CASE REPORT

# Catarrhal mastitis by *Staphylococcus simulans* in a nulliparous goat

## *Mastite catarral causada por* Staphylococcus simulans *em uma cabra nulipara*

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**Abstract**

The present paper is a case report of a one-year old nulliparous Alpine Goat belonging to a dairy goat farm in semi-arid region of Brazil. Both glands were naturally infected by α-hemolytic *Staphylococcus simulans* and evolved similar clinical signs. The mammary glands presented an acute catarrhal mastitis with systemic clinical signs that responded positively to treatment with gentamicin associated with amoxicillin. The present report suggests the importance of the pathogenic potential of *non-aureus Staphylococci* strains (NAS) as a cause of clinical mastitis also in nulliparous animals. The isolate showed resistance to tetracycline and contained staphylococcal toxin production genes (*sec, sec and TSST-1*). Moreover, it has been reported that *Staphylococcus simulans* is an emerging pathogen in humans causing cutaneous and osteoarticular infections, mainly in those in close contact with farm animals. To the best of our knowledge, this is the first report of a clinical mastitis in a nulliparous goat caused by *Staphylococcus simulans*.

**Keywords:** Intramammary infection. Coagulase-negative staphylococci. Small ruminants.

**Resumo**

O presente trabalho é o relato do caso de uma cabra nulípara da raça Parda Alpina, de um ano de idade, pertencente ao Setor de Caprinocultura da Universidade Federal da Paraíba – Bananeiras - Brasil. Ambas as glândulas foram naturalmente infectadas por *Staphylococcus simulans* α-hemolítico. As glândulas mamárias apresentaram mastite aguda catarral com envolvimento sistêmico, respondendo positivamente ao tratamento sistêmico com gentamicina associada a amoxicilina. O presente relato sugere a importância do potencial patogênico de *Staphylococcus não-aureus* (SNA) como causador de mastite clínica também em animais nulíparos. O isolado mostrou resistência a tetraciclina e continha genes de produção de toxinas estafilocócicas (*sec*, *seg* e *TSST-1*). Além disso, tem sido relatado que *Staphylococcus simulans* é um patógeno emergente em seres humanos causando infecções cutâneas e osteoarticulares, principalmente naqueles que têm contato íntimo com animais de fazenda. Até onde sabemos, este é o primeiro relato de uma mastite clínica em uma cabra nulípara causada por *Staphylococcus simulans*.

**Palavras-chave:** Infecção intramamária. Estafilococos coagulase-negativos. Pequenos ruminantes.

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Mastitis is the most important and costly disease in dairy goat production. Animals can present physical, chemical, pathological and bacteriological changes in milk and glandular tissue. Even though *Staphylococcus aureus* is the most common agent involved in clinical mastitis cases (BERGONIER et al., 2003), *non-aureus*


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*Staphylococci* (NAS) strains play a significant role in goat mastitis. For instance, unlike in cows, NAS, especially novobiocin-sensitive NAS, such as *Staphylococcus (S.) simulans*, leads to a great increase in somatic cell count in small ruminants, and then can be regarded as major pathogens. The importance of staphylococci in dairy goat herds is not limited to animal production, but is also a relevant issue for its implications to public health and well-being (BERGONIER et al., 2003).

The present paper is a case report of a one-year old nulliparous Alpine Goat raised in intensive grazing management, housed in elevated goat shed with food (chopped elephant grass + concentrate + minerals) and fresh water ad libitum. The animal showed episcleral injection, light fever (40°C) and signs of udder inflammation (local pain and swelling). Inflammation of both halves of mammary gland evolved similarly. They presented increased temperature, edema and clumps in milk, symptoms characteristic of catarrhal mastitis (DELLA LIBERA et al., 2007). The goat was totally dried off and treated according to the following protocol: gentamicin 4 mg/kg + amoxicillin 15 mg/kg (IM-12/12 h for three days) and flunixin meglumine 2.2 mg/kg (IV- single dose). After treatment, the animal clinically recovered from infection. Microbiological culture of both mammary gland secretions was performed before treatment. The samples were streaked directly onto blood agar plates (BD, Heidelberg, Germany) and incubated at 37°C for 24 h. Pure homogeneous colonies, circular, pinhead, convex, and showing light gray color, were cultured showing α-hemolytic pattern. Those colonies were submitted to VITEK® 2 Compact (bioMérieux, Marcy-l'Étoile, France) for speciation and antimicrobial susceptibility test by the determination of the minimal inhibitory concentration (LIGOZZI et al., 2002). Bacteria was considered resistant or susceptible to antimicrobials according to CLSI (2013). *S. simulans* was identified and the results of antimicrobial susceptibility test are shown in Table 1. We also performed genotyping for important virulence genes (Table 2) (MEHROTRA et al., 2000). Briefly, the DNA was extracted from pure culture isolates, using a commercial kit (Wizard® Genomic DNA Purification Kit, Madison, Wisconsin, USA) according to manufacturer's instructions and stored at -20°C. The primers used for amplification of the virulence factors genes fragments are shown in Table 2. The PCR products were visualized by electrophoresis in 2% agarose gel stained with Blue Green Loading Dye I (LGC Biotecnologia, São Paulo, Brazil) and photographed under UV illuminator (Molecular Imaging L.PIX Loccus Biotecnologia, São Paulo, Brazil).

Table 1 – Antibiotic resistance pattern of *Staphylococcus simulans* isolated from a catarrhal mastitis case in a nulliparous goat – Bananeiras, Paraíba State, Brazil – 2018

| Antibiotics | MIC | Interpretation | Antibiotics | MIC | Interpretation |
|---|---|---|---|---|---|
| Fusidic Acid | > 32 | S | Kanamycin | < 4 | S |
| Ampicillin/sulbactam | 16 | S | Marbofloxacin | < 0,5 | S |
| Benzylpenicillin | > 0,5 | S | Nitrofurantoin | 256 | S |
| Clindamycin | > 8 | S | Oxacillin | > 4 | S |
| Chloramphenicol | 8 | S | Rifampicin | > 32 | S |
| Enrofloxacin | < 0,5 | S | Tetracycline | > 16 | R |
| Erythromycin | > 8 | S | Trimethoprim/Sulfamethoxazole | < 10 | S |

| | | | | | |
|---|---|---|---|---|---|
| Gentamicin | < 0,5 | S | Vancomycin | > 32 | S |
| Imipenem | 2 | S | | | |

S = sensitive; R = resistant. MIC: minimum inhibitory concentration

Table 2 – Oligonucleotides for the different virulence genes used in *Staphylococcus simulans* isolated from a catarrhal mastitis case in a nulliparous goat – Bananeiras, Paraíba State, Brazil – 2018

| Target gene | Name | Oligonucleotide sequence (5´- 3´) | Expected size | At (°C)[1] |
|---|---|---|---|---|
| *fnbA* | fnbA_R | ACTTCACCTGTCGCCATTAC | 539 pb | 61 |
| | fnbA_F | GCAGTACAAGCACCACAAAC | | |
| *fnbB* | fnbB_F | AGGCGACGGCAAAGATAAA | 317 pb | 57 |
| | fnbB_R | TAGTAACCTGACCACCACCT | | |
| *clfA*[2] | clfA_F | GATTCTGACCCAGGTTCAGA | 945 pb | 60 |
| | clfA_R | CTGTATCTGGTAATGGTTCTTT | | |
| *clfB*[2] | clfB_F | ATGGTGATTCAGCAGTAAATCC | 880 pb | 55 |
| | clfB_R | CATTATTTGGTGGTGTAACTCTT | | |
| *sea* | sea_F | CCGAAGGTTCTGTAGAAGTATG | 269 pb | 55 |
| | sea_R | GCTTGTATGTATGGTGGTGTA | | |
| *seb* | seb_F | CCCGTTTCATAAGGCGAGTT | 314 pb | 55 |
| | seb_R | ACGTAGATGTGTTTGGAGCTAAT | | |
| *sec*[3] | sec_F | AGATGAAGTAGTTGATGTGTATGG | 451 pb | 57 |
| | sec_R | CACACTTTTAGAATCAACCG | | |
| *sed* | sed_F | GTCACTCCACACGAAGGTAATAA | 255 pb | 57 |
| | sed_R | GAGACTTTAGACCCATCAGAAGAA | | |
| *see*[3] | see_F | GCTGGAGGCACACCAAATA | 301 pb | 55 |
| | see_R | CATAACTTACCGTGGACCCTTC | | |
| *seg* | seg_F | GCCAGTGTCTTGCTTTGTAATC | 491 pb | 57 |
| | seg_R | GAATGCTCAACCCGATCCTAA | | |
| *seh* | seh_F | CACATCATATGCGAAAGCAGAAG | 365 pb | 56 |
| | seh_R | CCCAAACATTAGCACCAATCAC | | |
| *sei* | sei_F | AGGCAGTCCATCTCCTGTATAA | 568 pb | 60 |
| | sei_R | TGCTCAAGGTGATATTGGTGTAG | | |
| *tsst-1*[3] | tsst_F | ACCCCTGTTCCCTTATCATC | 326 pb | 55 |
| | tsst_R | TTTTCAGTATTTGTAACGCC | | |

[1] Annealing temperature °C. [2] PCR primers sequence were designed by Sabat et al. (2003). [3] PCR primers sequence were designed by Mehrotra et al. (2000). cflA: clumping factor A (cflA); cflB: clumping factor B; fnbA: fibronectin-binding protein A; fnbB: fibronectin-binding protein B; TSST-1: toxic shock syndrome toxin-1; sea: staphylococcal enterotoxin A; seb: staphylococcal enterotoxin B; sec: staphylococcal enterotoxin C; sed: staphylococcal enterotoxin D; see: staphylococcal enterotoxin E; seg: staphylococcal enterotoxin G; seh: staphylococcal enterotoxin H; and sei: staphylococcal enterotoxin I

To the best of our knowledge, this is the first report of *S. simulans* strain causing clinical mastitis in goats. This bacteria isolate was positive for two staphylococcal enterotoxigenic genes (*sec* and *seg*) and also for toxic shock syndrome toxin-1 (*TSST-1*), but did not show the virulence genes for clumping factor A (*cflA*), clumping factor B (*cflB*), fibronectin-binding protein A (*fnbA*) and fibronectin-binding protein B (*fnbB*). There is little information of the potential risk of NAS isolated from goats for human health. One of the major concerns about staphylococcal infections is the potential to produce staphylococcal enterotoxins (SEs), such as *TSST-1*. *TSST-1* is one of the most prevalent genes in severe cases of bovine staphylococcal mastitis, and can cause

disease in humans, although investigations of staphylococcal enterotoxins (SEs) in goats are rare (FREITAS et al., 2008; PEIXOTO et al., 2010).

Despite NAS being one of the most isolated pathogens in cases of subclinical mastitis in goats and cows with high persistence rates compared to other species (RUEGG, 2009), *S. simulans* association with a clinical case in goats had not been previously reported. Since clinical mastitis must be carefully handled by veterinarians and dairy farms, the potential for transmission of *S. simulans* to humans should not discarded. Thus, from a public health point of view, recent cases of septic osteoarthritis (MALLET et al., 2011) and cutaneous infections (ROMERO et al., 2016) in humans have been reported mainly in people who had intimate contact with production animals, suggesting that the zoonotic potential of this pathogen cannot be neglected.

Finally, although mastitis is a great problem for goat dairy production, information about mastitis in nulliparous goats (JÁCOME et al., 2014) is scarce. Furthermore, beyond animal and public health implications that should not be neglected, our results strengthen the idea that the control measures for this disease in goats cannot wait until the beginning of lactation, since infection can occur before milk production and milking, as it has been discussed and highlighted in the last decade for dairy heifers (DE VLIEGHER et al., 2012).

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