



PREVALENCE OF SHEEP BRUCELLOSIS IN HERDS IN THE STATE OF BAHIA

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ABSTRACT

This study aimed to investigate the prevalence of ovine brucellosis caused by *Brucella ovis* in sheep herds in the state of Bahia, Brazil. Blood serum samples were collected randomly from rural properties and slaughterhouses with official sanitary inspection in Bahia, focusing on animals originating from the state itself. Laboratory analyses were conducted using the ELISA test, alongside a questionnaire to characterize the production system. Variables such as sex, age, participation in events and gatherings, rearing method, and breed were evaluated. Samples were processed following a Standard Operating Procedure, and reactive animals were identified both on farms and in slaughterhouse samples. The prevalence rates were 2.22% in rural properties and 1.76% in slaughterhouses. Ovine brucellosis was detected in seven out of the twenty-seven identity territories of the state, affecting both males and females. Health risks were assessed, and strategies for the control and eradication of this disease in the state of Bahia were outlined.

Keywords: Sheep; *Brucella Ovis*; ELISA; Epidemiology.

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INTRODUCTION

Ovine brucellosis is a disease that causes significant losses to the sheep farming industry, reducing both production and productivity. *Brucella ovis* induces clinical or subclinical infections in sheep, characterized by epididymitis and orchitis in rams, leading to reduced fertility, and placentitis and abortion in ewes, as well as increased perinatal mortality in lambs (WOAH, 2023). *B. ovis* can be present in semen approximately five weeks after infection, with epididymal lesions detectable by palpation around nine weeks post-infection (Quinn et al., 2011). In females, the disease typically results in abortions and increased offspring mortality (Xavier et al., 2009). Placental remains, milk, and genital secretions are the primary routes of disease transmission (Castro, Gonzalez, and Prat, 2005). The lesions result from the pathological processes of the disease, initiated by the pathogen's penetration through mucosal barriers.

Once inside, the bacterium is transported, either freely or within macrophages, via lymphatic pathways to regional lymph nodes, where it actively multiplies and remains for days to months. Subsequently, it enters the bloodstream, characterizing an acute phase and facilitating bacterial dissemination throughout the body, particularly to organs rich in phagocytic cells, such as the liver, lymph nodes, spleen, lungs, and kidneys. These sites often exhibit lymphoid hyperplasia, diffuse granulomas, splenomegaly, hepatomegaly, and endocarditis (Brasil, 2006; Lira and Megid, 2009). As the disease progresses, the microorganism may persist in the genital tract 30 days post-infection, resulting in a chronic condition (González and Prat, 2005; Brasil, 2006; Castro).

Not all affected animals display detectable clinical signs upon physical examination, complicating diagnosis. The disease must be differentiated from conditions caused by other bacterial agents such as *Corynebacterium pseudotuberculosis*, *Actinobacillus seminis*, *Actinobacillus actinomycetemcomitans*, *Histophilus ovis*, *Haemophilus spp.*, and *Chlamydophila abortus*, which are potential causes of epididymitis (Alves et al., 2010). In Brazil, the first diagnosis of this disease, correlating clinical alterations with agent isolation, was conducted in the 1960s by Ramos et al. (1996) in the state of Rio Grande do Sul. Since then, various studies have been carried out nationwide, confirming the disease's presence across all regions with varying prevalence rates.

The state of Bahia has the largest sheep population in Brazil, with 4.49 million head, representing 22.1% of the national flock. Sheep farming in Bahia plays a crucial role, not only economically in the semi-arid region but also socially, serving as an important tool for improving the Human Development Index (HDI). This is particularly relevant as sheep farming is a primary activity for many smallholder farmers in the state. Epidemiological surveys on ovine brucellosis have been conducted in Bahia using the IDGA diagnostic test, such as those by Silva et al. (2009) in the Recôncavo region, Souza et al. (2012) in the Juazeiro region, Ribeiro et al. (2013) in the Feira de

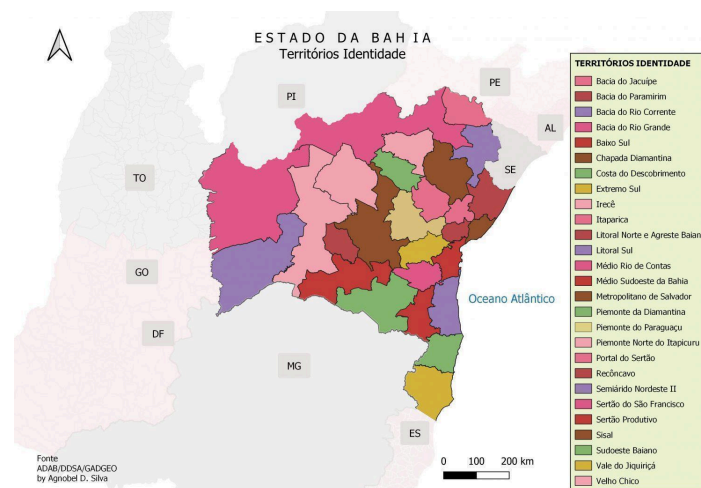
Santana region, and more recently by Carvalho et al. (2021) also in the Recôncavo region, with reported prevalence rates of 3.27%, 0.72%, 6.94%, and 0.52%, respectively.

Comparative studies of diagnostic techniques for ovine brucellosis demonstrate that the IDGA has a sensitivity of 96.4% to 97.10%. When the Complement Fixation (CF) test was used, sensitivity reached 92.7%. However, the ELISA (Enzyme-Linked Immunosorbent Assay) test proved to be the most sensitive (97.6%) with a specificity of 100% (Batista, 2012). This study aims to estimate the prevalence of ovine brucellosis in Bahia using the ELISA technique and to analyze potential risk factors associated with the occurrence of this significant disease.

MATERIALS AND METHODS

The sample size was calculated using the Integrated Program for Epidemiology (Epi Info). A 50% detection probability (for diseases of unknown occurrence in a given population), a 99% confidence interval, and a 3% statistical error were considered, resulting in a sample size of 1,834 animals. To account for potential losses, a 10% margin was added, totaling 2,018 samples. Sample collection was based on the sheep population in each identity territory (Fig. 1), selected randomly. If the chosen property no longer had sheep, sampling was conducted at the nearest property with an existing sheep herd.

Figure 1. Identity territories in the state of Bahia where blood samples were collected to determine the prevalence of ovine brucellosis.



Source: ABA/DDSSA/GADGEO_LADESA by Agnobel D. Silva.

On the selected properties, sampling prioritized the oldest male sheep in the flock when more than one ram was present. Similarly, the oldest ewe was sampled, followed by additional randomly selected animals from the flock until the required sample size for each property was met. A questionnaire was used to gather data on the variables to be studied, with information



collected in person from the property owners or those responsible for animal management.

In slaughterhouses, samples were collected randomly from the slaughter line, exclusively from animals originating from the state of Bahia, as verified by the animal transit guide (GTA). Sanitary aspects were considered to characterize the origin of the animals.

Figure 2. Map of identity territories in the state of Bahia showing the distribution of positive animals collected on farms, at official slaughterhouses, and in territories without disease occurrence.

Of the 2,018 samples collected, 396 originated from males and females in slaughterhouses, while 1,622 samples were collected from males and females on rural properties. The samples were processed at the Animal Health Defense Laboratory of the Bahia Agricultural Defense Agency (ADAB) using the ELISA test with an IDEXX kit approved by the Ministry of Agriculture, Livestock, and Supply (MAPA).

Sample processing was performed by ADAB's technical team, consisting of veterinarians and state agricultural inspectors trained and qualified to execute the technique. The entire laboratory analysis process followed the ISO 17.025 – 2017 standards. Each sample was individually identified by its registration number, and the property samples were identified through a service order (SO).

All samples, including those from slaughterhouses, were collected during the same period (July to August 2020) and processed immediately upon arrival at the sample reception sector of the Animal Health Defense Laboratory (LADESA) at ADAB. After blood collection, serum was extracted, stored under freezing conditions with daily temperature monitoring, and processed using the TP-READER microplate reader compatible with the IDEXX ELISA kit. Processing adhered to the manufacturer's instructions. All equipment used for sample processing was calibrated, meeting the technical requirements described in the ADAB laboratory quality manual.

This study was submitted to and approved by the ethics committee in a council meeting on April 13, 2022 (code PF389-2022), with an execution period from May 22, 2022, to May 22, 2024.

Statistical Analysis

For preliminary exploratory data analysis, data were organized into contingency tables. The prevalence of ovine brucellosis in the identity territories of Bahia, as determined by serologically positive results in the ELISA test, was assessed. Prevalence rates were calculated for animals from officially registered slaughterhouses in Bahia and rural properties in the state. Confidence intervals for proportions were calculated with 95% confidence using the PAST software (Hammer & Ryan, 2016).

Regarding the prevalence of ovine brucellosis by animal sex, the Z-test for the difference between proportions was applied with 95% confidence using Statistica software (StatSoft, Inc., 2011).

Forms were completed to include descriptions of each property, such as location/region, purpose of breeding, type of farming, and population data, including species affected, age groups, animal movement, outbreak chronology, vaccinations, and material collection records (species, sample number). Clinical signs for each animal on the property were also recorded.

RESULTS AND DISCUSSION

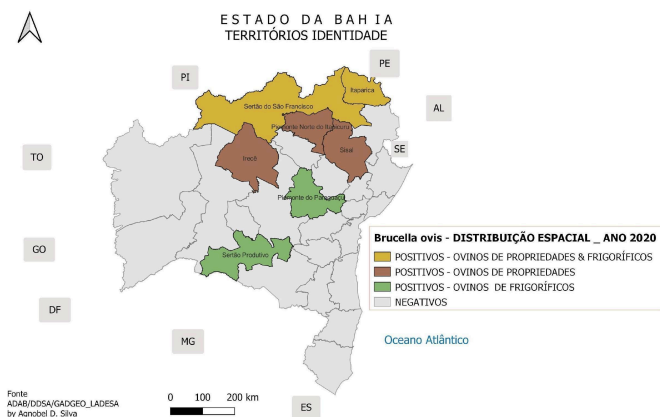
The study demonstrated the occurrence of ovine brucellosis in sheep herds in the state of Bahia, Brazil. The overall prevalence among males was 1.55% (10/644), while among females, it was 2.40% (33/1374) (Table 1).

Table 1. Prevalence of ovine brucellosis in Bahia by animal sex

Sex	Number of Animals	Nº de animais positivos	Prevalence
Males	644	10	1,55%
Females	1374	33	2,40%

The higher prevalence in females aligns with findings by Pinheiro Junior et al. (2009) and Souza et al. (2012). Araújo et al. (2013), however, did not report significant differences between sexes in the Feira de Santana region. This difference may be related to sanitary management, breeding methods, reproductive control, and the acquisition of breeding animals for introduction into rural properties.

For males, one positive animal originated from a slaughterhouse, while nine were from rural properties. Of these, six were breeding rams, and three were under six months of age. Alves et al. (2010) reported a 7.5% prevalence among animals at a municipal slaughterhouse in Patos, Paraíba. Dorneles et al.





(2020) found a prevalence of 24.4% using ELISA in a slaughterhouse in Minas Gerais, significantly higher than the results of this study. Figueiredo (2007), using IDGA in Patos, Paraíba, found a prevalence of 5% in males and 2.5% in females.

Among females, four of the thirty-three positive animals came from slaughterhouses, all older than six months. Rizzo et al. (2009) reported a 1.96% prevalence among females in São Paulo properties with a history of reproductive disorders. The remaining twenty-nine positive female samples were from rural properties, with twenty-five older than six months and four younger than six months.

These results suggest that animals destined for slaughter come from properties with varying production methods. In Bahia, the positive animals originated from confined properties, while in other studies, they came from properties with limited sanitary control. This highlights that even in intensive farming systems, proper sanitary care is often neglected, and protocols for confinement or technical slaughter are not always implemented.

Table 2. Prevalence of ovine brucellosis in Bahia

Source	Nº Animals	Nº Positive Animals	Herd/Positive Ratio	Prevalence
Rural properties	1622	36	1622/36	2,22 (36)
Official slaughterhouses	396	07	396/07	1,76 (07)
Average prevalence	2018	43	2018/43	2,13 (43)

In various studies, such as those by Araújo et al. (2013), Rizzo et al. (2014), Alves et al. (2010), and Salaberry et al. (2011), no clinical signs of the disease were observed, a situation similarly verified in the present study, where no clinical or macroscopic signs were identified in the animals examined on the slaughter line. When evaluating the technology applied to livestock management, only ten properties used technological methods such as artificial insemination (AI), fixed-time artificial insemination (FTAI), and embryo transfer. However, positive cases of the disease were detected in only one of these properties.

When the occurrence of the disease was assessed in relation to breed, no correlation could be established, as most of the sampled animals were of undefined breed (UB—undefined breed). The animals identified as positive were predominantly breeding rams in extensive farming systems, showing no correlation with breed susceptibility to the disease. Clementino et al. (2005) also found no statistical differences related to breed, as was the case in Carvalho et al. (2021), who studied Santa Inês and Dorper sheep in the Recôncavo region of Bahia. Regarding farming systems, the majority of the properties employed extensive systems, while intensive farming was observed in only

nine evaluated properties. Among the positive animals from these properties, cases were exclusively found in breeding males.

When analyzing the occurrence by identity territory, positive cases were identified only in properties within the territories of Sisal, Sertão do São Francisco, Piemonte Norte do Itapicuru, Itaparica, and Irecê, with the highest incidence observed in the Irecê territory. Positive animals originating from slaughterhouses were reported from the territories of Sertão do São Francisco, Itaparica, Piemonte Paraguaçu, and Sertão Produtivo. This indicates that out of the twenty-seven identity territories, positive cases were found in seven, leaving twenty territories with no evidence of the disease in the analyzed samples (Fig. 2).

Additionally, all properties with positive animals participated in events or animal gatherings, such as fairs and exhibitions, mostly at municipal and/or regional levels. Effective sanitary control for ovine brucellosis during livestock events and animal trade fairs is severely hindered by the lack of specific national legislation that ensures more stringent oversight of these gatherings. Furthermore, for some time, laboratory diagnostics were highly restricted.

Specific measures to control and eradicate this disease have been implemented in certain states. In Rio Grande do Sul, the State Sheep Health Program (PROESCO) was established as a structural program for the sheep farming sector, which includes controlling ovine brucellosis caused by *B. ovis*. Strategies under this program require serological testing for interstate animal transit and participation in livestock events and fairs, aiming to reduce disease incidence. In Bahia, strategies were developed to establish an operational technical base through Ordinance 207 of 2012, which created the State Program for Goat and Sheep Health (PESCO) (Bahia, 2012), and Ordinance 121 of 2017, which established the state laboratory network for diagnosing diseases of interest in small ruminant agricultural defense.

Due to the fragility of agricultural event inspection, data and scientific studies that could provide a better understanding of the sanitary risk at these events remain limited. However, from the collected samples that included positive animals, the sanitary risk is significant in events lacking proper oversight. One viable alternative for event inspection is conducting clinical examinations of animals, particularly males, by palpating the testicles. Nevertheless, as demonstrated in this study, the occurrence in females exceeds that in males, and in gatherings, the sanitary risk multiplies exponentially. Thus, requiring serological testing for animals of reproductive age is more appropriate.

In properties focused on animal production and genetic selection, positive cases were identified in only two properties, with cases found exclusively in males older than six months. In other prevalence studies, ovine brucellosis has been reported worldwide, with rates ranging from 2.4% to 26% (Torres et al., 1997). This prevalence range is consistent with the findings of this study. Ramos et al. (1966) reported a prevalence of 6.5%



using the IDGA technique. Compared to the present study, which used the ELISA technique, higher prevalence rates were found in some territories, while lower rates were observed in others. These differences may be explained by variations in diagnostic methods and management practices in the properties where samples were collected.

In Bahia, the relationship between livestock transit, farming models, and the number of properties trading animals at fairs is an important variable for risk analysis. In states with similar production characteristics and farming methods to the territories where positive animals were found, prevalence rates were reported as 16.25% in Pernambuco (Coletto et al., 2003), 7.5% in Paraíba (Alves et al., 2010), 11.3% in Rio Grande do Norte (Azevedo et al., 2004), and 3.1% in Alagoas (Pinheiro Junior et al., 2009). These findings are comparable to those of the present study, especially when considering similar edaphoclimatic conditions and farming methods.

In the twenty territories of Bahia without disease occurrence, farming practices and production characteristics predominantly included the use of technology, transit control, and animal access control, alongside more organized sanitary oversight. These findings align with studies conducted in other regions with similar production characteristics. For example, Salaberry et al. (2011) reported a 0% prevalence in Minas Gerais using the IDGA technique; Gomes et al. (2001) found 0% prevalence in Rio Grande do Sul using IDGA; and Schafer et al. (1997) also reported 0% prevalence in Santa Catarina using the same method.

When comparing the results of this study with previous findings in Bahia, Araújo et al. (2013) reported a prevalence of 6.94% in the Feira de Santana microregion, values close to those found in this study for neighboring areas. However, the microregion included municipalities from the Portal do Sertão and Bacia do Jacuípe territories, where no positive animals were identified in the present study, except in the Piemonte do Paraguaçu territory, which had positive cases.

CONCLUSION

This study concludes that ovine brucellosis caused by *B. ovis* is present in the state of Bahia, particularly in specific territories where animal movement, events, and gatherings are likely key risk factors. However, in regions where sheep farming does not play a significant role in the livestock sector, no positive cases of the disease were found.

Among animals from slaughterhouses, positive cases were observed in animals originating from intensive systems, such as confinement, in one instance, and from animals in border regions with limited sanitary control and extensive management practices in other instances. These territories exhibit greater deficiencies in sanitary control and the use of genetically improved breeding

Other factors and variables, particularly related to animal transit and participation in events and gatherings, suggest that the observed prevalence could be attributed to deficiencies in animal transit control and the high number of fairs lacking proper sanitary oversight. In the semi-arid region of Bahia, Souza et al. (2011) found a 0.72% prevalence using the IDGA technique in the Juazeiro microregion. When compared to the prevalence found in the same region using ELISA in this study, it can be confirmed that ovine brucellosis is present in this area of Bahia.

The prevalence observed during this period suggests a significant increase, primarily caused by irregular transit, an increase in events and gatherings without adequate sanitary control, inadequate laboratory diagnostics, and the use of a more sensitive and specific diagnostic technique in this study. The use of ELISA, recommended by the OIE and licensed by MAPA, is essential for improving diagnostics.

In a recent study by Magalhães et al. (2022), using ELISA in the Bacia do Jacuípe region of Bahia and in the states of Paraíba and Pernambuco, prevalence rates of 0.64%, 2.53%, and 5.53%, respectively, were reported. These results are similar to those observed in the Bacia do Jacuípe in this study. Additional variables are being analyzed to better assess the laboratory results, preliminary sanitary risk studies, and potential agro-defense measures to improve disease control.

Further analysis of risk factors is underway to better evaluate the laboratory results and identify potential actions for agricultural defense that enhance the control of this disease. It is important to note that some territories showed evident increases in prevalence, while others reported no cases. Internal technical aspects must be understood to prevent an increase in cases in affected territories and avoid the spread to others, some of which have little to no previous studies.

animals from properties with adequate sanitary measures. Most often, these animals are purchased at fairs and events with little or no participation from agricultural inspection authorities.

Therefore, this study highlights the necessity of controlling ovine brucellosis in the state of Bahia. Sheep farming holds significant economic importance in the semi-arid regions of the state and plays a vital social role in improving the Human Development Index (HDI), as it is a primary activity for many smallholder farmers. With the largest national sheep herd and the highest number of smallholder farmers in the country, Bahia has much to gain from enhanced disease control measures in the sheep farming sector.



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