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Gangrenous mastitis in sheep caused by multidrug-resistant *Staphylococcus haemolyticus*[1]

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ABSTRACT.- Moura G.S., Mota R.A., Marques M.F.S., Abad A.C.A., Costa L.B.B.C., Souza F.N., Almeida V.M., Silva Filho G.B., Bom H.A.S.C., Klaumann F., Souza F.A.L. & Mendonça F.S. 2020. **Gangrenous mastitis in sheep caused by multidrug-resistant *Staphylococcus haemolyticus*.** *Pesquisa Veterinária Brasileira 40(12):947-954.* Laboratório de Diagnóstico Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: fabio.mendonca@ufrpe.br

Mastitis is a multifactorial disease and considered one of the most critical problems in the dairy industry worldwide. The condition is characterized by reduced milk and several abnormalities in the mammary gland. This study aimed to report an outbreak of gangrenous mastitis caused by multidrug-resistant *Staphylococcus haemolyticus* in a Santa Inês sheep herd. Eighteen sheep were affected, and five of them with severe clinical pictures were examined. The clinical and pathological picture were variable and characterized by apathy, anorexia, emaciation, opaque and brittle hair, apparent and congested episcleral vessels, and hyperthermia. These ewes had enlarged, firm, and painful mammary glands. Macroscopically, these lesions consisted of severe gangrenous mastitis, and microscopically, the primary lesions consisted of necrosis, thrombosis, and fibrosis of the mammary parenchyma. Milk samples from one of the five severely affected ewes were collected and cultured under aerobic or microaerophilic incubation at 37°C for 24 hours on sheep blood agar. The obtained colonies were then submitted to MALDI-TOF for speciation. The colonies were also submitted to an antimicrobial susceptibility test, genotyping of virulence factors and resistance genes were also performed. The isolates showed antimicrobial multiresistance since they were resistant to seven out of 13 tested antibiotics. The isolates were also positive for two staphylococcal enterotoxigenic genes (*sec* and *see*) and fibronectin-binding protein B (*fnbB*).

INDEX TERMS: Gangrene, mastitis, sheep, multidrug-resistant, *Staphylococcus haemolyticus*, non-*aureus* staphylococci, small ruminant, ovine.

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RESUMO.- [**Mastite gangrenosa em ovinos causada por *Staphylococcus haemolyticus* multirresistente.**] A mastite é uma doença multifatorial e é considerada um dos problemas mais importantes na indústria de laticínios no mundo todo. A condição é caracterizada pela redução de leite e várias anormalidades na glândula mamária. O objetivo deste estudo foi relatar um surto de mastite gangrenosa causada por *Staphylococcus haemolyticus* multirresistente em um rebanho ovino Santa Inês. Dezoito ovelhas foram afetadas e cinco delas com quadro clínico severo foram examinadas. O quadro clínico-patológico era variável quanto a severidade e consistia em apatia, anorexia, magreza, pelos opacos e quebradiços e vasos episclerais aparentes e ingurgitados. As ovelhas apresentavam glândulas aumentadas, firmes e

dolorosas. Macroscopicamente, as principais lesões consistiam em mastite gangrenosa e microscopicamente havia necrose do parênquima glandular, trombose e fibrose. Amostras de leite de uma das cinco ovelhas severamente afetadas foram coletadas e cultivadas sob incubação aeróbica ou microaerofílica a 37°C por 24 horas em ágar sangue de ovelha. As colônias obtidas foram então submetidas ao MALDI-TOF para especiação. Além disso, as colônias foram submetidas a um teste de suscetibilidade antimicrobiana e foi realizada a genotipagem de fatores de virulência e genes de resistência. Os isolados apresentaram multirresistência antimicrobiana por serem resistentes a sete dos 13 antibióticos testados. Os isolados também foram positivos para dois genes enterotoxigênicos estafilocócicos (*sec* e *see*) e proteína B de ligação à fibronectina (*fnbB*).

TERMOS DE INDEXAÇÃO: Mastite, gangrena, ovinos, *Staphylococcus haemolyticus*, multirresistente, estafilococos não-aureus, pequenos ruminantes.

INTRODUCTION

Mastitis is an inflammation of the mammary gland tissue frequently found in ruminants during lactation. In addition to in cattle, mastitis in small ruminants is one of the most critical problems causing significant economic losses in sheep flocks and the dairy industry (Giadinis et al. 2012, Gelasakis et al. 2015). The disease, characterized by reduced milk, pain, anxiety, restlessness, and feeding behavior changes, is developed based on three processes: invasion of an organism, infection, and inflammation (Murphy 1947). Several pathogens have been associated with clinical or subclinical mastitis in ewes (Contreras et al. 2007), in most cases, different bacteria species. For dairy ruminants, *Streptococcus agalactiae* and *Staphylococcus aureus* were considered the primary causes of the disease during previous decades (Ruegg 2017). Currently, pathogens such as *Mannheimia haemolytica* are also included as mastitis causal agents (Omaleki et al. 2010).

In subclinical disease, non-*aureus* staphylococci (NAS) are the most prevalent pathogens found in udders of ewes affected by mastitis (Bergonier et al. 2003, Mørk et al. 2005, Contreras et al. 2007, Gelasakis et al. 2015). Although less pathogenic than *S. aureus*, NAS can also produce persistent subclinical mastitis progressing to clinical disease (Deinhofer & Pernthaner 1995, Contreras et al. 1997, Ariznabarreta et al. 2002). Among NAS, *Staphylococcus epidermidis* is a common species associated with ovine mastitis (Onni et al. 2011), followed by *Staphylococcus chromogenes*, *Staphylococcus simulans*, and *Staphylococcus xylosus*. Other infrequently isolated species, including *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus cohnii*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lentus*, *Staphylococcus muscae*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, and *Staphylococcus warneri*, were reported in other studies (Fthenakis 1994, Bergonier et al. 2003).

There are no NAS reports causing gangrenous mastitis in sheep to the best of our knowledge. Several studies using molecular tools have suggested that NAS are acquiring virulence factors that might be able to facilitate their transformation into potential clinical mastitis agents (Cunha et al. 2006, Pyörälä & Taponen 2009, Dando et al. 2014). Therefore, this study aimed to report an outbreak of gangrenous mastitis caused by multidrug-resistant *S. haemolyticus* in a sheep flock.

MATERIALS AND METHODS

Sheep farm and animals. This study was performed on a herd of 60 Santa Inês adult sheep located in Limoeiro, Pernambuco, northeastern Brazil (07°52'29" S 35°27'0" W). Epidemiological and clinical data were obtained from the owner and veterinarian during technical visits. A total of eighteen ewes were affected by clinical mastitis at different levels of intensity. Five of them, which were severely affected, were selected and examined in detail according to the clinical condition. Additionally, parameters such as behavior, appetite, mucous membrane color, rectal temperature, heart and respiratory rates, abdominal morphology, reticulum-rumen motility, and the physical appearance of their feces, urine, and skin were also observed. The physical examination identified a disease based on the mammary gland by palpation and visible abnormalities, including the milk's coloration and consistency concerning clinical mastitis.

As the clinical signs evolved, three sheep worsened and died spontaneously, so the owner allowed the necropsy to be performed.

Milk sampling, bacterial speciation and antibiotic resistance patterns. Two milk samples from one ewe were aseptically collected after scrubbing the teat ends with 2% iodine solution. Ten microliters of samples were cultured on 5% sheep blood agar (Oxoid) and incubated for 24 hours at 37°C. We considered an intramammary infection (IMI) to be present if more than 100 colony forming units / mL were detected in the bacteriological culture. Then, colonies were submitted to matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) for speciation. Bacterial samples were prepared as previously described (Cameron et al. 2017). Measurements were performed on a Microflex LT mass spectrometer (Bruker Daltonics) with a standard pattern-matching algorithm (BioTyper 2.0 Software).

The isolate was submitted to an antimicrobial susceptibility test through a VITEK® automated system using the methodology previously described (Spoor et al. 2013). All the minimum inhibitory concentrations (MIC) were interpreted in accordance with performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals of the Clinical and Laboratory Standards Institute (CLSI 2018).

DNA extraction and conventional PCR to detect virulence genes. Sixteen virulence genes (*fnbA, fnbB, clfA, clfB, sea, seb, sec, sed, see, seg, seh, sei, tsst, blaZ, mecA, mecC*) were analyzed (Table 1). Briefly, the DNA was extracted from 300µL of milk using a Wizard® Genomic DNA Purification kit (Promega, Madison/WI) according to the manufacturer's instructions. To detect virulence factor gene fragments in the tested samples, a conventional PCR assay was performed based on the protocol previously described (Mehrotra et al. 2000). All primers (Mehrotra et al. 2000, Sabat et al. 2003) used for amplification are displayed in Table 1. The PCR products were run on a 2% TAE agarose gel stained with Blue Green Loading Dye I (LGC Biotecnologia, São Paulo, Brazil) and photographed under a UV illuminator (L.PIX Molecular Imaging- Loccus biotecnologia, São Paulo, Brazil).

Gross and microscopy lesions. At necropsy, tissue sections from the central nervous system, thorax, abdominal organs and mammary gland were obtained and fixed in 10% buffered formalin. For histopathological observations, sections of the fixed tissues were processed routinely, embedded in paraffin, sectioned (4µm), stained with hematoxylin and eosin (HE) and examined under a light microscope.

RESULTS

Clinical, gross and histopathological findings

Eighteen out of 60 (30%) ewes showed clinical signs characterized by apathy, anorexia, emaciation (Fig.1), opaque and brittle hair, apparent and congested episcleral vessels, and hyperthermia. The owner reported that mammary glands were enlarged and that a purulent discharge was observed when ewes were milked. The ewes were treated for two weeks with oxytetracycline (10mg/kg/12h, IM) and amoxicillin with clavulanic acid (10mg/kg/12h, IM) without an efficient response. Thirteen out of 18 sheep (72.2%) were moderately affected, and five out of 18 sheep (27.8%) were severely affected by the clinical signs described above. Five sheep somewhat affected presented unilateral mastitis, and in these ewes, the uninfected sides were also swollen and tense, with reduced secretion. In general, the affected udders were swollen and tense, hot and firm, and very painful. There was complete stagnation of secretion in severe cases, and only a few milliliters of brown, blood-stained, or purulent secretion was observed when sheep were milked. In these cases, the clinical picture evolved to sternal recumbency, adipsia, cold extremities, mean temperature of 36°C, tachycardia, tachypnea, and comatose state. The signs progressed to spontaneous death in five affected ewes.

Macroscopically, the primary lesions consisted of severe gangrenous mastitis that presented an intense necrohemorrhagic characteristic. In the external examination of three ewes that

Fig.1. Ewe with gangrenous mastitis exhibiting a clinical picture characterized by apathy, prolonged recumbency, anorexia, emaciation and opaque and brittle hair.

Table 1. Target gene, initiator oligonucleotides and size of the amplification product of virulence genes for *Staphylococcus*

Target gene	Name	Oligonucleotide sequence (5´- 3´)	Expected size (bp)	Tm (°C)
fnbA	fnbA_R	ACTTCACCTGTCGCCATTAC	539 bp	61
	fnbA_F	GCAGTACAAGCACCACAAAC		
fnbB	fnbB_F	AGGCGACGGCAAAGATAAA	317 bp	57
	fnbB_R	TAGTAACCTGACCACCACCT		
*clfA**	clfA_F	GATTCTGACCCAGGTTCAGA	945 bp	60
	clfA_R	CTGTATCTGGTAATGGTTCTTT		
*clf**	clfB_F	ATGGTGATTCAGCAGTAAATCC	880 bp	55
	clfB_R	CATTATTTGGTGGTGTAACTCTT		
sea	sea_F	CCGAAGGTTCTGTAGAAGTATG	269 bp	55
	sea_R	GCTTGTATGTATGGTGGTGTA		
seb	seb_F	CCCGTTTCATAAGGCGAGTT	314 bp	55
	seb_R	ACGTAGATGTGTTTGGAGCTAAT		
sec†	sec_F	AGATGAAGTAGTTGATGTGTATGG	451 bp	57
	sec_R	CACACTTTTAGAATCAACCG		
sed	sed_F	GTCACTCCACACGAAGGTAATAA	255 bp	57
	sed_R	GAGACTTTAGACCCATCAGAAGAA		
see†	see_F	GCTGGAGGCACACCAAATA	301 bp	55
	see_R	CATAACTTACCGTGGACCCTTC		
seg	seg_F	GCCAGTGTCTTGCTTTGTAATC	491 bp	57
	seg_R	GAATGCTCAACCCGATCCTAA		
she	seh_F	CACATCATATGCGAAAGCAGAAG	365 bp	56
	seh_R	CCCAAACATTAGCACCAATCAC		
sei	sei_F	AGGCAGTCCATCTCCTGTATAA	568 bp	60
	sei_R	TGCTCAAGGTGATATTGGTGTAG		
tss†	tsst_F	ACCCCTGTTCCCTTATCATC	326 bp	55
	tsst_R	TTTTCAGTATTTGTAACGCC		
blaZ	blaZ_F	AAGAGATTTGCCTATGCTTC	102 bp	55
	blaZ_R	GCTTGACCACTTTTATCAGC		
mecA	mecA1	AAAATCGATGGTAAAGGTTGG	533 bp	52
	mecA2	AGTTCTGCAGTACCGGATTTGC		
mecC	$mec_{ALGA251}$ 1A	CATTAAAATCAGAGCGAGGC	188 bp	59
	$mec_{ALGA251}$ 1B	TGGCTGAACCCATTTTTGAT		

bp = Base pairs, Tm = primer melting temperature; * Primers described by Sabat et al. (2003); † Primers described by Mehrotra et al. (2000).

were severely affected, the udders very firm, enlarged and asymmetric (Fig.2A). The color of the skin was blue, ranging from black. The skin aspect was soft; ulcerations were also observed, and one or both teats were absent due to necrosis (Fig.2B). The affected glandular tissue was swollen and turgid, whereas the lobular parenchyma was necrotic, hemorrhagic, and contained bloody-purulent discharge (Fig.2C). The periductal tissues were white, rigid due to the lack of glandular tissue and fibrosis. The lactiferous sinus and larger ducts had a thickened lining and small rounded polypoid projections into the lumen (Fig.2D). Additionally, reactive supramammary lymph nodes were also observed. Other lesions consisted of congestion of the small intestine's blood vessels; the livers were enlarged, congested, and friable and had rounded edges, with enlarged gallbladders. In the epicardium, areas of petechiae were observed, and both sides of the cranial lobes of the lungs generally had a dark-red coloration with a smooth and shiny surface. Microscopically, the main lesions were characterized by necrosis, thrombosis and fibrosis of the mammary parenchyma (Fig.3A). The periacinar epithelium was vacuolated, necrotic, and large amounts of intralesional bacterial colonies widespread into proteinaceous eosinophilic material were noted. In the necrotic areas, severe inflammatory cell infiltration is composed mainly of degranulated neutrophils, macrophages, epithelioid macrophages, and Langhans giant cells, as well as a small number of lymphocytes and plasma cells (Fig.2C and 3B). Lymphocytes and plasma cells were observed mainly in the interstitium, and sometimes lymphoid follicles were noted, especially around the lactiferous sinus and ducts. Granulation and fibrosis were noted, obliterating many of the acini and lobules, and in these areas, severe thrombosis was remarkable (Fig.3D). The glandular ducts and lactiferous sinus were filled with many neutrophils and necrotic epithelioid macrophages. Other lesions less specific

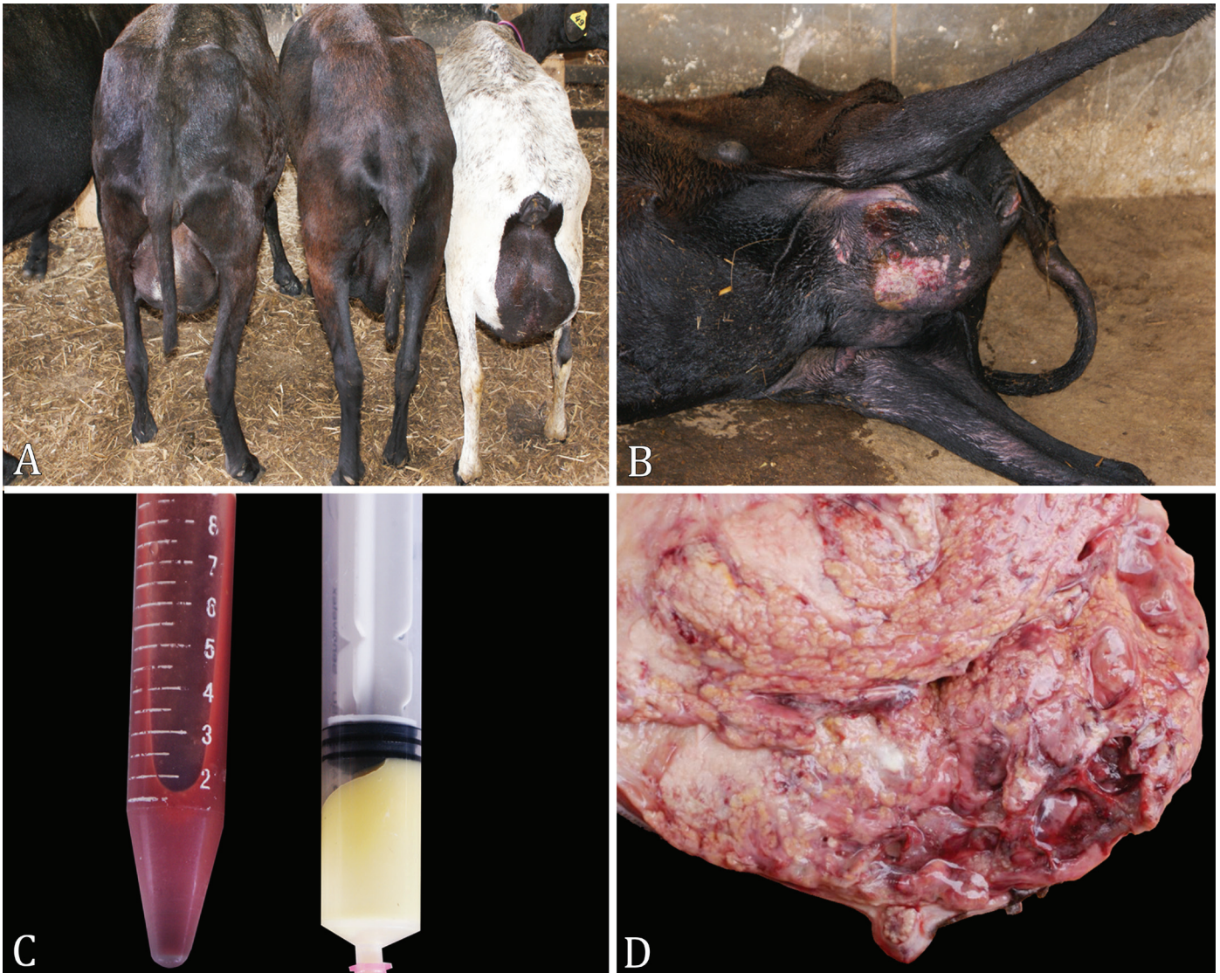

Fig.2. Gangrenous mastitis in ewes. (**A**) Three ewes showing enlarged and asymmetrical udders. (**B**) Ewe with gangrenous mastitis. The aspect of the skin was friable and ulcerated; the udder was firm and one teat is lacking due to necrosis. (**C**-**D**) The mammary gland had a bloody to purulent content and the affected glandular tissue was swollen and turgid whereas the lobular parenchyma was intensely necrohemorrhagic. The lactiferous sinus is thickened and lining and obstructed by polypoid projections into the lumen.

included mild to moderate infiltration of neutrophils and lymphocytes and plasma cells that were usually seen in the perisinusoidal spaces and among hepatocytes. They were often focally concentrated in sites of mild necrosis at the centrilobular areas. In the lungs, a severe neutrophilic and lymphoplasmacytic inflammatory infiltrate was frequently observed. The alveolar spaces, septa, and bronchioles contained scattered neutrophils, lymphocytes, plasma cells, fibrin, few erythrocytes (hemorrhage), and necrotic cellular debris. Additionally, there was dilated lymphatics and amorphic, homogenous, eosinophilic material (edema) expanding the alveolar lumen.

Bacteriological results

The bacteriological results revealed a pure round, gram-positive, catalase positive and coagulase negative colony in the growth conditions. The obtained isolate was identified by MALDI-TOF as *Staphylococcus haemolyticus*, which showed antimicrobial multiresistance characteristics since resistance was observed in nine out of 15 (60%) tested antibiotics (benzylpenicillin, clindamycin, erythromycin, oxacillin, rifampicin, tetracycline and vancomycin). MIC values and the antimicrobial resistance pattern are shown in Table 2.

Virulence factors

PCR results revealed that the isolate was positive for two staphylococcal enterotoxigenic genes (*sec* and *see*) and fibronectin-binding protein B (*fnbB*). In contrast, the isolate was negative for *fnbB, clfA, clfB, sea, seb, sed, seg, seh, sei, tsst, blaZ, mecA* and *mecC*.

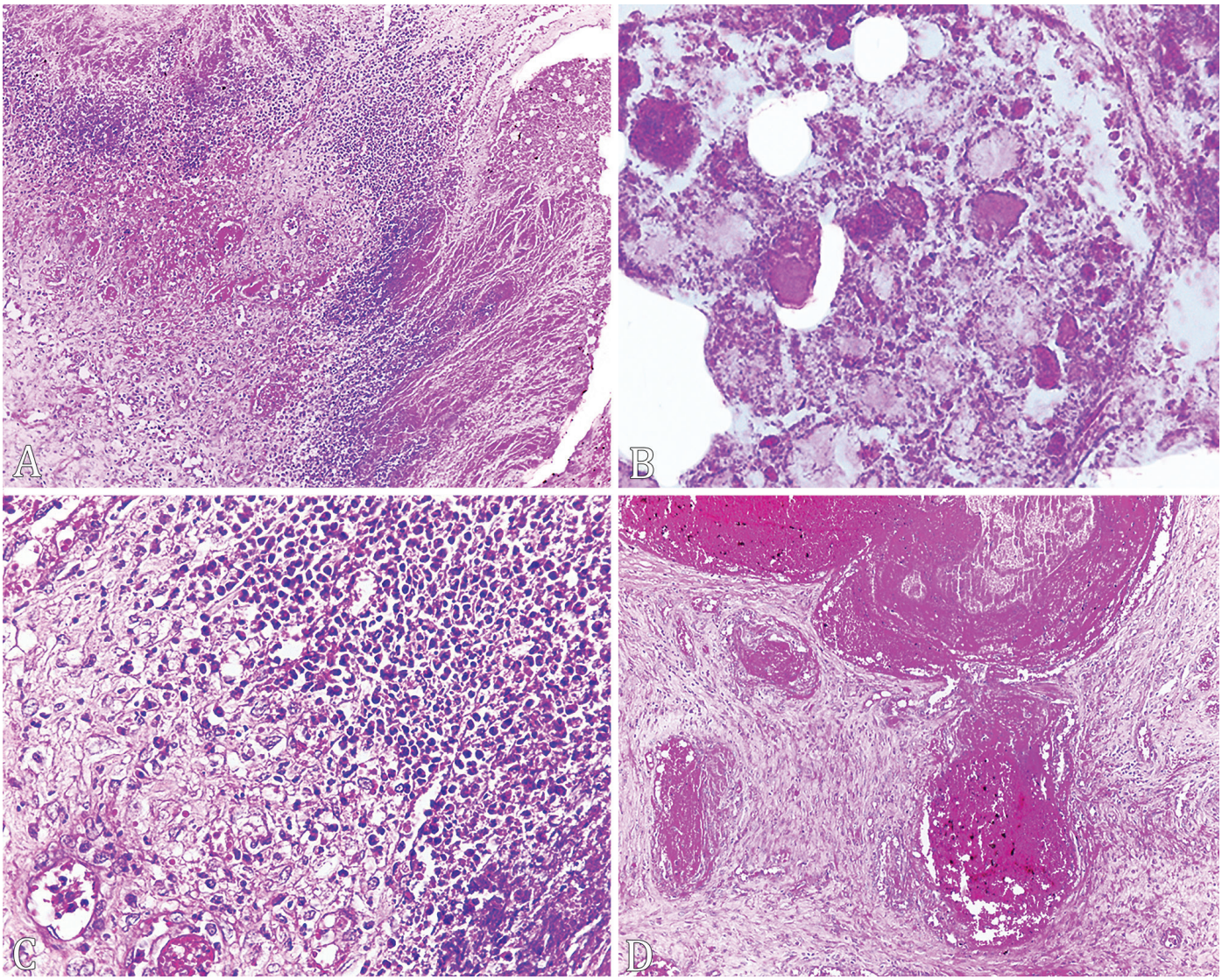

Fig.3. Ewe mammary gland affected by gangrenous mastitis. (**A**) Predominantly, the main lesions were characterized by necrosis, thrombosis and fibrosis of the mammary parenchyma. HE, obj.10x. (**B**) The periacinar epithelium was necrotic and large amounts of intralesional bacterial colonies widespread into proteinaceous eosinophilic material was noted. HE, obj.10x (**C**) Necrotic areas had severe inflammatory cell infiltration composed mainly by degranulated neutrophils, macrophages, lymphocytes and plasma cells. HE, obj.40x. (**D**) Replacement of the glandular parenchyma by fibrous connective tissue and a remarkable thrombosis. HE, obj.10x.

Table 2. Antibiotic resistance pattern of *Staphylococcus haemolyticus* isolated from a mastitis in a sheep

Antibiotics	MIC	Interpretation
Benzylpenicillin	≥0.5	R
Clindamycin	≥8	R
Chloramphenicol	8	S
Enrofloxacin	≤0.5	S
Erythromycin	≥8	R
Gentamicin	≤0.5	S
Kanamycin	≤4	S
Marbofloxacin	≤0.5	S
Oxacillin	≥4	R
Rifampicin	≥32	R
Tetracycline	≥16	R
Trimethoprim/ Sulfamethoxazole	≤10	S
Vancomycin	≥4	R

MIC = minimum inhibitory concentrations, R = resistant, S = sensitive.

DISCUSSION

This study reports a severe and uncommon clinical condition in a flock of meat sheep characterized by gangrenous mastitis and presented systemic clinical signs caused by *Staphylococcus haemolyticus*. The NAS have been isolated in many cases of sheep mastitis (Pyörälä & Taponen 2009). For this reason, the importance of this group of bacteria has been reviewed, and they are presently considered the most important etiologic agents of subclinical mastitis in sheep (Bergonier et al. 2003, Contreras et al. 2007). However, there have been no reports of gangrenous mastitis cases in sheep caused by multidrug-resistant non-*aureus* staphylococci.

In meat production systems, most clinical mastitis cases are associated with *Staphylococcus aureus* or *Mannheimia haemolytica* (Mavrogianni et al. 2007, Arsenault et al. 2008, Kopp 2010, Omaleki et al. 2010, Gelasakis et al. 2015), including gangrenous mastitis (Vautor et al. 2009). The proportion of NAS among bacteria isolated from clinical mastitis cases remains very low in many countries (Pyörälä & Taponen 2009). However, although less pathogenic than *S. aureus* and *M. haemolytica*, NAS can also produce persistent subclinical mastitis, significantly increasing milk somatic cell counts, which could cause clinical mastitis (Deinhofer & Pernthaner 1995, Contreras et al. 1997, Ariznabarreta et al. 2002), as well as producing thermostable enterotoxins (Meyrand et al. 1998, Udo et al. 1999).

Nevertheless, despite the accepted role of these bacteria as major intramammary infection-causing pathogens in small ruminants, the different NAS species' pathogenicity varies widely (Gonzalo et al. 2002, Bergonier et al. 2003). The most commonly isolated NAS species in persistent subclinical intramammary infection in goats and sheep are *Staphylococcus epidermidis*, *Staphylococcus caprae*, *Staphylococcus simulans*, *Staphylococcus chromogenes*, and *Staphylococcus xylosus* (Gonzalo et al. 2002, Bergonier et al. 2003, Contreras et al. 2003). *S. epidermidis* and *S. caprae* are among the most prevalent causal microorganisms in goats, and *S. epidermidis* and *S. simulans* are commonly found in ewes (Contreras et al. 2007).

MALDI-TOF identified the isolate as *S. haemolyticus*. MALDI-TOF was used to determine the *Staphylococcus* isolate at the species level. This approach is vital because this differentiation into species gives us essential information since, in veterinary medicine, we often assume that all NAS form a group with the same characteristics, which is not correct. *S. haemolyticus* is one of the most commonly isolated NAS from bovine mastitis (Hosseinzadeh & Dastmalchi Saei 2014); however, in small ruminants, they are not considered a significant pathogen (Leitner et al. 2009). Gangrene in the mammary gland is the result of marked necrosis caused by β-toxin and often progresses to septicemia. Most *S. haemolyticus* isolates show β-hemolytic activity that leads to an escape of bacteria from the host immune system, permitting the pathogen's survival (Pinheiro et al. 2015). In PCR results, the isolate was negative for the *mec*A and *mec*C genes but positive for the *bla*Z gene. We also detected two staphylococcal enterotoxigenic genes (*sec* and *see*) and fibronectin-binding protein B (*fnbB*).

S. haemolyticus isolates showed antimicrobial multiresistance characteristics, resistant to seven of the 13 antibiotics tested (Table 2). This feature is a result, among other factors, of the relationship between bacterial pathogenicity, host physical resistance, and the resulting selection of interventions such as antimicrobial therapy (Geisinger & Isberg 2017), a condition that hinders response to treatment and, under varying conditions, may become a risk to human health. This multidrug resistance pattern might be the result of selective pressure from the frequent use of antibiotics. These animals received indiscriminate treatment by the farmer. Resistance to benzylpenicillin is common in *S. haemolyticus* (Taponen et al. 2016), and resistance against β-lactams or aminoglycosides is the most common trait observed in *Staphylococcus* spp. Although the isolate was not favorable for the *mec*A and *mec*C genes, it showed phenotypic resistance to oxacillin and vancomycin, a trait that is not common. Multidrug and methicillin-resistant strains are particularly challenging because they narrow therapeutic options, increasing the risk of treatment failure and costs. In this study, genotypic testing for vancomycin resistance was not possible.

Interestingly, compiled data in the molecular analysis showed two staphylococcal enterotoxigenic genes (*sec* and *see*) and fibronectin-binding protein B (*fnbB*). The presence of the sec and see genes correspond to two of the five major types of staphylococcal enterotoxins (*sea, seb, sec, sed*, and *see*) (Johler et al. 2016). Staphylococcal toxins known to be pyrogenic and related to important human diseases, such as food poisoning and septic shock, are commonly associated with *S. aureus*. However, other species have already been shown to be enterotoxigenic (Frey et al. 2013), as in the case reported here. Fibronectin-binding protein B (*fnbB*) belongs to the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family, adhesins that are well described in *S. aureus* isolates and are known to mediate the adhesion of *Staphylococcus* spp. to fibrinogen, elastin, and fibronectin (Murai et al. 2016), mediating immune evasion mechanisms (Burke et al. 2010), hindering their action. However, the identification of MSCRAMM in non-*aureus* staphylococci (NAS) is poorly described.

Histopathological findings were possible to observe a granulomatous inflammatory process, chronic active in the mammary gland, in addition to areas of necrosis. This is also a characteristic observed in mastitis caused by coagulase-negative *Staphylococcus* in ruminants (Benites et al. 2002).

CONCLUSION

The current study provides evidence of gangrenous mastitis in sheep flocks caused by multidrug-resistant *Staphylococcus haemolyticus*, a non-*aureus* staphylococci. Further studies are needed to clarify the epidemiological, clinical, and histological association of bacterial presence in small ruminants in gangrenous mastitis cases.

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Conflict of interest statement.- The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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