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Isolation and identification of *Brucella suis* biovar 1 in pork samples (sus scrofa) from the municipality of Cachoeira do Arari, State of Para-Brazil.

Isolamento e identificação de *Brucella suis* biotipo 1 em amostras de suíno (*sus scrofa*) procedentes do município de Cachoeira do Arari, Estado do Pará- Brasil.

Aislamiento e identificación de *Brucella suis* biotipo 1 en muestras de cerdo (sus scrofa) del municipio de Cachoeira do Arari, Estado de Para-Brasil.

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Abstract
This work aimed to study the microorganisms present in lesions found in swine from the municipality of Cachoeira do Arari (Ilha do Marajó), sold at the Ver-o-Peso fair in Belém do Pará; were carried out the isolation, nucleotide sequencing and phylogenetic inference of the collected material. There was positive growth on brain-heart broth and blood agar and no growth on MacConkey agar. In the gram staining, grouped gram negative cocci were observed, and in the Modified Ziehl-Neelsen staining, grouped cocci stained in red were observed. These growth characteristics, as well as the observed microscopic results, were suggestive and similar to bacteria belonging to the genus Brucella sp. After a result suggestive of Brucella spp, the sample was sent for DNA extraction, nucleotide sequencing. Brucella suis biovar 1 was identified; with subsequent assembly of the phylogenetic tree. In addition to the sanitary issue, clandestine slaughter also involves the environmental issue, as it is usually carried out in places close to rivers or streams to facilitate the capture of water. The identification of bacteria with morphological characteristics similar to the Brucella genus of the swine abscess observed in this study confirms that the animal had swine brucellosis and that clandestine slaughterhouses can be important means of infection and dissemination of

these agents, if not have verification that guarantee the use sanitary surveillance measures of quality that come prevent this type of practice and the sale of the product for human consumption.
Keywords: *Brucella sp*; Brucellosis; *Brucella Suis*; Clandestine slaughter.

Resumo
Este trabalho teve como objetivo, estudar os microrganismos presentes em lesões encontradas em vísceras de suínos provenientes do município de Cachoeira do Arari (Ilha do Marajó), comercializados na feira do Ver-o-Peso em Belém do Pará; realizou-se o isolamento, sequenciamento nucleotídico e inferência filogenética do material coletado. Houve crescimento positivo no caldo cérebro coração e ágar sangue e ausência de crescimento no ágar MacConkey. Na coloração de gram observou-se cocos gram negativos agrupados, e na coloração de Ziehl- Neelsen Modificado observou cocos agrupados corados em vermelho. Essas características de crescimento, assim como os resultados microscópicos observados, foram sugestivos e semelhantes às bactérias pertencentes ao gênero *Brucella sp*. Após um resultado sugestivo a *Brucella spp*, a amostra foi encaminhada para extração de DNA, e sequenciamento nucleiotideo. Houve a identificação de *Brucella suis* biovar 1; com posterior montagem da árvore filogenética. Além da questão sanitária, o abate clandestino envolve ainda a questão ambiental, pois geralmente é realizado em locais próximos a rios ou córregos para facilitar a captação de água. A identificação de bactérias com características morfológicas semelhantes ao gênero *Brucella* do abscesso de suíno observado neste estudo, confirma que o animal apresentava brucelose suína e que abatedouros clandestinos podem ser importantes meios de infecção e disseminação desse agente, se não houver fiscalização que garantam a utilização de medidas de vigilância sanitária de qualidade que venham impedir esse tipo de prática e a venda do produto para o consumo humano.
Palavras-chave: *Brucella sp*; Brucelose*;* *Brucella Suis*; Abate clandestino.

Resumen
Este trabajo tuvo como objetivo estudiar los microorganismos presentes en lesiones encontradas en vísceras de cerdos del municipio de Cachoeirado Arari (Ilha do Marajó), vendidos en la feria Ver-o-Peso de Belém do Pará; Se realizó aislamiento, secuenciación de nucleótidos e inferencia filogenética del material colectado. Hubo crecimiento positivo en caldo cerebro-corazón y agar sangre y no hubo crecimiento en agar MacConkey. En la tinción de Gram se observaron cocos gram negativos agrupados, y en la tinción de Ziehl-Neelsen Modificada se observaron cocos agrupados teñidos de rojo. Estas características de crecimiento, así como los resultados microscópicos observados, fueron sugestivos y similares a bacterias pertenecientes al género Brucella sp. Ante un resultado sugestivo de Brucella spp, la muestra fue enviada para extracción, amplificación y secuenciación de ADN. Se identificó *Brucella suis* biovar 1; con el posterior ensamblaje del árbol filogenético. Además del tema sanitario, la matanza clandestina también involucra el tema ambiental, pues suele realizarse en lugares cercanos a ríos o quebradas para facilitar la captación de agua. La identificación de bacterias con características morfológicas similares al género Brucella del absceso porcino observado en este estudio confirma que el animal padecía brucelosis porcina y que los mataderos clandestinos pueden ser importantes medios de infección y diseminación de la enfermedad, si no se adoptan medidas de vigilancia sanitaria .de calidad para prevenir este tipo de prácticas y la venta del producto para consumo humano.
Palabras clave: *Brucella sp*; Brucelosis; *Brucella suis*; Sacrificio clandestino.

1. Introduction

Brucellosis is a zoonosis present throughout the world, especially in tropical and subtropical regions, with the exception of a few developed countries that have managed to eradicate it or reduce its prevalence rates. In developing countries, work is still being done to achieve this goal. It is a chronic infectious disease caused by bacteria of the genus Brucella that infect several species of domestic and wild animals, in addition to humans (Dean et al.; 2012).

It is an infectious disease caused by bacteria of the genus *Brucella*. It is endemic in many countries, resulting in significant economic losses to production systems and serious implications for animal and public health, given its zoonotic character (BRASIL, 2006). Brucellosis can be transmitted to humans through the ingestion of contaminated animal products, mainly milk and dairy products that have not undergone thermal processing. It can also be transmitted by direct or indirect contact with infected animals, aborted fetuses or fetal attachments, in addition to the handling of carcasses and viscera in the sanitary slaughter (Paulin et a., 2008).

The maximum exposure of the disease in humans is observed among veterinarians, insemination technicians, zootechnicians, rural producers and employees of meat processing companies, who can become infected directly, through contact with contaminated fomites or by ingestion of raw milk or its contaminated unpasteurized derivatives. The most

frequent routes of infection are mucous membranes, airways, the injured skin of the hands during contact with an infected placenta, aborted fetuses or amniotic fluid, the performance of gynecological procedures; as well as through interpersonal transmission, through sexual contact or bone marrow transplantation. Occupational brucellosis is related to laboratory professionals, either due to lack of biosafety or accidents in work facilities (Dean et al.; 2012).

Commonly caused by *Brucella suis*, infection by *B. abortus* is not ruled out when swine are raised promiscuously with cattle, which normally occurs in subsistence farms. Under these conditions, a sick animal represents economic damage due to the drop in productivity, risk to the health of other animals, in addition to concern about human contamination (Aguiar et al., 2006).

Infected animals acquired from farms without certification or prior knowledge (for example, a breeder) is usually the cause of the introduction of the disease in herds, mainly through the venereal route (Muys et al., 2004). The clandestine slaughter of swine, although an unacceptable practice, occurs throughout the country, so it is an important focus of infections and risk to public health by collective exposure to infectious agents, such as those that are transmitted to humans by contact with animals, by the ingestion of foods of suspect sanitary quality and by the contamination of the environment. However, despite the facts, the risk associated with these abattoirs does not seem to receive due attention (Hunter et al. 1994).

Thus, the places where pigs are illegally slaughtered in the country are important sources of infection and dissemination of *Brucella suis*, as well as risk factors for collective exposure in these environments to other infectious agents that are transmitted to humans through contact with animals. The ingestion of these foods without quality recognized by health agencies and even contamination of the environment, promote a high risk to public health. Although all the evidence pointed out about the risk related to the slaughter and clandestine commercialization of swine; this fact has been neglected as a risk factor for the permanence of zoonotic brucellosis (Taleski et al., 2002).

Considering the risk of transmission of swine brucellosis to humans and the conditions under which the animals are slaughtered and sold, this work aimed to study the microorganisms present in lesions found in swine from the municipality of Cachoeira do Arari (Ilha do Marajó), sold at the Ver-o-Peso fair in Belém do Pará; the phylogeny of samples positive for *Brucella suis* was also performed. This bacterium has great importance in public health and in the country's economy. It is believed that after the investigation, we will be able to use the results for a better analysis and planning of strategies for the promotion and prevention of diseases.

2. Methodology

This study was carried out using viscera samples that were acquired in a clandestine slaughterhouse. As these are commercially acquired parts, this study does not require approval by the Ethics Committee for the Use of Animals and will follow the Normative Resolution of the National Council for the Control of Animal Experimentation (Concea) No. 30, of February 2, 2016.

This is a descriptive study of the isolation of microorganisms present in lesions of swine viscera from the municipality of Cachoeira do Arari, State of Pará, Brazil. The viscera were acquired from a clandestine slaughterhouse for macro and microscopic studies in the Veterinary Microbiology laboratory of the Institute of Animal Health and Production of the Federal Rural University of the Amazon, for the analysis of lesions caused by the nematode Stephanurus dentatus, and in only one of these animals was the lesion in the form of an abscess near the urethra was detected, which was sent for microbiological analysis.

2.1 Isolation of microorganisms

2.1.1 Brain Heart Broth (BHI)

Brain Heart Infusion Agar (BHI) is a nutrient-rich liquid medium suitable for the cultivation of various strains of fastidious bacteria, fungi and yeasts. BHI medium is recommended in standard methods for water testing and antimicrobial susceptibility testing. It is a culture medium for the growth of cocci and other pathogenic microorganisms, including aerobic and anaerobic bacteria from various clinical and non-clinical materials. The medium was weighed and hydrated according to the manufacturer's instructions, mixed well and dissolved under frequent heating (Bunsen burner) and agitation. Boiling was carried out for one minute until dissolution was complete; then 3.0 ml was distributed in tubes with screw caps; and sterilized in an autoclave at 121°C for 15 minutes; then, the tubes were removed from the autoclave and cooled to room temperature. With the aid of a bacterial loop or thread, lesions of the abscess were inoculated to perform the test with pure colonies for 18 to 24 hours; was incubated at 37°C ±2 for 24 hours, under aerobic conditions. The original color of the medium is clear, pale yellow. For a positive result, turbidity will be present = bacterial growth. For negative result: absence of turbidity (Anvisa, 2004).

2.1.2 Blood Agar

Blood Agar (5%) medium offers optimal growth conditions for most microorganisms. The conservation of intact erythrocytes favors the formation of clear halos of hemolysis. It is used for the isolation of non-fastidious microorganisms; and to check for hemolysis. The medium was weighed and hydrated according to the manufacturer's instructions; then sterilization in an autoclave took place; the base was cooled to +/- 50°C; and 5 ml of defibrinated sheep blood is added for every 100 ml of base; it was gently homogenized so as not to form bubbles; was distributed in 90 mm diameter Petri dishes. Then, for inoculation, the striation of the surface of the medium was made, using the sowing technique for isolation; at the end of sowing, the medium was chopped with the handle to verify in-depth hemolysis.

2.1.3 MacConkey Agar

It aims to isolate Gram negative bacilli (enterobacteria and non-fermenters) and verify the fermentation or not of lactose. Crystal violet inhibits the growth of Gram positive microorganisms especially enterococci and staphylococci. The concentration of bile salts is relatively low compared to other media, so it is not as selective for Gram negatives. The medium was weighed and hydrated according to the manufacturer's instructions; then it was heated under stirring until the agar melted completely; was sterilized in an autoclave; cooled to 50°C and distributed 20 to 25 ml in sterile 90 mm Petri dishes; left at room temperature until cool; the plates were packed with transparent PVC plastic and stored in a refrigerator at 4 to 8°C. Then, the plates were inoculated and incubated for 24 hours at 37°C, under aerobic conditions.

2.2 Morphological and dyeing analysis of isolated microorganisms

2.2.1 Gram Technique

The Gram technique, also known as Gram stain, is a method of staining bacteria, which allows differentiating bacteria with different cell wall structures from the stains they acquire after treatment with specific chemical agents. The Gram stain method is based on the ability of the cell walls of Gram-positive bacteria to retain the crystal violet dye in the cytoplasm during an ethanol-acetone treatment, while the cell walls of Gram-negative bacteria do not. Initially, the smear was made; then it was stained with crystal violet for 60 seconds; then it was washed with a splash of distilled water; covered with lugol for 60 seconds; washed with a splash of distilled water; decolorized with 95% alcohol for 10-20 seconds; washed again with a splash of distilled water; stained with safranin for 60 seconds; washed with distilled water, dried and observed under a microscope.

The method consists of successively treating a heat-fixed bacterial smear with crystal violet, lugol, ethanol-acetone and safranin reagents. Bacteria that stain blue-violet are called Gram-positive and those that stain red are called Gram-negative.

2.2.2 Modified Ziehl-Neelsen Technique (ZNM)

This staining technique is more aggressive than the Gram technique and is used on bacteria that are not stained satisfactorily by Gram stain. There are bacteria that are resistant to staining, but when stained, they strongly resist discoloration, even when subjected to strongly diluted acids and absolute alcohol. These bacteria are called acid-fast bacilli (AFRB). In the Ziehl-Neelsen technique, after the sample staining process, Ziehl's fuchsin will stain all cellular elements red, but after discoloration with alcohol, only the acid-fast bacilli will continue to preserve the red color, the remaining cellular elements in the sample will be destained. So, in order to visualize the other (bleached) cellular elements in the sample, Methylene blue should be used, which will give a contrast, leaving the cellular elements in blue and the alcohol-acid-fast bacilli will remain in red. For this, a smear of the material was performed; was placed on a glass slide for microscopy, with heat fixation; Ziehl's fuchsin (reddish dye) was added all over the slide, heating it until the emission of vapors, repeatedly for 5 minutes; then the material was washed with running water, the substrate for decolorization was added (3% hydrochloric acid solution in ethyl alcohol), the slide was then washed. A 1% methylene blue solution was added to the slide, added from 4 to 6 drops of 1:500 sodium hydroxide solution at the time of staining. Let it act for three minutes. After, was washed in a thin stream of running water, falling outside the smear. Allowed to dry at room temperature (Siqueira et al., 1982).

2.3 DNA extraction

DNA extraction was performed using the phenol-chloroform methodology, it is an extraction method that is based on the difference in DNA solubility in water and organic solvents, according to pH. Lysis and homogenization are performed to release the DNA (JOSEPH et al., 2001). Chloroform phenol and isoamyl alcohol were added and stirred; followed by centrifugation. With centrifugation, the precipitation of DNA occurred, the supernatant was removed. What remained was resuspended in a buffered solution; at the end the evaluation of the quality of the extraction was carried out in the spectrophotometer. Then the DNA was quantified using the Qubit 2.0 instrument together with the Qubit™ dsDNA HS Assay Kit.

2.4 Clonal Amplification of the Genomic Library (emulsion PCR - emPCR)

DNA A for clonal amplification of the genomic library was performed using emulsion PCR (emPCR) using the emPCR Kit (Roche), in the Veterinary Parasitology laboratory of the Instituto da Saúde Produção Animal, Universidade Federal Rural da Amazônia.

This reaction requires the DNA sequence to be amplified, a pair of primers, nucleotides and an enzyme for DNA synthesis. This enzyme must be heat resistant in order to withstand the high temperatures used during each PCR cycle to denature the DNA. The emulsion reaction was carried out by mixing the products of the previous reaction with the enzymes and capture spheres soaked in a solution called MOCK and the reagents of the PCR reaction (water, buffer and enzyme). The DNA fragments from the genomic library were contacted with capture beads (Beads) required during the sequencing step. DNA fragments, enzymes and beads were soaked in water and injected into small deposits cylinders containing synthetic oil. The mixture of these reagents, combined with their vigorous agitation, produced an emulsion (small drops around the spheres, called microreactors). Each microreactor should contain an isolated DNA fragment amplified by PCR, producing millions of copies immobilized on the capture beads. At the end of the PCR reaction, the beads (beads) that did not present DNA

fragments were eliminated. However, the beads that presented amplified DNA were retained and used for the processing and reading of the sequencing signal.

2.5 Nucleotide Sequencing

The DNA amplified in the capture beads, from the previous step, were deposited in the holes of the plate called PicoTiterPlate, the plate was completely filled, and each hole allowed the inclusion of only one capture bead. After filling the plate with capture beads, the plate was inserted into the FLX GS 454 sequencer (Roche), and each sample received a different barcode. The nucleotides (A, C, T, G) migrated in a pre-established order over the holes on the plate, coming into contact with the capture beads containing the amplified DNA's. The addition of one or more complementary nucleotides to the target DNA resulted in a chemiluminescent signal, captured by a CCD camera (Charge Coupled Device) of the sequencer.

2.6 Phylogenetic Analysis

The phylogenetic inference was performed from the nucleotide sequences of nine gene regions (gap, aroA, glk, dnaK, gyrB, trpE, cobQ, omp25 and int-hyp) concatenated and with approximately 4,396 bp described by Whatmore et al 2007, together with sequences available in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov).

The dataset was generated together with the samples from the aforementioned study, to be submitted to the Multiple Sequence Alignment (MSA), using the Mafft v.7 program (Katoh & Standley, 2013), the result of the alignment was manually inspected for manual alignment corrections, when necessary, using the Geneious v.9.1.8 program (https://www.geneious.com/).

The aligned dataset was submitted to analysis to identify the best nucleotide substitution model, and then the construction of phylogenetic trees was performed using the Maximum Likelihood (MV) methodology (Myung, 2003). Both methodologies were used using the IQ-TREE v.1.6.12 program (Nguyen, 2015). Together with these analyses, the bootstrap test was used, setting 1000 replicates, in order to provide greater reliability to the values of the groups (Felsenstein, 1985).

The visualization of the phylogeny was performed by the program FigTree v.1.4.4 (https://github.com/rambaut/figtree/releases/tag/v1.4.4). For the dataset used, it was decided not to use a root sequence, for this reason the Midpoint rooting methodology was used, a tool available in the phylogeny visualization program.

After evaluating and editing the phylogeny, a file with the extension ".sgv" (Scalable Vector Graphics), for image editing and manipulation using the Inkscape v.1.1 program (https://inkscape.org/release/inkscape-1.1/).

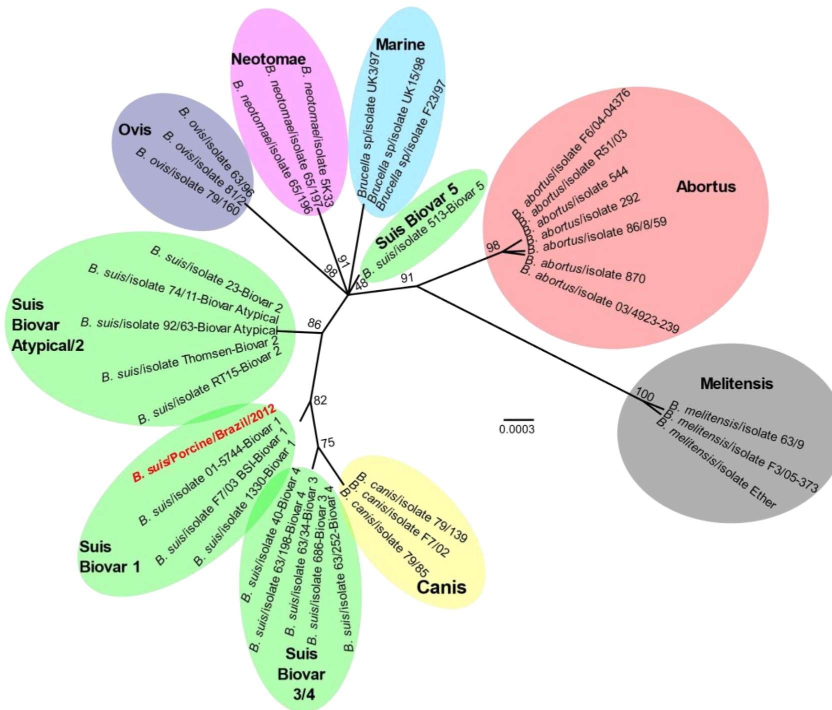
3. Results

There was positive growth on brain-heart broth and blood agar and no growth on MacConkey agar. Growth was observed on the fifth day after inoculation, of small, circular, mucusless, less shiny colonies and without hemolysis.

In the gram staining, grouped gram negative cocci were observed, and in the ZNM staining, grouped cocci stained in red were observed. These growth characteristics, as well as the observed microscopic results, are suggestive and similar to bacteria belonging to the genus *Brucella*.

The *Brucella* strain isolated in theses study was grouped in a monophyletic *Brucella suis* (Biovar 1) clade (Figure 1).

Figure 1. Phylogenetic tree of different species of *Brucella*. Using Method Maximum Likelihood (ML) based nine gene regions (gap, aroA, glk, dnaK, gyrB, trpE, cobQ, omp25 and int-hyp). Phylogenetic groups are labelled in different colors. Numbers at each main node of the tree correspond to bootstrap values in percent (1000 replicates). The scale bar corresponds of genetic divergence in nucleotide sequences.



Source: Authors.

4. Discussion

The pigs acquired for this study came from a clandestine breeding facility in Cachoeira do Arari (State of Pará/Brazil) and had inflammatory abscesses in the urethral region of the animal. They arrived in Belém, at the Mercado do Ver-o-Peso port, to go for clandestine slaughter and subsequent sale/consumption at fairs in the Municipality of Belém. The pigs were slaughtered and the researchers collected the viscera with the abscess. Only one positive swine was identified, suggestive of swine brucellosis, observed through microbiological and microscopic analysis of the abscess found in the urethral region of the animal. In contrast to what is observed in some studies, the presence of swine brucellosis in pigs from farms, wild, slaughterhouses and slaughterhouses (Pedersen et. al.; 2014).

Then, after a suggestive result for *Brucella sp*, it was necessary to confirm through molecular analysis, which was identified as contamination by *brucella suis* biovar 1.

In work describe by Cvetnić et al in 2009 also isolated morphologically suspicious colonies from lymph nodes and reproductive organs of domestic swine and wild boar and confirmed *Brucella sp* in 58.3% of pigs and 7.5% of wild boar by polymerase chain reaction (PCR), and six strains were identified as *B. suis*.

In the study by Stoffregen et. al., in 2007, *Brucella sp* was isolated from bacterial culture based on colony morphology and growth characteristics, in which, from a total of 77.5% (62) of the samples, 68.8% (55) were confirmed for *B. suis* by multiplex PCR. Clustered cocci, stained red by the ZNM technique, were also observed by microscopy. This

observation was important to complement the suspicion of bacteria of the genus Brucella. Both staining techniques serve as a safe and cost-effective method of presumptive identification of Brucella from fluid and tissue cultures.

Although an early and rapid detection of *Brucella sp* in culture is very important to initiate an appropriate antimicrobial treatment, the risks are increased to acquire brucellosis from the manipulation of these materials in the laboratory (Tilak et al., 2016). In addition, studies point out the risks inherent to people who work with these animals in breeding places, slaughterhouses and slaughterhouses, in view of handling without adequate care, such as the use of personal protective equipment, and consumers in general, since many of the animals do not show visible or indicative lesions of the disease (Rosa et. al.; 2012).

Molecular techniques, in addition to their importance in diagnosis, are useful in molecular typing and characterization of *Brucella sp.*, and can be used in studies of genetic diversity, in an evolutionary context, or in epidemiological surveillance and research in regions with a high incidence of brucellosis (Ferreira et.al., 2011). However, discrimination between *Brucella sp.* species proves to be a complicated task, since the Brucella genus is genetically conserved, with more than 90% homology between Brucella species (Lee et al.; 2013).

Studies have indicated that *B. suis* biovar 5 is distinct from other *B. suis* isolates and therefore it is unclear whether there is justification for including *B. suis* biovar 5 in a taxonomic group with *B. suis*. Indeed, the status of *B. suis* as a single species has been questioned in light of broader host specificity and because, in contrast to other classical species, no species-specific marker for *B. suis* has been identified (Ferreira et al., 2017).

In *B. suis* isolates, there is a strong congruence between the genotype and the biovar designation (Whatmore et. al., 2016), which differs according to geographic distribution and host range (Munõz, et. al., 2019). *B. suis* biovars 1 and 3 are zoonotic, and the preferred hosts are domestic pigs and occasionally wild boar (Godfroid et al., 2013). *B. suis* biovar 1 is common in South America and Asia (Cvetnić et. al., 2009), while biovar 1 and biovar 3 are mainly present in the United States, Australia and China (Deqiu et al., 2002). *B. suis* biovar 2, mainly restricted to hares and wild boar in Euroasia, also infects domestic pigs (Munõz, et. al., 2019; Ferreira et. al., 2017), but rarely humans (Mailles et. al., 2017). *B. suis* biovar 4 is a pathogen of reindeer and caribou and a zoonotic risk in the arctic region (Godfroid et. al., 2013). *B. suis* biovar 5 from eastern European rodents appears to be confined to its natural hosts and is rarely found in domestic animals or humans (Olsen & Tatum, 2016; Vergnaud et. al., 2018).

Similar to the distribution of biovars, two major clades of *b. south*genetically divergent were described by MLVA (Multiple Locus Variable-number Tandem Repeat Analysis), MLST (Multilocus sequence typing) and WGS (Whole genome sequencing) phylogenetic reconstruction: one included *B. suis* biovar 2 and the second *B. suis* biovars 1, 3 and 4 and *B. canis*. *B. suis* biovars 5 forms a separate branch (Whatmore et al., 2016).

The percentage of positivity observed in several seroepidemiological studies suggesting that swine brucellosis is present in the country in a significant way is worrying, but not exactly because of the notification of cases of disease in humans, but because of the percentage of positive properties and slaughter sites, presenting at least one animal reactive for brucellosis (Atluri et al.; 2011)

In the study by Vicente et. al., 2021, serological investigation was carried out for brucellosis in pigs, horses, dogs and cattle on a farm with a history of abortion in sows and necropsy of a wild boar with severe necrosuppurative orchitis. One sow, two cows, and two dogs tested positive on the Acidified Buffered Antigen Test (AAT), although only the sow had a confirmatory result on 2-mercaptoethanol (2-ME). The 2-ME-positive sow was sacrificed and the microbiological culture of lymph nodes and liver followed by biochemical characterization allowed the phenotypic characterization of *Brucella suis* biovar 1; performed using Bruce-ladder and Suis-ladder multiplex PCR.

In Argentina, Bence et. al., 2021 reported a case of human brucellosis caused by *B. suis* in a person who worked on a small pig farm. The farm had no history of clinical brucellosis and no signs of the disease were observed on clinical examination of the animals. Specific antibodies were detected in 53% (10/19) of the adult pigs, while all gilts sampled were seronegative. *B. suis* biovar 1 was isolated from a boar. In contrast, although the bacterium was not isolated from any tissue of a seropositive sow, it was detected by DFAT (direct immunofluorescence antibody test). From the bacteriological and serological evidence of endemic *B. suis* infection in the swine farm and the lack of preventive measures and biosecurity practices,

Pork is the most consumed protein source in the world, with Brazil being the fourth largest producer and exporter. In this context, Brazilian swine farming plays an important role in the country's agribusiness. This production chain is an important factor in national economic development, as it causes multiplier effects on income and employment in different sectors of the economy, requiring strict standards of disease control from the health surveillance system, especially zoonoses (ABIPECS, 2016).

Post-mortem inspection carried out in official slaughterhouses is vital for the discovery of lesions suggestive of such diseases and the application of preventive measures would prevent the spread of pathogenic agents among workers at the site (Dias, 2012). Most facilities need to have tiled walls, cold floors, waste treatment and an air exhaust system recommended by the Ministry of Agriculture, Livestock and Supply (MAPA, 2013). According to Federal Law No. 8,137/90, clandestine slaughter is an illegal activity considered a crime against consumer relations. And, despite the existence of laws that regulate inspection and sanitary surveillance services, in order to curb these slaughterhouses and improve the situation of meat sold in Brazil, the perpetuation of these establishments,

The Clandestine slaughter has been neglected as a risk factor in the occurrence of zoonotic brucellosis. The transmission of zoonoses can occur in several ways, among them, handling of animal carcass and contaminated viscera, in addition to blood, urine, feces and sharp material during the stages at the time of slaughter (Castro et al., 2014). And, despite the existence of laws that regulate inspection and sanitary surveillance services, in order to curb these slaughterhouses and improve the situation of meat sold in Brazil, the perpetuation of these establishments remains a serious problem in national public health for decades. Bánkuti e Azevedo, 2001 mention that clandestinity has two basic conditions: non-inspection by the sanitary inspection service and tax evasion.

The hygienic obtaining of the carcasses, from which the cuts of meat, viscera and meat derivatives result, is a primordial condition in the prevention of zoonoses and intoxications of enteric and nervous action. It is estimated that 60% of pathogens that affect humans are of zoonotic origin and that 80% of animal pathogens have multiple hosts (Cutler, 2010). The spread of these diseases is directly related to the ability of the etiologic agent to maintain itself in viable conditions at the source of infection.

The hygienic conditions found in clandestine slaughterhouses, the disregard for the health and protection of the people involved in the slaughter, the lack of sanitary inspection of meat and carcasses, animal welfare, are absent points in these killings, since it is completely out of legislation, according to Normative Instruction No. 3, of January 17, 2000, which deals with the methods of stunning for humane slaughter. It was also observed that they promote mistreatment of animals from transport to the time of slaughter, causing stress, hunger and thirst, directly affecting the quality of the meat (Castro et al., 2014).

There needs to be an intensification of the community work of clarification for the owner, workers and slaughterhouses, as well as for community leaders, animal health agents and consumers (Azevedo et al., 2012). In addition to the sanitary issue, clandestine slaughter also involves the environmental issue, as it is usually carried out in places close to

rivers or streams to facilitate the capture of water. However, blood and animal remains end up being dumped in these waters or even consumed by animals close to the point of slaughter, increasing the risk of spreading diseases (Costa et al., 2011).

The cases of diseases caused by zoonoses have been increasing gradually due to the constant changes caused by man in the environment, most of the time, the cases of diseases are in regions where the populations are deprived, have precarious habits of hygiene and low income, providing a space higher for infection by pathogens (Lima et al., 2017).

5. Conclusion

The identification of bacteria with morphological characteristics similar to the *Brucella* genus of the swine abscess observed in this study confirms that the animal had swine brucellosis and that clandestine slaughterhouses can be important means of infection and dissemination of the disease if there is no adoption of sanitary surveillance measures. quality to prevent this type of practice and the sale of the product for human consumption.

We suggest that more seroepidemiological and molecular studies be carried out in the State of Pará to identify positive properties for brucellosis, as well as surveillance measures to combat clandestine slaughter in the region and specific control projects for swine brucellosis to prevent infection and spread of the disease.

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