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Atypical Mandibular Osteomyelitis in an Ewe Caused by Coinfection of *Pseudomonas aeruginosa* and *Lactococcus raffinolactis*

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ABSTRACT

***Background*:** Osteomyelitis is defined as a bone inflammation involving the cortical and medullary regions, usually caused by the local invasion of opportunistic microorganisms. The inflammatory reaction of bone may extend to the periosteum and soft tissues, compromising adjacent structures far from the initially infected foci. Different classifications of transmission routes, gravity levels, and tissues involved in animal and human osteomyelitis are available. In humans, the infection can reach bone tissue by exogenous or hematogenous pathways. This paper reports an atypical case of mandibular pyogranulomatous osteomyelitis in an ewe caused by concomitant *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* infection. ***Case*:** The animal presented a 1-month history of progressive mandibular enlargement refractory to conventional therapy. In a physical examination, an increased volume located in the ventrolateral region of the right ramus of the mandible was observed. Fine-needle aspiration of the lesion enabled isolation in bacteriological culture of *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Besides support care procedures and antimicrobial treatment approaches for the sheep based on in vitro tests, the animal died due to the severity of the clinical signs and the progressive worsening of the general health status. The radiographic image examination of the mandibular region revealed a severe and infiltrative periodontal reaction, with a predominance of a great number of neutrophils and macrophages, necrotic areas, and bone destruction, characterized histologically as a pyogranulomatous rection. At *post mortem* examination, a large pyogranuloma was observed in the entire horizontal branch of the mandible as well, showing a dark yellowish content of coarse consistency, caseous appearance, and bone fragmentation.

***Discussion*:** Ovine mandibular osteomyelitis is a well-established bone inflammation involving the cortical and medullary regions, characterized clinically by local enlargement, asymmetry, pain sensitivity, edema, hyperthermia, infiltrate caseous or suppurative material, and bone rarefaction. In the current report, 1-month history of progressive enlargement of the mandibular region, prostration, and weight loss in an ewe were referred. Where clinical and epidemiological features, bacteriological, cytological, histological, and mass spectrometry diagnostic approaches were assessed to diagnostic. Most reports involving the etiology of ovine mandibular osteomyelitis have been diagnosed based on classical phenotypic tests. Here, the concomitant identification of *P. aeruginosa* and L. raffinolactis infection was possible using mass spectrometry (MALDI-TOF), highlighting the importance of molecular methods in the diagnosis of animal diseases. In addition, the differentiation between *Lactococcus* and *Enterococcus* species is difficult, which could underestimate the diagnosis of *Lactococcus* species as a primary pathogen from animal diseases. We report, for the first time, a fatal case of mandibular pyogranulomatous osteomyelitis in a sheep caused by *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection.

Keywords: sheep, ovine osteomyelitis, *Lactococcus* sp., *Pseudomonas* sp., MALDI-TOF MS.

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INTRODUCTION

Ovine mandibular osteomyelitis is a well-established condition related mainly to an endogenous route, caused by microorganisms that inhabit the oral microbiota [9].

The most common pathways of mandibular osteomyelitis in sheep include periodontal infections and lesions in oral mucosa secondary to ingestion of rough pasture or other food [2]. In turn, exogenous osteomyelitis in sheep results from direct contamination after a traumatic condition caused by hard and coarse fodder, inappropriate use of jet injectors, and surgical and iatrogenic complications [1,8]. In cases of septicemia from primary mandibular osteomyelitis, the animals may develop secondary complications due to the dissemination of the pathogens, including arthritis, pneumonia, and meningitis.

Clinically, mandibular osteomyelitis in sheep is characterized by local enlargement, asymmetry, pain sensitivity, edema, hyperthermia, and abscesses formation [2,5,9]. A progressive weight loss, prostration, and low body condition score have been intimately associated with the disease due to pain and difficulty in chewing food, which results in high mortality rates of ovine mandibular osteomyelitis [4].

Routine diagnosis of mandibular osteomyelitis in sheep has been based on bacteriological culture, cytological, histopathological, and images procedures [1-3].

Here, we report for the first time an atypical and fatal case of mandibular pyogranulomatous osteomyelitis-related *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection in an ewe, refractory to therapy, where clinical and epidemiological aspects, bacteriological, cytological, histological, and mass spectrometry examination were assessed to diagnostic.

CASE

A 2-year-old crossbred ewe with a 1-month history of enlargement of the mandibular region, prostration, and progressive weight loss was referred to the Veterinary Medical Teaching Hospital of the State University of Maringá, Brazil. The animal grazed on native pastures, drinking water *ad libitum*, and its diet was supplemented with hay, silage, and mineral salt. No history of predisposing conditions or concomitant diseases was mentioned by the owner.

On physical examination, fever (40.2ºC), reduced ruminal movements, lymphadenomegaly (submandibular, parotid-auricular parotitis), and submandibular edema, which extended over the entire ventrolateral face of the right horizontal ramus of the mandible was observed. The lesion had a firm consistency on palpation, with areas of crepitus and high pain sensitivity (Figure 1). Upon opening the oral cavity, fistulas projecting from the horizontal ramus of the mandible were observed, containing caseous material and bone fragments. A right lateral ventrodorsal and oblique ventrodorsal radiographic views of the mandibular lesion were performed.

The lesion was subjected to fine-needle aspiration in duplicate to microbiological culture. A sample was subjected to direct Gram staining and microbiological culture [13]. Another sample was simultaneously cultured on sheep blood agar (5%), MacConkey agar, and Sabouraud media[1]. The plates were incubated at 37°C under aerobic conditions for 120 h. In addition, the same sample cultured in aerobic conditions described above was subjected to culture in sheep blood (5%) and brain-heart infusion media, incubated at 37°C under anaerobic conditions for 120 h. Microorganisms were initially identified based on conventional phenotypic tests [13], whereas the conclusive diagnosis at the species level was based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)[2] [6].

The isolates were subjected to *in vitro* susceptibility testing based on the disk diffusion[3] method [7], using nine antimicrobials belonging to 7 classes

Figure 1. Extensive edema in submandibular region (arrow) in an ewe with mandibular osteomyelitis-related *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection.

as follows: 1) aminoglycosides [gentamicin 10 µg], 2) amphenicols [florfenicol 30 µg], 3) cefalosporins [ceftiofur 30 µg], 4) derivatives of penicillin [amoxicillin/clavulanic acid 30 µg, ampicillin 30 µg], 5) fluoroquinolones [enrofloxacin 5 µg, marbofloxacin, 5 µg], 6) tetracyclines [oxytetracycline 30 µg], and 7) sulfonamides [sulfamethoxazole/trimethoprim 25 µg].

Given the severity of the clinical signs and difficulty in chewing food, the animal worsened the corporal condition and died. Samples of the mandibular lesion and bone fragments were subjected to microbiological culture (as described above) and histological examination. Fragments of the lesion and adjacent tissues were collected, fixed in 10% formalin, and histologically stained with hematoxylin and eosin.

Fine-needle aspiration subjected to Gram staining revealed the identification of gram-positive cocci and gram-negative bacilli.

Bacteriological and mycological cultures of the mandibular lesion and bone fragments revealed isolation of gram-positive bacilli and *Pseudomonas* sp. in aerobic conditions. These isolates were subjected to MALDI-TOF MS and classified at species level as *Pseudomonas aeruginosa* and *Lactococcus raffinolactis*, respectively. No fungi or isolation of organisms in anaerobic conditions were obtained.

The *in vitro* antimicrobial pattern of *P. aeruginosa* and *L. raffinolactis* revealed susceptibility of isolates only to the fluoroquinolones group, i.e., enrofloxacin and marbofloxacin, which subsidized the treatment of the animal with enrofloxacin[4] [Kinetomax® - 5 mg/kg, im, SID, for 5 days].

The radiographic images revealed a severe, amorphous, mixed, predominantly lytic, osteoperiosteal reaction of the body of the right mandible from the angular process of the mandible to the lower incisor region. Destruction of the cortical bone, medullary canal, dental alveoli, and lamina dura of all right mandibular premolars (Figure 2) was seen, as well as marked soft-tissue edema throughout the lateral and ventral portion of the mandible, suggestive of chronic pyogranulomatous osteomyelitis.

Post mortem examination showed an extensive abscess area in the horizontal ramus of the mandible. On the section surface, this area contained a dark yellow secretion with a caseous appearance, which filled the spaces of the right mandible premolars and alveolar region. In addition, lytic bone tissue throughout the mandibular extension was seen, compatible with the radiographic aspect (Figure 3).

Figure 2. Radiographic images of mandibula in an ewe with osteomyelitis caused by *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection: A- Edema and severe lytic osteoperiosteal reaction of the angular process of right mandibula (laterolateral face). B- Destruction of cortical bone, medullar canal, dental alveoli, and lamina dura of all mandibular premolars (ventrolateral face).

Microscopically, the fragments of the lesion and adjacent areas revealed a necrotic lesion surrounded by a mixed infiltrative population of inflammatory cells, particularly neutrophils, macrophages, epithelioid cells, and lymphocytes, as well as rosettes formed by bacterial colonies in the center and Splendore-Hoeppli

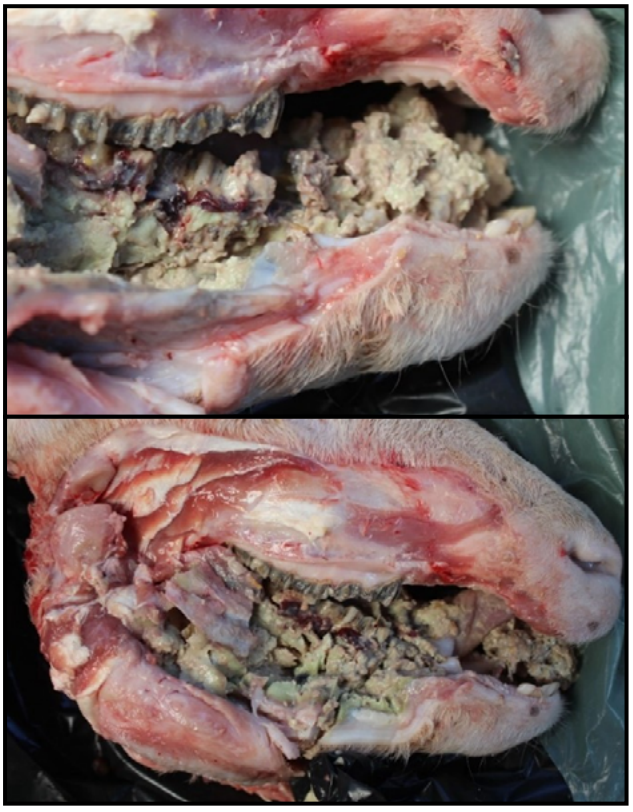

Figure 3. Extensive pyogranulomatous reaction in an ewe with mandibular osteomyelitis-related *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection.

bodies in the periphery, surrounded by fibrous tissue. Also, exuberant fibrosis around foci of the lesion and irregular bone trabeculae were noted. Based on the histopathological features, the mandibular lesion was characterized as pyogranulomatous osteomyelitis.

DISCUSSION

Ovine mandibular osteomyelitis is a well-established bone inflammation involving the cortical and medullary regions, characterized clinically by local enlargement, asymmetry, pain sensitivity, edema, hyperthermia, infiltrate caseous or suppurative material, and bone rarefaction [8]. In the current report, one-month history of progressive enlargement of the mandibular region, prostration, and weight loss in an ewe were referred. At clinical examination, regional lymphadenomegaly, and submandibular edema in the ventrolateral face of the right horizontal ramus of the mandible with a firm consistency on palpation were seen, as well as areas of crepitus, and pain sensitivity, containing caseous material and bone fragments. These clinical features have been observed in similar reports of ovine mandibular osteomyelitis elsewhere [5,14] and Brazil [1].

The mandibular osteomyelitis in sheep is intimately related to the local invasion of microorganisms that inhabit the oral microbiota [8]. *Trueperella pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Fusobacterium necrophorum*, *Actinomyces bovis*, staphylococci, and streptococci are the most common agents related to ovine mandibular osteomyelitis [1,4,9,15,16]. In the present report, *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection is described as causing mandibular osteomyelitis in an ewe, detected based on MALDI-TOF MS diagnosis.

Pseudomonas species are well-known gram-negative bacilli. These bacteria are opportunistic in nature, related to infections in both humans and animals, particularly in hospital-acquired infections and among immunosuppressed patients. The pathogen has been described in a set of clinical manifestations, *e.g.*, osteomyelitis, otitis, cystitis, endometritis, encephalitis, lymphadenitis, mastitis, dermatitis, abscesses, pneumonia, enteritis, and septicemia [13,16,17]. The organism is widely found in the environment, mainly in the water and soil, and occasionally in plants, whereas in domestic animals they can be isolated from mucous membranes and feces [13]. Likewise, the present report, *Pseudomonas* species, particularly *P. aeruginosa*, have been reported as a primary agent of mandibular osteomyelitis in sheep [1,5], which may be credited to oral infection secondary to contamination of water or feeds, due to opportunistic lifestyle of the pathogen and ubiquitous presence in the farm environment [13].

Lactococcus species are gram-positive facultative anaerobic cocci bacteria. The genus comprises 5 well-known species: *L. lactis*, *L. garvieae*, *L. piscium*, *L. plantarum*, and *L. raffinolactis*. They have been found in mucocutaneous surfaces of humans and animals, including the intestine and bovine mammary gland, and also in plants, particularly grasses. The organism has been commonly used in the dairy and biotechnology industry for manufacturing cheese and other fermented foods [10,18].

Human infections by *Lactococcus* species have been reported in the last 2 decades, mainly among immunocompromised hosts [11], and the microorganism has been currently proposed as an emerging zoonotic pathogen [18]. Endocarditis, osteomyelitis, liver abscess, septic arthritis, septicemia, cerebellar abscess, deep neck infection, necrotizing pneumonia, cholangitis, and subdural empyema have been reported in

human patients [10]. Conversely, descriptions of *Lactobacillus* infections and the pathogenicity of the agent to domestic animals remain unclear [12]. *L. garvieae* have seen reported as an occasional primary agent of different clinical manifestations in cattle, sheep, goats, buffalo, and pigs, and rarely in companion animals, horses, camels, turtles, snakes, and crocodiles [18]. To our knowledge, the atypical concomitant *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* concomitant infection described herein, causing mandibular osteomyelitis in an ewe is reported for the first time. Similar to *P. aeruginosa*, the oral infection of an ewe by *L. raffinolactis* may be attributed to ingestion of contaminated feed or water, since *Lactococcus* species have been found in plants, particularly grasses [10,18].

Most reports involving the etiology of ovine mandibular osteomyelitis have been diagnosed based on classical phenotypic tests [1,2,5,14]. Here, the concomitant identification of *P. aeruginosa* and *L. raffinolactis* infection was possible using mass spectrometry (MALDI-TOF), highlighting the importance of molecular methods in the diagnosis of animal diseases [18]. In addition, the differentiation between *Lactococcus* and *Enterococcus* species is difficult, which could underestimate the diagnosis of *Lactococcus* species as a primary pathogen from animal diseases [18].

Previous reports of ovine mandibular osteomyelitis have observed an infiltrative periodontal reaction, with a great number of neutrophils, macrophages, lymphocytes, and epithelioid cells, necrotic areas, and bone destruction, surrounded by fibrous tissue that characterize a typical pyogranulomatous rection, which agree with the cyto- and histological findings of the present report.

Cytological, bacteriological, histopathological, and images were assessed for the diagnosis of mandibular osteomyelitis in the present report, reinforcing the importance of a combination of methods to confirm a diagnosis, establish prognosis, and adopt therapy and control approaches [1,2,5,9].

Besides support care and antimicrobial therapy based on *in vitro* susceptibility test, the animal worsened the corporal conditions and died, which is consistent with similar reports of mandibular osteomyelitis related-*Pseudomonas aeruginosa* [14] probably due to high pathogenicity of the agent, a set of virulence mechanisms, and well-known multidrug resistance pattern of the pathogen [13].

No specific prophylactic measures are recommended to control mandibular osteomyelitis in sheep, except offering a feed of good quality to avoid oral lesions that could predispose the infections by opportunistic agents from oral microbiota or same microorganisms that may contaminate water or food [1].

Overall, we report for the first time, an atypical and fatal case of mandibular pyogranulomatous osteomyelitis-related *Pseudomonas aeruginosa* and *Lactococcus raffinolactis* coinfection in an ewe, refractory to therapy, where clinical and epidemiological features, bacteriological, cytological, histological, and mass spectrometry diagnostic approaches were combined to diagnostic purpose.

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