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# New area of occurrence of human brucellosis in Brazil: serological and molecular prevalence and risk factors associated with Brucella abortus infection

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# Abstract

**Background** Brucellosis is a re-emerging underdiagnosed zoonosis that has significant relevance, especially in developing countries. This study aimed to report the first cases of human brucellosis in the state of Paraíba, Brazil, and to determine the prevalence and risk factors associated with Brucella abortus infection in slaughterhouse workers.

Methods A cross-sectional study was carried out from December 2021 to February 2022 in public and private slaughterhouses in the state of Paraíba. Blood samples were collected from 188 people and tested using the Rose Bengal Test (RBT), a screening test, and the 2-mercaptoethanol (2-ME) and standard tube agglutination test (STAT), which are confirmatory tests. Samples that tested positive were subjected to molecular analysis using gPCR. Risk factors were determined through multivariate analysis based on epidemiological questionnaires administered to the workers. After the diagnoses, results were provided to the participants, and health education campaigns were conducted for all workers in the evaluated slaughterhouses.

Results The study found a serological prevalence (RBT + 2-ME + STAT) of 4.2% (16/188) (95% CI = [1.28; 6.96], sensibility 90% specificity 95%) among workers. Five samples were positive in gPCR with Ct (Cycle threshold) values ranging from 31.58 to 38.11. The ingestion of undercooked meat was identified as a risk factor, with a 2.95 times higher chance of contamination. Among the 16 individuals who tested positive in the serological tests, 15 (p<0.0001) reported in the epidemiological guestionnaire that they did not know what brucellosis is.

**Conclusion** These are the first published cases of human brucellosis in the state of Paraíba, reinforcing the hypothesis that the bacterium is circulating among slaughterhouse workers. This underscores the need to develop public policies that provide adequate medical support to the population.

Keywords Zoonoses, Infectious diseases, Health

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# Introduction

Brucellosis is a re-emerging zoonosis that is rarely reported in humans in Brazil. However, it often occurs in cattle. Due to this reality, it is believed that the disease is highly underreported, highlighting its importance in public health [1-3].

Transmission can occur directly or indirectly through contact with mucous membranes, the respiratory tract, and injured skin of susceptible individuals. This involves contact with placental remains, aborted fetuses, amniotic fluid, meat, dairy products, and their products and byproducts from infected animals without heat treatment [4].

The infection is also associated with occupational and livelihood hazards, affecting veterinary professionals, butchers, slaughterhouse employees, and individuals who regularly work with livestock. Brucellosis in humans and animals has a worldwide distribution, with a higher prevalence of cases in tropical and subtropical regions. The incidence of *Brucella* spp infections varies according to the sanitary, socioeconomic, and environmental conditions of each region [5–7].

In Brazil, *Brucella abortus* is the only species reported and is widely distributed in cattle herds [2]. In the state of Paraíba, a prevalence of 4.6% was observed in herds, with a higher frequency in properties located in the Zona da Mata region, and a risk factor associated with infection in zebu cattle [8].

Human brucellosis is a neglected zoonosis, underdiagnosed, and little recognized by health professionals. Additionally, the disease is endemic in the Brazilian cattle herd, suggesting that individuals can be infected without a definitive diagnosis. Therefore, this study aims to characterize brucellosis in slaughterhouse workers in the state of Paraíba, northeastern Brazil.

# Methods

# Place of study

The research is a cross-sectional observational study carried out from December 2021 to February 2022 in public and private slaughterhouses located in the municipalities of Sapé, Campina Grande, Mamanguape, Itapororoca, Santa Rita, and Patos, in the state of Paraíba, Northeast Brazil (Fig. 1).

## Study design, population, and sample size determination

The sample size was determined by simple random sampling, applying the following formula:

$$N = \frac{Z^2 \cdot p(1-p)}{d^2}$$

N = number of individuals sampled;

Z = value of the normal distribution for the 95% confidence level;

p = expected prevalence of 15%; [7].

d = absolute error of 5.2%.

A total of 188 human blood samples were collected. Participation in the study was voluntary, so the number of participants varied by slaughterhouse. People of both sexes, aged between 18 and 65 years, who worked directly in the slaughter and processing of animal products at the evaluated sites were eligible to participate. Only individuals in the gestational period were excluded. Blood samples (4 mL) were collected by vacuum cephalic venipuncture. Participants answered an epidemiological questionnaire, administered by a single trained researcher, covering eating habits, hygiene, health, work routine, and contact with animals.

# Serological diagnosis

The blood samples were centrifuged at 3000 RPM for 15 min, and the serum obtained was stored in 1.5 mL plastic containers and frozen at  $-20^{\circ}$ C. The diagnosis for brucellosis was made using the Rose Bengal Test (RBT) as the screening test, and 2-mercaptoethanol (2-ME) associated with the standard tube agglutination test (STAT) as the confirmatory test. To be considered positive for brucellosis, samples had to show a reaction in the RBT, STAT, and 2-ME tests. Samples that tested positive were subjected to molecular analysis using qPCR. All processing was carried out at the Histopathological Processing Laboratory of the Department of Morphology (DMORF) of the Health Sciences Center (CCS) of the Federal University of Paraíba (UFPB) [8].

#### Molecular diagnostics

For DNA extraction and purification, 250  $\mu$ L of serum were incubated with 220  $\mu$ L of lysis solution (containing 20 mg/mL of proteinase K [PROMEGA]) at 56°C for 10 min. Subsequently, the volumes were transferred to the cartridges of the Maxwell<sup>®</sup> RSC TNA (PROMEGA) Purification Kit in the MAXWELL 48 RSC (PROMEGA) automated system, following the manufacturer's instructions. Detection of Brucella spp was performed through a qPCR reaction targeting a previously described genetic target [9].

The reactions were configured in a final volume of 20  $\mu$ L, containing 0.1  $\mu$ M of each primer oligonucleotide, 0.25  $\mu$ M of the FAM-labeled oligonucleotide probe (SIGMA-ALDRICH), 10  $\mu$ L of reaction mixture (GoTaq probe qPCR system, PROMEGA), and 5  $\mu$ L of purified DNA. Amplifications were performed on the Quantstudio 3 platform (THERMO-FISHER) with a program of 95°C for 2 min, followed by 40 cycles of denaturation at



Fig. 1 Highlighted municipalities in the state of Paraíba, where blood samples were collected from 188 workers in public and private slaughterhouses and the proportion of positive cases in serological tests (RBT + 2-ME + STAT) and in real-time PCR in the period from December 2021 to February 2022, in detail the image of Brazil

95°C for 15 s, and annealing/extension at 60°C for 1 min. All reactions were performed in duplicate, using a negative amplification control (ultrapure water) and a positive amplification control (a synthesized target oligonucleotide, SIGMA-ALDRICH) [10].

# Data collection and analysis

Data from the epidemiological questionnaire were recorded in a spreadsheet, and an analysis was performed to examine the association between the dependent variable (brucellosis test result: negative or positive) and various independent variables. These variables included: sex (female/male), type of slaughterhouse (public/private), education level (illiterate/elementary school/high school/ higher education), length of work in the activity (up to 7 years/between 8 and 25 years/over 25 years), contact with other animals (yes/no), consumption of raw or undercooked milk (yes/no), knowledge about brucellosis (yes/no), presence of joint pain (yes/no), occurrence of recurrent fever (yes/no), ingestion of undercooked meat (yes/no), contact with fetuses of other species (yes/no), presence of comorbidities (yes/no), difficulties conceiving children (yes/no), frequency of cuts at work (frequent/ infrequent), use of personal protective equipment (yes/ no), and contact with other animals (yes/no).

The chi-square test or Fisher's exact test was used to evaluate the association between these variables, with a significance level of p < 0.2. Variables showing significant associations were selected for further analysis and subjected to binary multiple logistic regression to determine their independent effects. A significance level of p < 0.05 was adopted for the logistic regression analysis. The quality of the model's fit was assessed using the Hosmer and Lemeshow test, with a significance value of p > 0.05 indicating a well-fitting model. The associated risk analysis was performed considering the positive results for 2-mercaptoethanol. All statistical analyses were performed using SPSS 25 for Mac software.

# Results

A total of 188 serological samples were collected from humans and tested using the Rose Bengal Test (RBT). Of these, 24 samples were positive, representing a true prevalence of 9,06% (95% CI=[4.96.; 13.16] sensibility 90% specificity 95%). The positive samples were subsequently submitted to the confirmatory tests, 2-mercaptoethanol (2-ME) and the standard tube agglutination test (STAT), resulting in 16 positive results. This corresponds to a final true prevalence of 4.2% (16/188) (95% CI=[1.28; 6.96], sensibility 90% specificity 95%). Among the positive samples in the confirmatory test, 31.2% (5/16) (95%

**Table 1** Result of brucellosis diagnoses of slaughterhouseworkers in Paraíba organized according to the municipality,patient identification and qPCR CT performed from December2021 to February 202

City	Patient	Results	Cycle	
		Serological	qPCR	threshold (Ct)
Sapé	104	+	-	0
	122	+	-	0
Campina Grande	202	+	-	0
	203	+	-	0
	204	+	-	0
	208	+	+	35,5
	209	+	+	38,11
	203	+	+	31,58
	207	+	-	0
	212	+	-	0
	214	+	+	35,57
Santa Rita	403	+	-	0
	518	+	+	36,91
Itapororoca	602	+	-	0
	604	+	-	0
	611	+	-	0

CI = [14.16; 55.60]) (Table 1) had a significant amount of *Brucella abortus* DNA in qPCR. We observed that the variable "Consumption of undercooked meat?" presented odds ratio 2.95 ([CI: 1.02-8.52] p=0.04) (Table 2).

# Discussion

Brucellosis is recognized as one of the most prevalent zoonoses worldwide, particularly in developing regions such as Brazil. This study provides the first reports in the literature on the occurrence of human brucellosis in the state of Paraíba. It is believed that the cases observed are associated with the poor working conditions found in some of the slaughterhouses visited, such as the inadequate use of personal protective equipment (PPE) by workers (overalls, rubber boots, goggles, gloves, and disposable caps). According to those who evaluated the hygiene and sanitation conditions in slaughterhouses in Paraíba, it was found that 80.4% of the workers in these environments did not use any type of PPE during their activities. This is a recurrent condition in slaughterhouses in the interior of the state and can contribute to the occurrence of zoonoses such as brucellosis. Notably, in several cases, the workers involved in the slaughter of infected animals only recognized rubber boots as a form of PPE, emphasizing the need for permanent health education in the slaughterhouses [6, 11, 12].

Table 1 presents the factors associated with *Brucella abortus* infection in slaughterhouse workers in Paraíba. It is observed that the intake of undercooked meat increases the probability of testing positive for brucellosis by 2.95 times. Consumption of meat and dairy products without proper heat treatment is considered a risk factor for infection, as bacteria can survive in raw or undercooked food. In Brazil, the law allows the consumption of cattle that test positive for brucellosis, except for udder, blood, and genitalia. However, the consumption of undercooked genitals and udders as a snack is a common practice in northeastern Brazil [12–14].

**Table 2** Risk factors associated with diagnostic test 2-mercaptoetanol, detected by univariate and multivariate analysis, the infection by *Brucella abortus* in workers of slaughterhouses in the state of Paraíba performed in the period December 2021 to February 2022

Variable	Category	Total people	Total de positives (%)	Ρ	Odds Ratio	Confidence of interval	Ρ
Consumption of raw milk?	No	141	9 (6,4)	0,12*	-	-	-
	Yes	47	7 (17,9)				
Consumption of undercooked meat?	No	116	6 (5,2)	0,03*	2,95	[1,02-8,52]	0,04**
	Yes	72	10 (13,9)				
Frequent cuts at work?	No	106	6 (5,7)	0,11*	-	-	-
	Yes	82	10 (12,2)				

\* Univariate analysis (P < 0.2)

\*\*Multivariate analysis (P<0.05)

The seroprevalence of positive farms has a heterogeneous distribution throughout the country, ranging from 0.91% to 30.60%. In the state of Paraíba, bovine brucellosis has an outbreak prevalence of 4.6% (95% CI = [3.2-(6.5%) and infected animals (2.5%) (95%) CI = (1.1-3.9%)[8]. In this study, the prevalence of positive cattle was 53.8% (28/52) (95% CI=[38.56; 69.15]). The animals tested did not present characteristic lesions of brucellosis (inflammatory nodule in the nuchal ligament) during slaughter, and there was no information on the antemortem diagnosis of brucellosis in the positive animals. Despite the low number of samples collected from other researchers, it is believed that the high number of reactive animals is related to the fact that rural producers know that their animals are in a positive condition and, even so, send them to slaughter without any type of notification [4].

The situation becomes more alarming because tests for brucellosis in cattle in Brazil are predominantly concentrated in females. However, not only are females' important transmitters, but males can also transmit *Brucella abortus*. Additionally, the use of equipment and utensils contaminated with *Brucella* spp can transfer the bacteria to other meats or foods, as they survive well at the temperatures reached by refrigerators or freezers. Given this perspective, it is believed that these animals can be sources of infection (16).

The answers to the epidemiological questionnaire were evaluated only from positive individuals, revealing that 15 of the 16 participants (P < 0.0001, Chi square = 12,25) reported not knowing what brucellosis is. This highlights the lack of awareness among the population about this zoonosis. The results of the tests were delivered to all participants by the medical team, and those who tested positive received information about brucellosis and were referred to health services for treatment. Simultaneously, the work team visited all the slaughterhouses and carried out educational activities involving all workers. The objective was to understand the realities of the work environment and identify critical points in the transmission of zoonoses. The importance of using personal protective equipment (PPE) to prevent the transmission of diseases was emphasized. It is known that multidisciplinary and integrated multiprofessional approaches, such as the One Health approach, which aims at the comprehensive control of infectious diseases, can be effective tools in containing the spread of human and animal brucellosis [3].

The cases of human brucellosis in this study were concentrated in the municipalities of Campina Grande (9 cases, 4 qPCR), Santa Rita (3 cases, 1 qPCR), Sapé (2 cases), and Itapororoca (2 cases), all located in the agreste or zona da mata regions of Paraíba (Table 2). According to Clementino and Azevedo (2016), these

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areas have the highest number of brucellosis-positive cattle in Paraíba. This situation suggests greater circulation of the brucellosis agent and possible zoonotic transmission [8].

The combination of several factors, including the lack of full use of PPE, insufficient health education among workers, the slaughter of infected cattle for consumption, and the lack of diagnosis in humans, has contributed to the spread of brucellosis to populations that previously did not have the disease.

## Conclusions

The prevalence of human brucellosis found in slaughterhouse workers in the state of Paraíba is likely related to the occupational nature of their activities and their eating habits, as indicated by the analysis of risk factors. Additionally, negligence in the use of personal protective equipment (PPE) is a factor to be considered. The risk factors associated with the positivity of the disease suggest that the consumption of raw and undercooked meat may be primarily responsible for the high human prevalence found in the study.

Human and animal brucellosis is considered a One Health problem and generates an alert for the community and relevant government institutions. This emphasizes the need for public policies focused on education, control, and treatment of brucellosis.

#### Authors' contributions

TSM: Collection of biological material, Performed diagnostic serological and qPCR, Wrote the manuscript, Guidance to workers. IB: Collection of biological material, Performed diagnostic serological and qPCR, Wrote the manuscript, Guidance to workers. ALPF: Collection of biological material, Performed diagnostic serological, Wrote the manuscript. ASMB: Collection of biological material, Performed diagnostic serological, Wrote the manuscript. WGLB: Collection of biological material, Performed diagnostic serological, Wrote the manuscript. WGLB: Collection of biological material, Performed diagnostic serological, Wrote the manuscript. WGLB: Collection of biological material, Performed diagnostic serological and qPCR, Wrote the manuscript, Guidance to workers, ESSS: Guidance to workers, Revision of the manuscript, Wrote the manuscript, Wrote the manuscript. WPP: Performed diagnostic qPCR, VLRV Translation Review, Revision of the manuscript, RNP Study design, Creation of the manuscript, Revision of the manuscript.

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#### Data availability

All the data available is included in the manuscript.

#### Declarations

#### Ethics approval and consent to participate

According to the approval of the Ethics Committee for Research with Human Beings (CEP), under registration number 46809121.0.0000.8069.

#### Consent for publication

Not applicable.

#### **Competing interests**

The authors state that they have no known conflicting financial interests or personal relationships that could have influenced the findings presented in this article.

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