport to slaughterhouses constitutes an important part of total animal transport. Thus, direct or indirect contact resulting from sending cattle to slaughter needs to be further investigated in order to define the transmission routes of infectious diseases among animals (León et al., 2006). Thus, when an animal is infected and transported to another municipality, it will become a source of infection in the new location, and, even if briefly, it will be able to transmit the agent (Coelho et al., 2008). There are reports of *Brucella* spp. persistence in meat preserved in cold rooms (Mafra, 2008). Therefore, it is essential to control animal transit and perform diagnostic tests (Leal Filho et al., 2016), in addition to establishing a health surveillance system based on epidemiological and traceability studies of seropositive animals with routine examinations performed at slaughterhouses, dairy shops, or farms where animals are commercialized (Ragan, 2002).

It is worth noting that, in addition to an occupational character, affecting workers in direct contact with infected animals, brucellosis also has a populational character as the consumption of contaminated animal products can transmit these bacteria (Meirelles-Bartoli et al., 2014). This pathogen can survive in infected animal products, e.g., milk, dairy products, and raw or rare meat, thus highlighting the need for pasteurization or high temperature and/or boiling sterilization methods in order to eliminate bacteria that can persist for long periods on such products (Kishida, 2008; Sola, et al., 2014). Therefore, surveillance needs to be rigorous and effective not only due to the economic importance of the disease but also to decrease its impacts on the human population (Mamisashvili et al., 2013). Controlling human infections depends almost entirely on disease control among animals (Nicoletti, 2010; Rubach et al., 2013).

According to the Hospital Information System of the Unified Health System (SIH/SUS) of the Ministry of Health of Brazil, there were 181 admissions due to brucellosis from January 2012 to December 2019, 49 in the South region, 47 in the Southeast region, 36 in the Northeast region, 29 in the North region, and 20 in the Central-West region. Six deaths were recorded during this period: four in the South region and two in the Northeast region (Brasil, 2019). A study conducted by Santos et al. (2007) reported that 10% of the workers of a municipal slaughterhouse in São Luís, MA, were infected by brucellosis. The state of Tocantins, in 2008, notified twelve cases of brucellosis at a slaughterhouse in Araguaína, with data referring to only a single month of the year (Santos, 2010).

Considering the epidemiological variables, the seropositive cases for bovine brucellosis in this mesoregion possibly occur due to the extensive cattle raising that predominates in the region. A study conducted by Campos (2020), which analyzed the prevalence of buffalo brucellosis in the Federal District, reported an incidence rate of 17.65%. The authors discussed the importance of wild animals in their territories, especially deer, capybaras, marsupials, felids, and primates, which can contribute to spreading the disease. The data generated by the characterization of cattle transport in this study are essential to assess the risk of spread of infectious diseases such as brucellosis, contributing to controlling them. These data may provide information to direct epidemiological strategies for disease control, constituting a benefit for the safety of the production chain of animals and animal products in the state of Ceará.

Conclusion

The present study highlights a low prevalence of brucellosis in the studied region, although pathogen circulation has been observed in the state of Ceará. It is worth noting that the surveillance of animals that participate in agglomerated events and animals from locations with disease reports or with unknown status, considering that vaccination or a negative certificate for brucellosis are not required for cattle slaughter in the state, constitutes a relevant factor for the increase of brucellosis cases. Finally, control and eradication measures are also necessary, as established by the National Program instituted by the Ministry of Agriculture, Livestock, and Supply of Brazil. The municipalities and the state, in association with MAPA, should direct an awareness program aimed at producers and intensify the enforcement of preventive actions aiming at the control and later eradication of brucellosis, thus reducing its economic and social impacts.

Ethics statement

Blood collection was approved by the Ethics Committee on Animal Use of the Federal University of Vale do São Francisco (CEUA/UNIVASF) under protocol number 0004/121218 and by the Ethics Committee on Human Research (CEP/Plataforma Brasil) under protocol number 05375718.1.0000.8052.

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The research was carried out with its own resources.

Conflict of interests

LCSSCA, MMC, JANJ, FDAS and RMP – No conflict of interest.

Authors' contributions

LCSSCA, FDAS and RMP - Development of methodology; preparation and writing the initial draft. LCSSCA and RMP - Application of statistical study data, Review and Editing manuscript. MMC and JANJ - Writing, Review and Editing manuscript.

Availability of complementary results

Ag Data Commons (ADC)

Data deposit type: *Restricted

Data access type: Open / *Embargo

http://data.nal.usda.gov

Ag Data Commons (ADC) provides access to a wide variety of open data relevant to agricultural research.

AgBase

Data deposit type: *Restricted
Data access type: Open
http://agbase.arizona.edu
AgBase is a curated, open-source, Web-accessible resource for functional analysis of agricultural plant and animal gene products.
Legume Information System (LIS)
Data deposit type: Restricted
Data access type: Open
http://legumeinfo.org
The Legume Information System (LIS) is a collaborative, community resource to facilitate crop improvement by integrating genetic, genomic, and trait data across legume species.

The screening test was carried out at Veterinarian Laboratory enabled by Agência de Defesa Agropecuária do Estado do Ceará, Iguatu, CE, Brazil, and the confirmatory test by the Laboratório Federal de Defesa Animal, Ministério da Agricultura Pecuária e Abastecimento, Pedro Leopoldo, MG, Brazil.

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