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[*Comunicação*]

# Prevalence of *Brucella melitensis* in creole goats raised in extensive systems in the tropical dry forest of Amazonas, Peru



[*Prevalência de* Brucella melitensis *em cabras Crioulas criadas em sistemas extensivos na floresta seca tropical amazônica, Peru*]

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*Brucella melitensis* (*B. melitensis*) is the primary cause of brucellosis infections in goats and sheep, representing a zoonotic disease with a significant economic impact on the livestock industry and public health (World…, 2022). Globally, the goat population has increased by more than 20% over the last decade, reaching approximately one billion animals (Rossetti *et al.*, 2017). Brucellosis in goats is characterized by abortions in the second or third trimester of pregnancy and the birth of weak or stillborn offspring. Transmission in goats can occur through close contact with infected animals, contaminated environments where the bacteria persist, or semen from infected breeding males (Sánchez, 2007).

Historically, goats have been carriers of *B. melitensis*. While many developed countries have successfully controlled the disease, it remains a significant issue for goat and human health in regions such as the Mediterranean, the Middle East, Central and Southeast Asia, Sub-Saharan Africa, and specific areas of Latin America, where approximately 3.5 billion people are at risk (Rossetti *et al.*, 2017). Human transmission can occur through blood transfusions or organ transplants, contact with contaminated environments, inhalation of contaminated dust, dried manure, or water sources, and occupational exposure through contact with the placenta, fetus, or vaginal discharges of infected animals, affecting farm workers or laboratory personnel. For those without direct contact with animals, brucellosis is mainly transmitted through the consumption of unpasteurized milk and dairy products. Meat can also be a considerable source of infection, especially in cultures that prefer raw or undercooked meat products (Corbel, 2006).

In humans, the disease can present with a wide range of symptoms, including asymptomatic infections. When symptoms do appear, they may develop gradually or suddenly, typically beginning with acute fever and other nonspecific signs resembling flu, such as headache, general malaise, muscle pain, and back pain. Severe night sweats, splenomegaly, and hepatomegaly may also occur. Gastrointestinal symptoms such as loss of appetite, nausea, vomiting, and changes in bowel function are more common in adults than children (Corbel, 2006).

In Peru, various studies have focused on this disease, primarily in dairy cattle (Huguet *et al.*, 2005; Meza *et al.*, 2010; Reyes *et al.*, 2017) and goats (Garro *et al.*, 2005; Taboada *et al.*, 2005; Rojas *et al.*, 2006; Toledo *et al.*, 2007). In the

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Amazonas region, a study conducted in the Upper Imaza basin revealed a 0% prevalence of brucellosis in cattle (López, 2021). However, human brucellosis cases have been reported, placing this region in third place nationally in 2022 (SENASA, 2023). Despite the lack of previous research on this disease in goats in this region, the only available information comes from a SENASA report in 2019.

This study aimed to determine the prevalence of *Brucella melitensis* in native goats in the Utcubamba province, Amazonas region.

The research was conducted in May 2023 in the districts of Bagua Grande, Cumba, and El Milagro, located in the Utcubamba province in the Amazonas region (Fig. 1). Sampling sites were situated at altitudes ranging from 458 m above sea level in El Milagro to 1026 m above sea level in Bagua Grande.

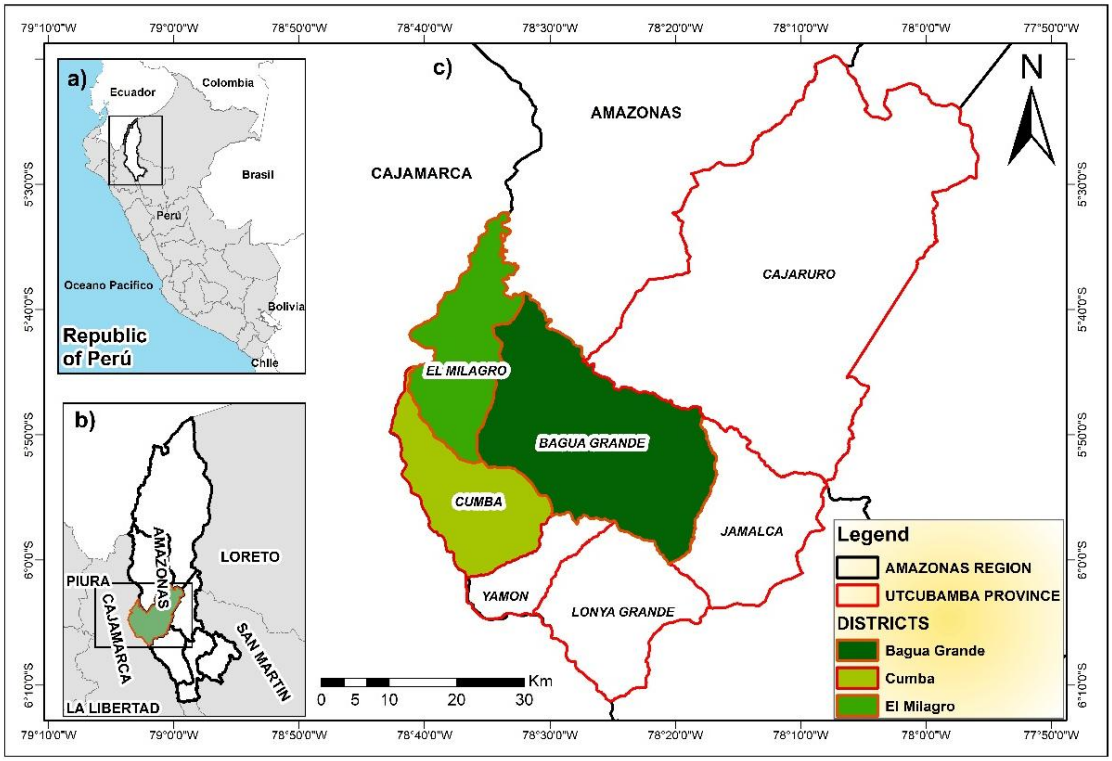


Figure 1. Location of the sampled districts in the Utcubamba province, Amazonas region, Peru.

Three hundred fifty-four blood samples were collected from native goats, including 306 females and 48 males, aged three months to five years, from herds raised under extensive systems in a tropical dry forest. Producers confirmed that the goats had not been vaccinated against caprine brucellosis. The sampling was conducted by selecting producers with a significant number of animals. A survey assessed the herd's technical, reproductive, and health management.

Approximately 4 ml of blood was drawn from each animal via jugular venipuncture using sterile vacutainer tubes. These tubes were labeled with a code corresponding to each animal and kept upright in a container with ice at 5°C to promote clotting and serum separation. The samples were transported to the Laboratory of Infectious and Parasitic Diseases of Domestic Animals at the National University Toribio Rodríguez de Mendoza of Amazonas within 24 hours of collection. Serum was obtained by centrifugation at 3000 rpm for 3 minutes using a Hettich EBA 200 centrifuge (Germany) and stored at -20 °C in properly labeled cryotubes for subsequent analysis.

Serological detection of *B. melitensis* antibodies was done using an indirect multi-species ELISA kit for brucellosis from Innovative Diagnostics

(iD.vet, rue Louis Pasteur, Grabels, France). The test was performed according to the manufacturer's instructions, and readings were taken using an ELISA microplate reader (BIO-RAD iMark, Japan) at an optical density (OD) of 450 nm. Positive (PC) and negative (NC) control wells were duplicated on each microplate, aiding in the validation of the assay. The OD readings were analyzed with the ID Soft™ software (France).

The S/P% of each sample was calculated using the following formula:

$$\frac{S}{P}\% = \frac{OD\ sample - OD_{NC}}{OD_{PC} - OD_{NC}}\ X\ 100$$

Table 1. Interpretation of results for *B. melitensis*

| Result | State |
|---|---|
| S/P% ≤ 110% | Negative |
| 110% < S/P% < 120% | Inconclusive |
| S/P% ≥ 120% | Positive |

The data analysis was performed using Microsoft Excel, where the frequencies of the studied variables were calculated. Because there were no positive cases, no statistical tests were conducted.

No positive cases of *B. melitensis* were identified in the Amazonas region (Table 2). This indicates that, based on the tests conducted, there is no evidence of brucellosis infection in the evaluated goat population. This result suggests that the goats were unaffected by this disease at the time of evaluation, which is favorable for herd health and sanitary management.

This study established the prevalence of *B. melitensis* in goats using indirect ELISA tests. Numerous prior studies in the country have concentrated on the Rose Bengal test. As Díaz-Aparicio *et al.* (1994) noted, the ELISA test employing smooth lipopolysaccharide (S-LPS) and conjugates that detect Immunoglobulin G may be suitable in areas lacking vaccination. Recent investigations have adopted both methods; the Rose Bengal test is used for initial screening, and a positive result leads to confirmation via ELISA (Rodríguez, 2021). Among the serological tests for identifying Brucella, the Rose Bengal test is quick and straightforward yet less specific; the complement fixation test offers greater specificity and sensitivity, making it ideal for epidemiological research, while ELISA is both highly sensitive and specific, making it suitable for large-scale detection as it efficiently processes multiple samples (Díaz-Aparicio *et al.*, 1994; Sadhu *et al.*, 2015).

Table 2. Number of herds and goats sampled by sex for caprine brucellosis diagnosis in the Utcubamba province, Amazonas districts.

| District | Sampled herds | Sex | | Total (n=354) | Positive cases |
|---|---|---|---|---|---|
| | | Female (n=306) | Male (n=48) | | |
| Bagua Grande | 1 | 85 | 15 | 100 | 0 |
| Cumba | 3 | 163 | 12 | 175 | 0 |
| El Milagro | 3 | 58 | 21 | 79 | 0 |

In 2019, the National Agrarian Health Service (SENASA) reported a case of caprine brucellosis in the district of Bagua Grande during monitoring activities conducted in the Amazonas region (SENASA, s.f). Our findings are consistent with previous studies undertaken in Lima Province and Huarochirí in 2004 (Rojas *et al.*, 2006), as well as in Barranca in 2004 (Garro *et al.*, 2005), both of which indicated a prevalence of 0% for caprine brucellosis. Conversely, these results differ from those observed in Cañete in 2004, which reported a prevalence of 0.26% (Toledo *et al.*, 2007), and in Callao, where a prevalence of 6.8% was documented (Taboada *et al.*, 2005). The observed variations in prevalence are likely attributable to the control and eradication program implemented by the National Agrarian Health Service since

the year 2000 (Decreto Supremo N°032-2000-AG) (SENASA, 2000), in conjunction with the limited movement of stud animals from other regions since breeding practices are conducted using natural mating with sires sourced from the herd or nearby producers.

Brucellosis is a substantial public health concern in Peru due to its zoonotic characteristics. Between 2018 and 2022, 1,612 human cases of brucellosis were documented. During the same timeframe, the Amazonas region ranked third nationally regarding the incidence of human brucellosis, accounting for 8.33% of reported cases (SENASA, 2023). The primary transmission pathways to humans encompass the consumption of unpasteurized milk or dairy products, direct interaction with infected animals, and the inhalation of aerosols in areas experiencing significant contamination (Corbel, 2006). Consequently, these findings cannot be exclusively attributed to consuming raw milk or unpasteurized dairy products from bovines, given that goat milk is not prevalent in this region. Instead, they stem from direct exposure of agricultural producers to infected animals and insufficient management of placentas and aborted fetuses.

Although there are no documented positive cases of brucellosis in goats in this region, continuous surveillance remains paramount. Brucellosis, a zoonotic disease, can pose significant public health threats if it manifests within an animal population. Sustained monitoring facilitates the early identification of potential occurrences and aids in preventing disease transmission, even in locales where infections have not been previously identified (Corbel, 2006).

Educating producers and the public about brucellosis, including its symptoms and preventive measures, is paramount. This initiative ensures the maintenance of appropriate management and biosecurity practices in case the disease manifests in the areas where this research has been undertaken. Controlling brucellosis in goats necessitates vaccination, regulation of animal movements, and consistent health monitoring. Although no cases have been documented in goats, with occurrences solely reported in humans, adherence to robust practices in these domains is essential to preclude the disease's emergence and ensure optimal animal health. The lack of caprine brucellosis within the region guarantees the safety of goat-derived food products, particularly milk. This food holds significant importance for economic and nutritional stability in developing nations, playing a vital role in children's and adults' health and nutrition (Ribeiro and Ribeiro, 2010).

Research concerning the prevalence of *Brucella melitensis* in goats in Peru underscores the significance of employing diagnostic methodologies such as ELISA instead of relying exclusively on the Rose Bengal test to attain more precise outcomes. Despite the absence of reported positive cases of caprine brucellosis within the Amazonas region, ongoing surveillance remains imperative due to the zoonotic threat this disease poses to public health. The elevated incidence of human brucellosis throughout the nation, particularly in the Amazonas area, highlights the necessity of educating producers regarding appropriate management and biosecurity protocols and implementing control strategies such as vaccination and animal health monitoring. This will not only aid in averting the propagation of brucellosis but also safeguard animal and public health from potential future outbreaks.

Keywords: *Brucella melitensis*, creole goats, ELISA Test, Amazonas

## RESUMO

*Este estudo teve como objetivo avaliar a prevalência da* Brucella melitensis *em populações de cabras nativas de Utcubamba, situada na região amazônica do Peru. Foram coletadas 354 amostras de sangue de cabras de vários sexos e idades de 18 produtores dos distritos de Bagua Grande, El Milagro e Cumba. Cada produtor preencheu um questionário sobre a presença da doença. As amostras de sangue foram centrifugadas para extrair o soro, armazenadas a -20°C e posteriormente analisadas por meio de um teste ELISA indireto. Os resultados não indicaram nenhum caso positivo de* Brucella melitensis *em Utcubamba. Esse resultado pode ser atribuído, em grande parte, à ausência de movimentação de animais*

*reprodutores de outras regiões e à implementação de um programa de controle e erradicação pelo Serviço Nacional de Saúde Agrária (SENASA) desde 2000. Utcubamba é considerada livre de* Brucella melitensis *devido a dois fatores principais: o consumo limitado de leite de cabra na região e a ausência de animais reprodutores introduzidos de áreas potencialmente infectadas. Essas condições atenuam substancialmente o risco de transmissão e refletem um ambiente favorável à saúde das populações caprinas locais.*

*Palavras-chave:* Brucella melitensis, *cabras crioulas, teste ELISA, Amazonas*

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