

A Case Series on *Streptococcus agalactiae* Infection in Llamas and Alpacas in a Semi-Intensive Breeding System in Southern Brazil

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ABSTRACT

The South American Camelids (CSA), including llamas (*Lama glama*) and alpacas (*Vicugna pacos*), are primarily concentrated in the Andean countries of Argentina, Bolivia, Chile, and Peru, where they represent an important source of income for the local population. In Brazil, their captive breeding, although recent, has gained significant attention. These animals can act as potential carriers and transmitters of diseases, especially those with zoonotic potential. For many years, *Streptococcus agalactiae* was considered a strictly contagious bacterium among bovine herds. However, it has now been identified in various other animal species. This study aims to describe the clinical aspects of an outbreak of *S. agalactiae* in a herd of llamas and alpacas in southern Brazil. Two llamas and one alpaca presented chronic formation of encapsulated suppurative abscesses on the flank region. After conducting microbiological cultures, the pathogen was confirmed using MALDI-TOF MS. An antibiogram was performed, demonstrating antimicrobial sensitivity to most evaluated agents but indicating resistance to tetracycline, oxacillin, and orbifloxacin. The animals fully recovered after treatment with penicillin, with no recurrence of the infection. This study describes the first cases of *S. agalactiae* infection in llamas and alpacas in Brazil, highlighting the importance of further research on the health, breeding, and management of these animals.

1. Introduction

The Camelidae family is divided into two tribes: the Old World camelids, which include dromedary camels (*Camelus dromedarius*) and bactrian camels (*Camelus bactrianus*), and the tribe of South American camelids (SAC) or New World camelids, which are further divided into guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*), considered wild, and llamas (*Lama glama*) and alpacas (*Vicugna pacos*), considered domestic animals (Ledesma et al., 2017; Konieczny & Pomorska-Mól, 2023).

These animals have a wide distribution but are mainly concentrated in the Andes of Argentina, Bolivia, Chile, and Peru, where they represent an important source of income. In Brazil, the maintenance of SAC in captivity has gained considerable attention for various purposes, such as exhibitions, ornamental purposes, and the development of animal products (Rodrigues et al., 2016; Santos et al., 2023).

Besides their contact with humans, they are often raised in contact with other domestic species, allowing the transmission of diseases between the animals, including those with zoonotic potential (Sting et al., 2022; Konieczny & Pomorska-Mól, 2023). The *Streptococcus agalactiae* is a pathogen of great importance that can affect animals and humans. This bacteria is a gram-positive coccus, facultative anaerobe, and encapsulated, also classified as Group B *Streptococcus* - Lancefield, 1930. This microorganism is known as one of the main causing agents of mastitis in cattle, and its contamination in herds can result in severe productive and economic losses (Cobo-Angel et al., 2019).

For many years, it was considered an obligatory intramammary and strictly contagious agent among cattle herds, with the udder as the only reservoir in the body. However, its occurrence has now been reported in different body sites of various animal species, leading to different clinical manifestations (Barsi et al., 2022).

Cases of *S. agalactiae* infections have been widely documented in Old

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World camelids (Tibary et al., 2006; Younan & Bornstein, 2007; Fischer et al., 2013; Tigani-Asil et al., 2020; Seligsohn et al., 2021; Hussien, 2021; Crestani et al., 2022). In contrast, reports of this infection in SAC remain scarce. The first documented case in a llama was reported by Tavella et al. (2018), and, to date, no cases have been reported in Brazil. This study aims to document the occurrence and describe the clinical aspects of a case series of *S. agalactiae* infections in a herd of llamas and alpacas in southern Brazil.

2. Materials and methods

2.1. Clinical history

The infection outbreak occurred on a property located in the municipality of Francisco Beltrão (26°04'51"S, 53°03'17"W), in the south-west region of Paraná, in southern Brazil. The herd, consisting of 15 llamas and seven alpacas, was kept on pasture in the morning, while in the afternoon, they were brought to the stable, where they remained overnight. Sometimes, the animals shared paddocks with rheas, cattle, and deer. All camelids were vaccinated against anthrax and dewormed.

Two llamas, one male and one female, and one female alpaca developed subcutaneous abscesses at different points on the abdominal region. The abscesses showed slow and progressive enlargement, evolving into ulcerated suppurative lesions. The animals did not exhibit other clinical signs besides the lesions and were not isolated from the rest of the herd, remaining in contact with the other herd animals.

Furthermore, it was reported that other animals in the herd had previously exhibited similar lesions to those in these cases; however, the nodule spontaneously reduced following abscess drainage. In one of the llamas, this was the second occurrence of clinical manifestation of the disease. The remaining animals were sold, precluding determination of recurrence history.

2.2. Biological sample collection

Purulent discharge samples were collected by aspiration from the lesions of the alpaca and one of the llamas. The animals were physically restrained during the procedure, and a 20 mL syringe with a 25 × 8 mm needle was used. Samples were obtained from non-ulcerated areas overlying subcutaneous, encapsulated and non-adherent tissue. The needle was inserted into the lesion, and approximately 5 mL of subcutaneous content was aspirated from each animal. The samples were immediately transported to the laboratory for processing. Sedatives were not required, as the procedure was minimally invasive and well-tolerated by the animals. In the other llama, sample collection was not possible due to the spontaneous rupture of the abscess nodule one day before the scheduled procedure, which resulted in reduced nodule volume and size.

2.3. Pathogen identification

Initial screening of bacteria was performed by Gram stain of the samples. The same swabs were then plated on blood agar (Himedia®) with 5 % defibrinated sheep blood and incubated aerobically at 35 °C for 72 h. The bacterial isolates were subjected to initial genus identification by morphotintorial and biochemical characteristics as described by Quinn et al. (2005).

After phenotypic characterization, bacterial isolates were identified using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass-Spectrometry (MALDI-TOF MS). For this analysis, bacterial colonies were transferred onto a target plate, overlaid with a matrix solution, and subjected to laser desorption/ionization to generate protein mass spectra. The resulting spectra were compared to a reference database, and identification results were expressed as scores. Scores above 1.7 indicate reliable genus identification, scores above 2.0 indicate reliable genus and probable species identification, and scores above 2.3 suggest

highly probable species identification (Lartigue et al., 2009).

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk-diffusion method. Bacterial colonies were selected to prepare a 0.5 McFarland suspension for inoculation of Mueller-Hinton (MH) agar (Himedia®), and MH agar supplemented with 5 % blood for *Streptococcus* spp. testing, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). *Staphylococcus aureus* ATCC 25,923 strain was included as quality control. The isolates were tested against 11 antimicrobials: penicillin, ampicillin, ceftiofur, oxacillin, marbofloxacin, norfloxacin, orbifloxacin, erythromycin, clindamycin, chloramphenicol and tetracycline. The resulting zone diameter break-points were translated into clinical categories of susceptible, intermediate, and resistant following the VET-08 CLSI recommendations.

3. Results

In all three cases reported in this study, the only clinical manifestation observed was the formation of subcutaneous lesions in the flank region, characterized by encapsulated and suppurative abscesses (Fig. 1). In one of the llamas, spontaneous rupture of the abscess occurred one day prior to biological material collection, resulting in reduced volume and size of the nodule, thus making sample collection unfeasible. Despite this limitation, the lesions observed in the two llamas and the alpaca shared similar morphological characteristics, suggesting a common etiology.

After bacterial culture of the lesion samples, small translucent beta-hemolytic colonies were observed. Gram staining and biochemical tests revealed gram-positive cocci, catalase-negative, consistent with *Streptococcus* spp. (Fig. 2). The pathogen identification was further confirmed by MALDI-TOF MS, which provided scores of 2.245 and 2.076, supporting a high-probability identification of *S. agalactiae*.

Following the identification of *S. agalactiae*, antimicrobial susceptibility testing revealed that the isolates were sensitive to most antibiotics tested. However, intermediate resistance to orbifloxacin and full resistance to tetracycline and oxacillin were observed (Table 1).

4. Discussion

This is, to the best of our knowledge, the first report of infection by *Streptococcus agalactiae* in alpacas and llamas, in Brazil. Infections by *Streptococcus agalactiae*, although classically associated with subclinical mastitis, can also present with diverse clinical manifestations, often



Fig. 1. Clinical presentation of a llama with a suppurative abscess on the flank region (arrows).

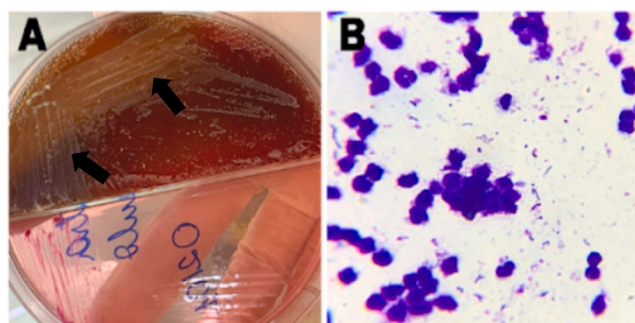


Fig. 2. (A) Bacterial cultures showing translucent beta-hemolytic colonies on blood agar (arrows). (B) Gram stain of isolated strains revealing gram-positive cocci.

Table 1

Sensitivity profile of *S. agalactiae* against 11 antimicrobial agents. All animals tested exhibited the same susceptibility pattern.

Antimicrobial agent	Susceptibility
Penicillin	Susceptible
Ampicillin	Susceptible
Ceftiofur	Susceptible
Oxacillin	Resistant
Marbofloxacin	Susceptible
Norfloxacin	Susceptible
Orbifloxacin	Intermediate
Erythromycin	Susceptible
Clindamycin	Susceptible
Chloramphenicol	Susceptible
Tetracycline	Resistant

involving superficial lesions such as subcutaneous abscesses and suppurative infections. This lesion pattern has been documented in camels, affecting areas such as the submandibular region and udder (Tavella et al., 2018; Zhou et al., 2023). In addition to these localized manifestations, systemic involvement has also been reported, including hyperthermia, confusion, depression, anorexia, enlarged superficial lymph nodes, sero-fibrinous ascites, thoracic effusion, interstitial pneumonia, pericardial swelling and sudden death. (Zhou et al., 2023). In this study, however, no systemic signs were observed, with clinical manifestations limited to the formation of the subcutaneous lesions.

Given the clinical presentation of suppurative abscesses, differential diagnoses must be considered to identify or rule out other potential causative agents. Differential diagnoses should initially encompass bacterial infections commonly associated with abscess formation in these species, such as *Corynebacterium pseudotuberculosis*, *Mycobacterium tuberculosis* complex, or even other *Streptococcus* spp. agents (Konieczny & Pomorska-Mól, 2023).

However, other non-bacterial causes must also be considered in the differential diagnosis. For instance, sterile abscesses or injection site reactions, often resulting from vaccine adjuvants or medications, may present similarly in the absence of systemic signs. Foreign body reactions and traumatic injuries with secondary inflammation can also mimic bacterial abscesses. In cases where abscesses are the primary clinical sign, these differential diagnoses must be evaluated carefully, as microbiological testing is required to confirm or exclude bacterial etiology. Before confirmatory tests, clinical observations such as lesion location, consistency, and presence of systemic signs may help prioritize likely causes (Misk et al., 2020).

Once the agent is identified, evaluating its antimicrobial susceptibility is essential for understanding resistance patterns. Antimicrobial resistance in *S. agalactiae* is becoming an increasingly recognized concern, particularly due to its potential to reduce treatment efficacy (Hayes et al., 2020). Penicillin is widely used as a first-line treatment for

these infections and is generally effective against this pathogen. However, there is an increasing number of reports indicating a reduction in the pathogen's susceptibility to penicillin, highlighting the importance of monitoring antimicrobial resistance in clinical cases (Hayes et al., 2020).

An important factor to highlight is the tetracycline resistance observed in the *S. agalactiae* isolates analyzed in this study. Tetracycline resistance may be directly related to some mutations in adapted human strains (Simões et al., 2021). This finding raises concerns about zoonotic transmission, a significant risk given the potential of *S. agalactiae* to infect humans. It was not possible in this study to determine the specific bacterial strain affecting these animals, but this possibility should still be considered.

The zoonotic potential of *S. agalactiae* is a significant issue. Several studies indicate the possibility of pathogen transmission and adaptation from animals to humans. This infection can lead to severe respiratory and urinary tract infections, bone and joint infections, endocarditis, meningitis, and skin infections. Moreover, this agent can also severely affect pregnant women, resulting in complications such as intrauterine fetal death and neonatal infections (Cobo-Angel et al., 2019).

The risk of *S. agalactiae* transmission to humans in close contact with infected animals is widely reported, especially due to the high incidence of this infection in cattle herds (Konieczny & Pomorska-Mól, 2023). The pathogens that affect cattle were once restricted to this species but have adapted to humans over time (Botelho et al., 2018). Therefore, similar processes may occur with *S. agalactiae* strains affecting camels. Additionally, studies indicate that the pathogen strains affecting camels are more similar to those affecting humans than those affecting cattle, suggesting that breeders and other people in close contact with camels may be more susceptible to *S. agalactiae* infections (Tigani-Asil et al., 2020).

After transmission, *S. agalactiae* can be considered an opportunistic pathogen, remaining asymptomatic in different tissues. It can, however, cause severe infections when the host's immune system is compromised (Zhou et al., 2023). Studies report that animals with gastrointestinal parasites are more susceptible to this infection (Tavella et al., 2018). In this study, the camels had no contact with other infected animals, so it is possible that they were already asymptomatic carriers of the bacterium and began to show clinical signs due to a drop in immunity.

This hypothesis is supported by the fact that the two infected females were pregnant, a condition that may have contributed to a decrease in their immunity. Pregnancy is associated with immunological shifts that prioritize fetal tolerance, potentially increasing susceptibility to infections in these camels. This immune modulation could favor bacterial proliferation and exacerbate the clinical presentation of the disease (Velázquez et al., 2019).

Another factor that must be considered is the climate. Llamas and alpacas are historically adapted to high-altitude environments, such as the high Andes, marked by a cold and dry climate (Brown, 2000). The southern region of Brazil, however, has a humid subtropical climate, characterized by hot summers with high rainfall concentrations (Marcolin & Calegari, 2020). These factors may contribute to animal stress, compromising their immunity and overall health (Carroll et al., 2012; Lambert et al., 2014; Baena et al., 2019).

A major issue encountered in these cases was the lack of isolation of the diseased animals. In cases where there was spontaneous drainage of the lesions, bacteria present in the secretion may have contaminated the environment where camels interacted with each other and other livestock. Another limitation of this study was the absence of molecular testing, which could have provided more precise identification and characterization of the pathogen, including potential virulence factors or genetic resistance mechanisms. Additionally, other potential risk factors, such as detailed nutritional deficiencies, stress levels, or prior exposure to other infectious agents, were not thoroughly investigated. By the end of this study, no new cases of animals showing clinical signs of the disease had been reported in the herd. Future studies should

address these limitations by incorporating molecular diagnostics, assessing broader risk factors, and implementing strict biosecurity measures to minimize environmental contamination and disease transmission.

5. Conclusions

For decades, *Streptococcus agalactiae* has posed a significant challenge to animal production health and public health. Despite this, its occurrence in llamas and alpacas has been rarely reported and investigated. Given the increasing number of these camelids in Brazil, it is essential to develop more studies on the manifestation of *S. agalactiae* in these animals to improve the understanding of the infectious agents that can affect these herds, thereby developing better breeding and management practices to help combat its occurrence and spread among animals and humans.

CRediT authorship contribution statement

Vitor Eduardo Mamgüe: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Letícia Trevisan Gressler:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Andriel Gustavo Felichak:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paulo Henrique Braz:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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