CASE REPORT

Corynebacterium pseudotuberculosis Associated Caseous Lymphadenitis in a Goat from India: First Confirmation Case Identified by MALDI-TOF Mass Spectrometry

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Caseous lymphadenitis (CLA) is a chronic, contagious disease that mainly affects small ruminants and is caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*). CLA constitutes a major threat to sheep and goat populations worldwide (Smith and Sherman, 2023). The significant economic losses caused by CLA are associated with poor reproductive performance, reduced wool and milk production, progressive emaciation, carcass condemnation at slaughterhouses and culling of affected animals (Ruiz *et al*., 2020; Bettini *et al*., 2022). The primary mode of spread of CLA is through direct contact of healthy animals with purulent discharges from draining abscesses of infected animals, or indirectly through exposure to a contaminated environment (Underwood *et al.,* 2015).

CLA is manifested clinically in two forms: external and internal (Costa *et al*., 2020). The external form is more frequently observed in goats, whereas the internal form is more commonly seen in sheep (Williamson, 2001). Although rare, *C. pseudotuberculosis* has been reported as a potential zoonosis, primarily affecting veterinarians, abattoir personnel, and livestock handlers causing lymphadenitis and abscess formation in affected individuals (Torres *et al*., 2013). The disease is characterized by the formation of abscesses of varying sizes in superficial lymph nodes, skin and internal organs (Fontaine and Baird, 2008). Parotid, submandibular, prescapular, prefemoral, popliteal, and supramammary lymph glands are the most commonly involved superficial lymph glands (Al-Gaabary *et al*., 2009). Clinical reports on CLA in goats remain limited in India (Muthukumar *et al*., 2020). Identification of the etiological agent has primarily been based on conventional bacteriological techniques. Molecular detection of *C. pseudotuberculosis* using PCR has been reported from goats in India (Mohan *et al*., 2008, Kumar *et al*., 2012; Sunder *et al*., 2024). However, there are no documented cases of CLA in goats from Gujarat and application of MALDI-TOF for confirmatory identification of *C. pseudotuberculosis* to the best of authors', hence documented in this report.

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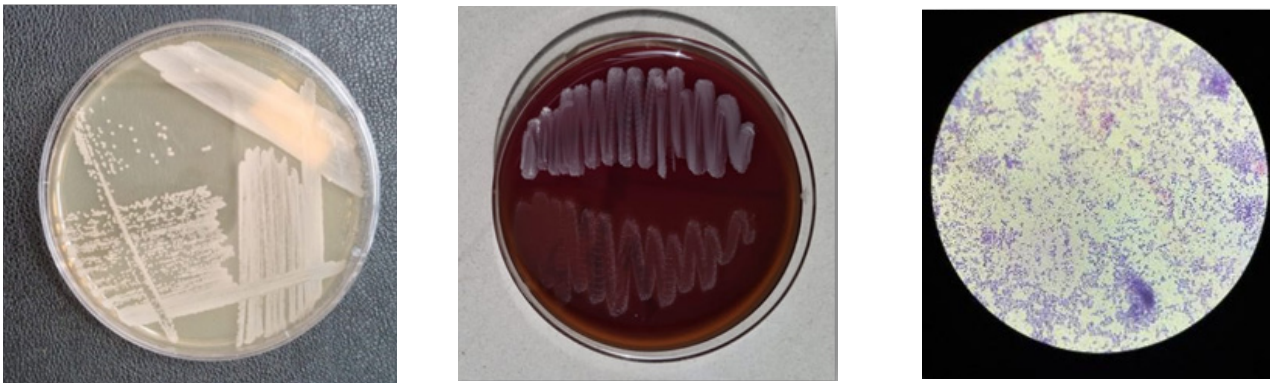
Case History and Observations

A 2-year-old male goat was presented to the Veterinary Clinical Complex, Sardarkrushinagar, Gujarat, India, with a two-week history of partial anorexia, progressive weight loss, and a swelling in the submandibular region. Clinical examination revealed a rectal temperature of 101.5°F, slight congested conjunctival membrane, emaciation, dullness, and an enlarged superficial submandibular lymph node (Fig. 1). Fine-needle aspiration of the enlarged lymph node produced thick, white, viscous pus with a cheese-like consistency (Fig. 2), without signs of pain on palpation. Based on history and clinical findings, CLA was suspected.

Approximately 1 mL of whole blood was aseptically collected via jugular venipuncture into EDTA vacutainer tube for haematology using an auto-analyzer (Exigo, Boule Medical AB, Sweden), which revealed decreased haematocrit (19.1%) and increased platelet count (660 × 10^3/µL), monocytes (7.8%), and neutrophils (68.6%). Pus was aseptically

Fig. 1: A 2-year-old male goat with submandibular lymph node swelling

Fig. 2: Fine-needle aspiration of submandibular lymph node abscess in the affected goat



A B C

Fig. 3: Colony and bacterial morphology of *C. pseudotuberculosis*: (A) Dry, crumbly, cream-colored colonies on BHI agar; (B) Similar colony morphology on blood agar; (C) Gram-positive coccobacilli (100X), small with rounded ends, arranged in bundles.

collected from the abscess of the superficial submandibular lymph node, clinically suspected for CLA, using a sterile disposable 18-gauge needle and syringe and transported to the microbiology laboratory for bacterial isolation and antimicrobial susceptibility testing, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Initial culture done in Brain Heart Infusion (BHI) broth (HiMedia, India) was subsequently streaked onto BHI and blood agar. After incubation, dry, crumbly, cream-colored colonies were observed on both media (Fig. 3A, B). Microscopic examination revealed Gram-positive coccobacilli arranged in bundles (Fig. 3C). The isolate was catalase-positive, oxidase-negative, coagulase-negative, and fermented D-mannitol, consistent with *Corynebacterium pseudotuberculosis*.

Confirmation of the etiological agent to the species level was performed using MALDI-TOF mass spectrometry (Bruker MALDI Biotyper, Bremen, Germany) following the manufacturer's protocol (Pranada *et al.,* 2016). Spectral analysis was conducted using Biotyper software (version 3.0.66). A log score value of ≥2.0 was considered reliable for species-level identification, while values between 1.7 and 1.99 indicated genus-level identification as per Bruker's interpretation criteria. In the present case, *Corynebacterium pseudotuberculosis* was identified with a score value of 2.16, indicating secure genus and probable species identification.

Antibiotic susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method. The isolate demonstrated resistance to all tested antibiotics, categorizing it as a multidrug-resistant (MDR) strain of *Corynebacterium pseudotuberculosis*.

Treatment and Discussion

The treatment involved abscess drainage, administration of Inj. Dicrysticin @ 10,000 IU/kg b.wt, IM, BID; Inj. Melonex @ 0.5 mg/kg, IM, OD; and Inj. Avil @ 0.5 mg/kg, IM, OD for seven days, along with supportive therapy. Although the isolate was subsequently identified as multidrug-resistant, empirical antimicrobial therapy was initiated to prevent systemic progression of the disease and to alleviate clinical signs. The response to treatment was poor, and the goat later presented with an elevated body temperature (106.0°F). Despite treatment, the animal showed poor clinical response and died 15 days after initial presentation, likely due to internal or systemic dissemination of the infection.

Submandibular lymph node involvement observed in the present case is consistent with reports indicating that *C. pseudotuberculosis* more commonly affects the superficial lymph nodes of the anterior half of the body in goats (Al-Gaabary *et al.,* 2009). The presence of an abscess in the peripheral lymph nodes of small ruminants is highly suggestive of CLA (Dorella *et al.,* 2006), however, confirmatory diagnosis is necessary.

The haematological alterations observed were indicative of the chronic inflammatory response characteristic of CLA. Reduced feed intake might be also one of the contributing factors altering the haemodynamics of the animal body. *Corynebacterium pseudotuberculosis* produces phospholipase D (PLD), a key virulence factor that increases vascular endothelial permeability and disrupts normal haematopoietic function, thereby contributing to the observed haematological deviations and facilitating systemic dissemination of the bacterium (Mahmood *et al*. 2015).

MALDI-TOF MS provides a rapid, accurate, and high-throughput method for bacterial identification. Similar applications of MALDI-TOF MS have been reported for species-level identification of *C. pseudotuberculosis* in camelids (Hiller *et al.,* 2024) and novel *Corynebacterium* spp. associated with fatal diphtheritic stomatitis in endangered yellow-eyed penguins (Saunderson *et al.,* 2021). Although PCR is highly sensitive, it lacks the speed and comprehensive species differentiation offered by MALDI-TOF mass spectrometry, while genome sequencing is time-consuming and requires highly specialized personnel.

Previous studies have reported varied antimicrobial sensitivity patterns of *C. pseudotuberculosis* (Robaj *et al*., 2017; Sunder *et al*., 2024). The resistance observed in the present case may be attributed to delayed clinical presentation and indiscriminate use of antibiotics at the field level, emphasizing the importance of early diagnosis. Reports also suggest that *C. pseudotuberculosis* isolates often exhibit resistance to several clinically important antimicrobial classes, including cephalosporins, aminoglycosides, and macrolides - agents classified by the World Health Organization as critically important for human medicine (Collignon *et al.,* 2016). The owner was advised to keep the animal isolated from the flock and to observe similar symptoms, if any, in other animals. Reports on the occurrence of CLA in goat populations in India are available from Haryana (Mittal *et al*., 2010), Punjab (Hussain *et al*., 2013), Rajasthan (Kumar *et al.,* 2012), Kerala (Mohan *et al.,* 2008), Tamil Nadu (Muthukumar *et al*., 2020), and Andaman and Nicobar Islands (Sunder *et al*. 2024).

To the best of the authors' knowledge, this is the first reported case of *C. pseudotuberculosis*-associated caseous lymphadenitis in a goat from India confirmed using MALDI-TOF mass spectrometry, exhibiting multidrug resistance. This finding underscores the importance of continuous surveillance to monitor antimicrobial resistance trends and the need for refined diagnostic strategies such as MALDI-TOF MS for early diagnosis and effective disease control in small ruminants.

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