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REGULAR ARTICLES

Pathological and microbiological characterization of mastitis in dairy cows

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Abstract

Mastitis may be caused by a wide range of microorganisms able to induce distinct lesions in mammary tissues. This study aims to characterize the gross and microscopic features of mastitis in dairy cows and to correlate them with the pathogens involved. The udders of slaughtered dairy cows were inspected and milk samples from each mammary quarter or fragments of the parenchyma were sent for microbiological analysis, and tissue collected for histopathological evaluation. A total of 148 cows and 592 mammary quarters were collected. From these, 432 quarters (73%) had mastitis and in 160 (27%), no changes were observed. Mastitis was classified into seven patterns based on the histopathological findings, of which mixed, lymphoplasmacytic, and suppurative mastitides were the most prevalent with 35.9% (155/432), 27.1% (117/432), and 14.3% (62/432) of the cases, respectively. These patterns were associated with the same set of pathogens: *Streptococcus* spp., coagulase-negative *Staphylococcus* (CNS), *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Corynebacterium bovis*. The pyogranulomatous pattern represented 7.2% (31/432) of the cases with distinct distribution based on the agent involved, mostly *S. aureus* and *Nocardia* sp. Abscedative mastitis accounted for 6.0% (26/432) of the cases; it was characterized by multiple abscesses in the parenchyma and was mainly caused by *Trueperella pyogenes*. Necrosuppurative mastitis represented 5.8% (25/432) of the cases which were characterized by severe parenchyma necrosis and were caused by bacteria such as CNS and *Escherichia coli*. The granulomatous pattern represented 3.7% (16/432) of the cases and was occasionally associated with *Mycobacterium* sp.

Keywords Mammary gland · Dairy cattle · Mammary pathology · Bacteria

Introduction

Mastitis is an important disease of dairy cattle and represents a challenge for the dairy industry since it causes losses associated with the reduction of production and quality of milk, treatment expenses, milk discard, and cattle mortality (Hazlett et al. 1984; Bradley 2002; Acosta et al. 2016; Busanello et al. 2017). The disease is often caused by contagious or environmental pathogenic microorganisms (Oviedo-Boyso et al. 2007), mainly bacteria (Bandeira et al. 2013; Foster 2017), capable of inducing various lesions in mammary tissues (Zhao and Lacasse 2008; Akers and Nickerson 2011). Studies that performed an evaluation correlating these pathological lesions with the pathogenic agents causing mastitis are scarce (Macadam 1958; Hazlett et al. 1984; Benites et al. 2002; Hussian et al. 2012). Surveys are usually conducted to determine the frequency of the major agents involved in mastitis cases (Bandeira et al. 2013; Cunha et al. 2015; Acosta et al. 2016; Busanello et al. 2017); in addition to studies on the description of case reports or outbreaks related to a single microorganism (Schiefer et al. 1976; Shibahara and Nakamura 1999; Pisoni et al. 2008; Tessele et al. 2014). Therefore, this work aims to characterize the pathological aspects of inflammatory lesions in the mammary glands of slaughtered dairy cows in the southern region of Brazil and determine their correlations with the pathogens involved.

Materials and methods

From August 2016 to March 2017, the slaughter of dairy cows was carried out in two slaughterhouses located in the state of

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Rio Grande do Sul, Brazil. The mammary glands were randomly selected and inspected. After inspection, milk samples were collected by manual milking using aseptic technique from each mammary quarter and kept under refrigeration in sterile tubes. In cases where it was not possible to obtain the milk sample, a sample of the mammary parenchyma was collected. After that, fragments of mammary tissue were collected and kept in 10% neutral buffered formalin.

All collected samples, either 0.01 ml of milk or a loopful of parenchyma, were cultured in both 5% Sheep Blood agar (Kasvi®) and MacConkey agar (Kasvi®). Plates were incubated in a CO^2-enriched atmosphere (~ 5%) at 37 °C and examined after 24, 48, and 72 h. Bacterial species were identified by their cultural, morphological, tinctorial, and biochemical characteristics using a simplified scheme based on National Mastitis Council manual guidelines (1999). Hemolytic, catalase-positive, coagulase-positive, maltose-positive and mannitol-positive Gram-positive cocci in cell clusters were classified as *Staphylococcus aureus*. Remaining isolates of coagulase-negative, catalase-positive Gram-positive cocci were grouped as "coagulase-negative *Staphylococcus*" (CNS). Catalase negative, Gram-positive cocci arranged in individual cells or in pairs were identified as *Streptococci*. Based on the results for CAMP reaction and esculin hydrolysis, the isolates were presumptively identified as *Streptococcus agalactiae* (CAMP+, Esculin-), *S. uberis* (CAMP±, Esculin+), and *S. dysgalactiae* (CAMP-, Esculin-). Complementary fermentation tests of inulin, lactose, mannitol, raffinose, salicin, sorbitol, and trehalose (MARKEY et al. 2013a), either confirmed the preliminary identification or when biochemical discrepancies were found, reclassified an isolate as *Streptococcus* spp. Straight, catalase-positive, large-sized Gram-positive rods from strongly hemolytic colonies were classified as *Bacillus* sp. Irregular shaped, lipophilic, Gram-positive rods were classified as *Corynebacterium bovis*. Slow-growing (48 h or more), hemolytic pin-point colonies, highly proteolytic (on Loeffler's medium slants) Gram-positive cocci or irregular rods were deemed as *Trueperella pyogenes*. Slow-growing (> 72 h), white, powdery, firmly adherent to the medium colonies displaying branching Gram-labile filaments on microscopy were identified as *Nocardia* sp. Gram-negative rods were tested for catalase, oxidase, and lactose fermentation on MacConkey Agar and then more comprehensively characterized using API20E strips (BioMerieux). In cases where the simplified identification scheme was unable to result in a proper species discrimination, additional tests were conducted following Markey et al. (2013a).

The formalin-fixed material was routinely processed for histopathology and stained by hematoxylin and eosin (H&E). Histopathological analysis was carried out, and the inflammatory alterations were classified according to their morphological aspect in mixed, lymphoplasmacytic, suppurative, pyogranulomatous, abscedative, necrosuppurative, and granulomatous mastitis. In cases in which granulomatous and pyogranulomatous lesions were observed without the identification of agents by microbiological examination, the histochemical techniques of Grocott Methenamine Silver and Ziehl-Neelsen (ZN) were performed. Additionally, sections of mammary parenchyma with pyogranulomatous or necrosupurative lesions suggestive of *Nocardia* sp. were submitted to immunohistochemistry (IHC). A polyclonal anti-*Nocardia* spp. antibody (non-commercial) was used at a dilution of 1:50. Amplification signal was achieved by using the Mack 4 Universal HRP polymer (Biocare Medical®) and the reaction was revealed with the 3-amino-9-ethyl-carbazole chromogen (Biocare Medical®). Furthermore, a section of one mammary quarter was submitted to IHC for *Fusobacterium necrophorum* with a polyclonal antibody produced in rabbit (strain ATCC25286) (Shibahara et al. 2002).

Results

A total of 148 mammary glands were collected and 592 mammary quarters were analyzed. Of these, 432 (73%) showed inflammatory lesions and in 160 (27%), no changes were observed. However, when the distribution of the inflammatory lesions in the mammary gland of each cow was analyzed, it was verified that 5.4% (8/148) of the cows presented mastitis in at least one quarter, 13.5% (20/148) in two quarters, 24.3% (36/148) in three quarters, 46.6% (69/148) in all quarters, and 10.2% (15/148) had no inflammatory lesions.

The inflammatory lesions (mastitis) observed in the mammary quarters were morphologically classified into mixed (35.9% [155/432]); lymphoplasmacytic (27.1% [117/432]), suppurative (14.3% [62/432]), pyogranulomatous (7.2% [31/432]), abscedative (6.0% [26/432]), necrosuppurative (5.8% [25/432]), and granulomatous (3.7% [16/432]). The agents identified in the lesions are listed in Table 1 and were subdivided according to the histological pattern of the associated mastitis. Of the 432 bacteriological cultures, correlated to the mammary quarters with mastitis, pure and mixed cultures, were isolated, respectively, in 45.8% (198/432) and in 14.8% (64/432), while in 39.4% (170/432), no significant bacterial growth (< 10 CFU [< 103/ml]) or no bacterial growth was detected.

Grossly, the mixed mastitis was characterized by the pronounced lobular mammary pattern, with small yellowish nodules (0.2–0.5 cm in diameter) in the middle of the parenchyma and projecting towards the lumen of the ducts and gland cistern, which were interspersed by thin white septa (Fig. 1a). The histological pattern was constituted of a discrete to moderate inflammatory infiltrate, composed of neutrophils within the alveoli and ducts as well as a multifocal infiltrate of neutrophils, lymphocytes, plasma cells, and macrophages in the interstitium.

Table 1 Morphological aspects of bovine mammary lesions and pathogenic agents identified

Agent	No. of quarters
Suppurative mastitis	
Streptococcus spp.	18
Coagulase-negative *Staphylococcus*	9
Staphylococcus aureus	3
Other streptococci (S. *agalactiae*, *S. uberis* e *S. dysgalactiae*)	5
Others (*C. bovis*, *E. coli*, *Klebsiella* sp., *T. pyogenes*, *Proteus* sp., and *Bacillus* sp.)	13
Necrosuppurative mastitis	
Coagulase-negative *Staphylococcus*	6
Escherichia coli	4
Gram-negative rod-shaped	4
Nocardia sp.	3
Klebsiella sp.	2
Bacillus sp.	2
Streptococcus spp., *S. agalactiae*, *Proteus* sp., and *C. bovis*.	6
Abscedative mastitis	
Trueperella pyogenes	16
Staphylococcus aureus	2
Streptococcus spp. and *S. dysgalactiae*	4
Corynebacterium bovis	1
Lymphoplasmacytic mastitis	
Streptococcus spp.	21
Coagulase-negative *Staphylococcus*	21
Corynebacterium bovis	21
Staphylococcus aureus	8
Streptococcus uberis	8
S. agalactiae, *Proteus* sp., *Escherichia coli*, and *Bacillus* sp.	5
Mixed mastitis	
Streptococcus spp.	36
Staphylococcus aureus	22
Coagulase-negative *Staphylococcus*	22
Corynebacterium bovis	18
Streptococcus agalactiae	8
S. uberis and *S. dysgalactiae*	5
Others (Gram-negative rod-shaped, *Bacillus* sp., *Nocardia* sp., *T. pyogenes*, and *E. coli*	16
Pyogranulomatous mastitis	
Staphylococcus aureus	11
Nocardia sp.	5
Pseudomonas aeruginosa	2
Fusobacterium necrophorum	1
Filamentous fungus	1

Bacterial agents isolated from mammary quarters without histopathological lesions were disregarded

It was commonly associated with hyperplasia and degeneration of epithelial cells and discrete fibrosis (Fig. 1b). Occasionally, bacterial myriads, squamous metaplasia of the glandular epithelium, alveolar dilatation, and formation of fibrous polyps in the ductal lumina were observed. *Streptococcus* spp., coagulase-negative *Staphylococcus* (CNS), *Staphylococcus aureus,* and *Corynebacterium bovis* were the main agents identified in pure or mixed cultures, associated with each other.

Suppurative mastitis presented a gross pattern similar to that described for mixed mastitis (Fig. 1c). Histologically, mild to moderate amounts of intact and degenerate neutrophils were observed in the alveoli, ducts, and the interstitium. They were often associated with hyperplasia and degeneration of epithelial cells (Fig. 1d). Bacterial myriads were occasionally visualized in association with the inflammatory infiltrate, as well as squamous metaplasia of the glandular epithelium, alveolar dilatation, and fibrosis. *Streptococcus* spp. and CNS were the main bacterial agents isolated, either pure or mixed cultures, mainly in association with *C. bovis*.

Grossly, the lymphoplasmacytic pattern was characterized by firm mammary quarters with a decrease of mammary lobulations and thick white septa dissecting the parenchyma (Fig. 2a). Histopathologically, it consisted of discrete to moderate interstitial inflammatory infiltrate composed of lymphocytes and plasma cells, with occasional macrophages, associated with moderate fibrosis (Fig. 2b). Sometimes, nodular, white, and polypoid structures (Fig. 2c) were observed macroscopically, which in histology corresponded to dilated alveoli covered by hyperplastic epithelium (Fig. 2d). In this category, the main microorganisms isolated in pure or mixed culture and associated with each other were *Streptococcus* spp., CNS, and *C. bovis*.

In the pyogranulomatous mastitis, three bacteria were identified in pure cultures (*S. aureus*, *Pseudomonas aeruginosa*, and *Nocardia* sp.), and a filamentous fungus was detected by histopathology. Similarly, three distinct macroscopic patterns were observed according to the agent involved. In the first pattern (associated with *S. aureus* and *P. aeruginosa*), nodular, yellowish, and firm structures (0.5–1.5 cm in diameter) were found in the middle of the mammary parenchyma with purulent material at the center (Fig. 3a). In the second pattern (associated with *Nocardia* sp.), some quarters were firm, yellowish, and interspersed with dark red areas, with pronounced mammary lobular pattern (Fig. 3b). In the third pattern (associated with the fungus), multifocal to coalescing nodules of 0.5 to 3.0 cm in diameter were observed, as well as markedly distended ducts filled with purulent material (Fig. 3c). Histologically, multiple pyogranulomas were observed in the mammary parenchyma characterized by areas of necrosis, surrounded by a marked inflammatory infiltrate of intact and degenerate neutrophils, epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells, with peripheral fibrosis (Fig. 3d, e, f). In addition, in 14 of the 25 cases, a strongly eosinophilic, radiated material (Splendore-Hoeppli

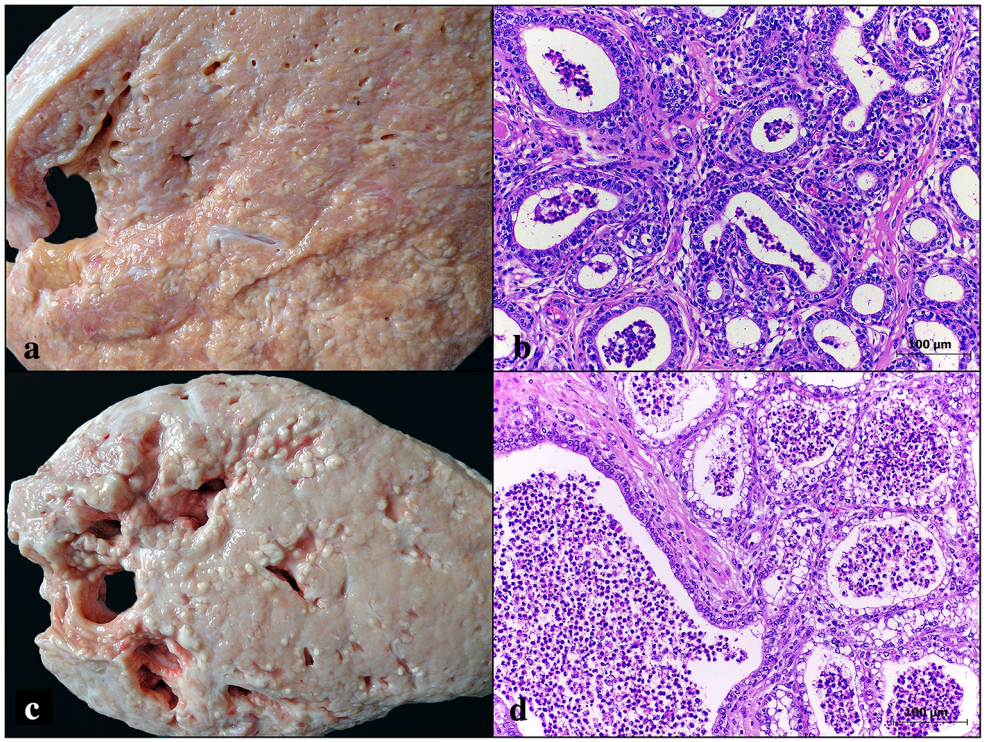

Fig. 1 Patterns of mastitis in dairy cows. **a** Mixed mastitis. This is an evidence of the mammary lobular pattern with the presence of yellowish nodules in the middle of the parenchyma ranging from 0.2 to 0.5 cm in diameter and interspersed by thin white septa. **b** Mixed mastitis. Image is showing a moderate inflammatory infiltrate composed of neutrophils within the alveoli, in addition to multifocal infiltration of neutrophils, lymphocytes, plasma cells, and macrophages in the interstitium interspersed by discrete fibrosis. Hematoxylin and eosin (H&E). **c** Suppurative mastitis. Gross pattern similar to that described in **a**, with nodules protruding into the lumen of the lactiferous ducts and the cisternae of the mammary gland. **d** Suppurative mastitis. There is a marked infiltration of intact and degenerate neutrophils within the alveoli and ducts associated with pronounced vacuolization of epithelial cells (degeneration). H&E

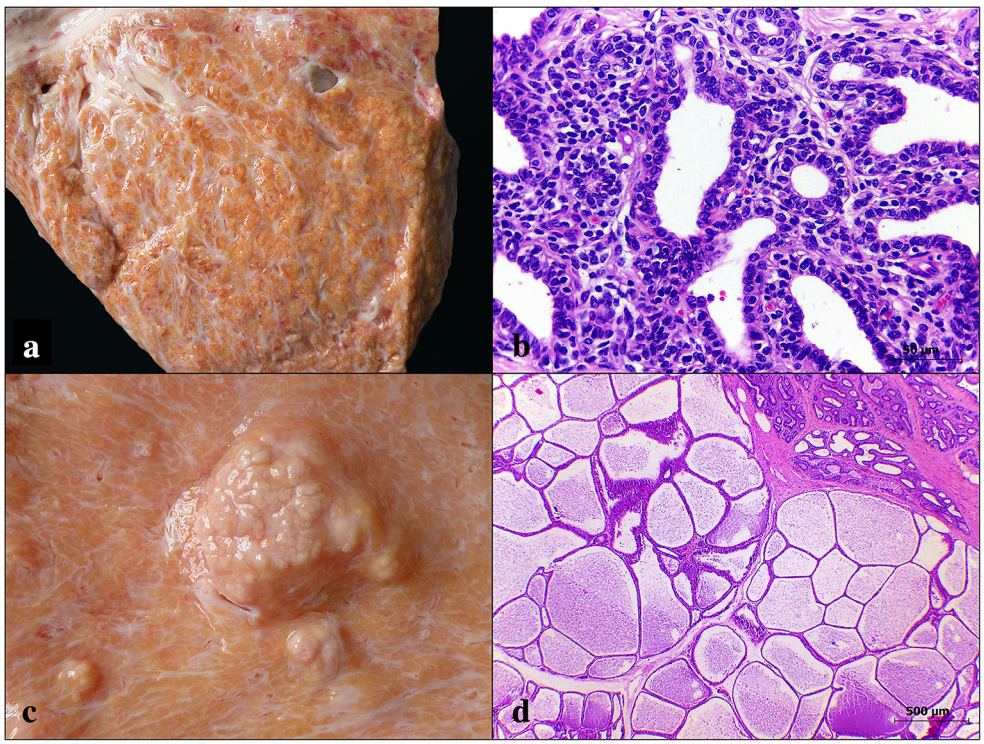

Fig. 2 Lymphoplasmacytic mastitis in dairy cows. **a** Mammary quarter with diminished lobulations and thick white septa that dissect the parenchyma. **b** There is moderate, multifocal, and interstitial inflammatory infiltrate composed of lymphocytes and plasma cells associated with moderate fibrosis. H&E. **c** Nodular, white, and polypoid-like formations are seen in the middle of the mammary parenchyma. **d** Several markedly dilated alveoli, sometimes covered by hyperplastic epithelium, are noted. H&E

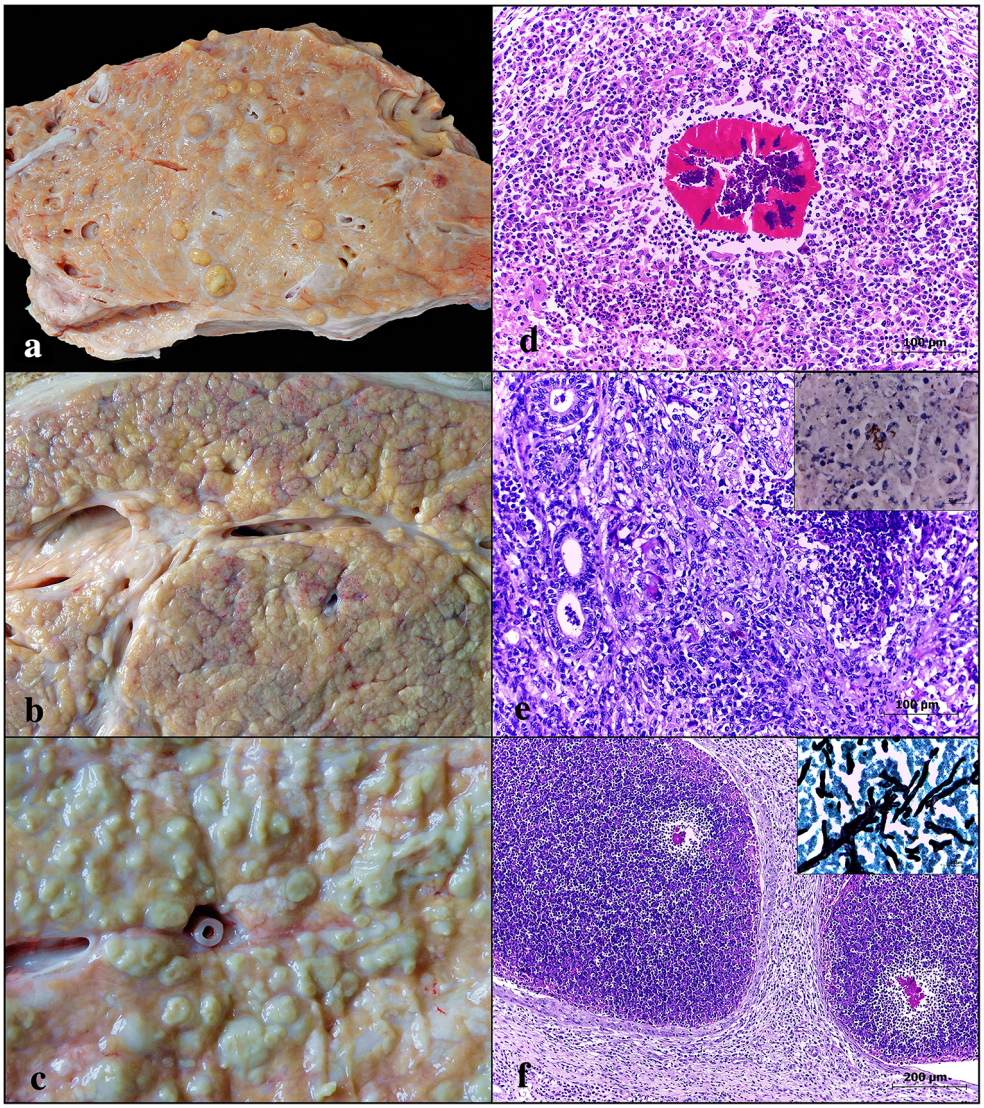

Fig. 3 Pyogranulomatous mastitis in dairy cows. **a** Nodular, yellowish, and firm structures are found in the middle of the mammary parenchyma, ranging from 0.5 to 1.5 cm in diameter, with purulent material at the center (lesion associated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*). **b** There is a firm and yellowish mammary quarter interspersed with dark red areas with evidence of the lobular pattern (lesion associated with *Nocardia* sp.). **c** Multifocal to coalescing nodules, of varying sizes, filled with purulent contents (fungal mastitis) are observed in the middle of the mammary parenchyma. **d** A marked inflammatory infiltrate of intact and degenerate neutrophils, epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells is seen in the mammary parenchyma. There is also a strongly eosinophilic, radiated material (Splendore-Hoeppli phenomenon), which contains large basophilic cocci in the center (*S. aureus*). H&E. **e** A focal area of necrosis associated with a marked inflammatory infiltrate of intact and degenerate neutrophils, macrophages, lymphocytes, plasma cells, and occasional multinucleated giant cells is observed in the middle of mammary parenchyma. H&E. Inset, positive immunolabeling for *Nocardia* sp. immunohistochemistry. **f** There are marked dilated alveoli, filled by intact and degenerate neutrophils, as well as necrotic debris, associated with Splendore-Hoeppli phenomenon. Marked fibrosis interspersed by a moderate inflammatory infiltrate of macrophages, lymphocytes, plasma cells, and occasional multinucleated giant cells is seen in the interstitium. H&E. Inset, there are septate and branched fungal structures, strongly impregnated by silver. Grocott Methenamine Silver

phenomenon) was observed at the center of the pyogranulomas. In the middle of this material, in 11 of the 14 cases, large basophilic cocci were observed (*S. aureus*) (Fig. 3d); in two cases, small basophilic bacilli (*P. aeruginosa*); and in one case, septate and branched fungal structures, better visualized by the Grocott technique (Fig. 3f).

In both pyogranulomatous and necrosuppurative patterns associated with the isolation of *Nocardia* sp. (8/10) or showing evidence of filamentous bacteria in lesions compatible with the agent (2/10), the bacteria were better visualized by the ZN technique in 40% (4/10) of the cases and 80% (8/10) of the cases were positive in the IHC (Fig. 3e). In a case of pyogranulomatous mastitis, the H&E staining revealed bacillary bacteria that formed small clusters, negative in IHC for *Nocardia* sp. and positive in IHC for *F. necrophorum*.

Grossly, abscedative mastitis was characterized by the formation of single or multiple abscesses in the middle of the mammary parenchyma (Fig. 4a). At histopathology, these were composed of areas of necrosis with a large number of bacteria associated with a marked inflammatory infiltrate of intact and degenerate neutrophils, as well as macrophages and lymphocytes, surrounded by a thick fibrous capsule (Fig. 4b). Squamous metaplasia of the glandular epithelium and multifocal areas of hemorrhage were frequently observed adjacent to the abscess. *Trueperella pyogenes* was the predominant agent in this category and was isolated in pure cultures (15/16).

On macroscopic examination, necrosuppurative lesions were characterized by a moderate evidence of the mammary lobular pattern with yellowish areas in the middle of the parenchyma, occasionally filled by purulent material, commonly associated with subcutaneous edema (Fig. 4c). Histologically, there were multifocal areas of coagulation necrosis in the mammary parenchyma associated with a marked inflammatory infiltrate of intact and degenerate neutrophils, as well as

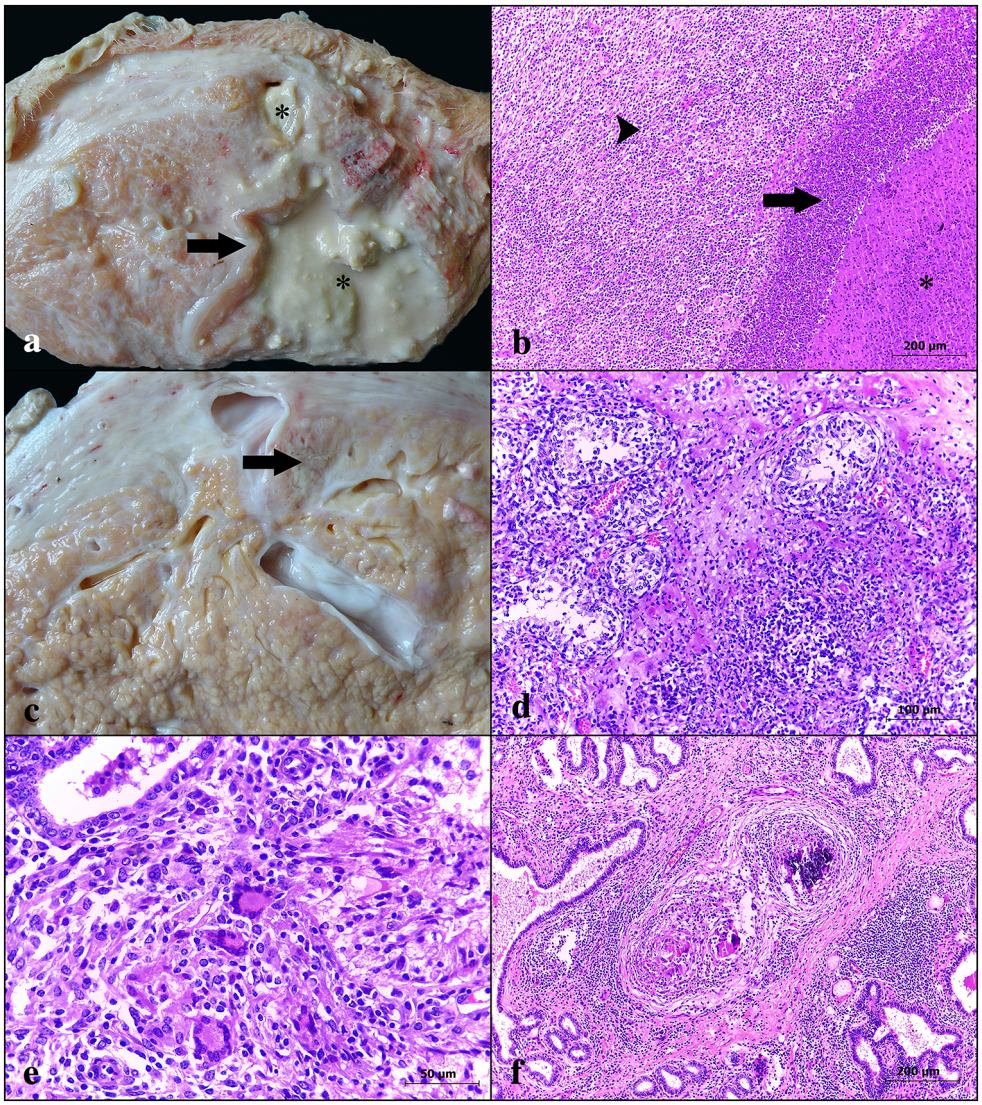

Fig. 4 Patterns of mastitis in dairy cows. **a** Abscedative mastitis. In the middle of the parenchyma are observed cavitations filled with purulent content (asterisks) and surrounded by a fibrous capsule (arrows). **b** Abscedative mastitis. The layers of an abscess are observed: a necrotic area (asterisk) is surrounded by a marked amount of intact and degenerate neutrophils (arrow) and more externally marked fibrosis interspersed by macrophages, lymphocytes, and plasma cells (arrow head). H&E. **c** Necrosuppurative mastitis. There is moderate evidence of the mammary lobular pattern, with a yellowish area in the middle of the parenchyma (arrow). **d** Necrosuppurative mastitis. Multifocal areas of necrosis of the mammary parenchyma are observed, associated with a marked inflammatory infiltrate of intact and degenerate neutrophils with an abundant deposition of fibrin. H&E. **e** Granulomatous mastitis. In the mammary parenchyma, there is a marked inflammatory infiltrate composed of epithelioid macrophages, multinucleated giant cells, lymphocytes and plasma cells. H&E. **f** Granulomatous mastitis. Multifocal granulomas are found in the middle of the mammary parenchyma, characterized by areas of mineralized necrosis surrounded by an infiltrate, similar to that described in **e**, with moderate peripheral fibrosis. H&E

fibrin deposition (Fig. 4d), vascular fibrinoid necrosis, thrombosis, and a large amount of bacteria. Hyperplasia and degeneration of epithelial cells and proliferation of fibrovascular tissue were frequently observed. CNS and *E. coli* were the main etiological agents isolated in this category.

Granulomatous lesions presented a macroscopic pattern similar to that observed in the lymphoplasmacytic mastitis, but two histological patterns were noted. The first pattern was characterized by an inflammatory infiltrate composed of epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells distributed in a multifocal to coalescent form and associated with discrete fibrosis (Fig. 4e). In the second pattern, multiple granulomas were observed in the middle of the mammary parenchyma and were characterized by small areas of necrosis, sometimes mineralized, surrounded by a moderate inflammatory infiltrate similar to that described in the first pattern, and by a fibrous capsule (Fig. 4f). There was no bacterial growth in this category. However, in three quarters of the same cow, acid-fast bacilli could be identified through the ZN stain in the middle of the mammary parenchyma and in the cytoplasm of multinucleated giant cells. These acid-fast bacilli were morphologically compatible with *Mycobacterium* sp.

The main agents involved in cases of mastitis and their correlation to the type of lesion observed in the mammary gland are described in Table 2.

Discussion

Mastitis is one of the primary diseases of dairy cows and is responsible for considerable economic losses due to the decrease of the volume and quality of milk produced and the early culling of the cows (Bandeira et al. 2013; Acosta et al. 2016; Busanello et al. 2017). In this study, inflammatory lesions, involving at least one mammary quarter, were detected in 133 out of 148 (89.9%) cows, and these lesions may have contributed to the culling of these animals. Infection by bacterial agents, such as *S. aureus* and *Nocardia* spp., causes the destruction of the secretory mammary epithelium with replacement by fibrous connective tissue, which leads to a decrease in milk production and makes it impossible to maintain the cow in the productive cycle (Benites et al. 2002; Barkema et al. 2006; Zhao and Lacasse 2008).

Bacteria are the main cause of bovine mastitis, acting through the ascending infection of the mammary gland. However, the presentation forms of the disease can be influenced by factors related to the host, the microorganism involved, and the environment (Benites et al. 2002; Schlafer and Foster 2016; Foster 2017). This explains the great variety of the isolated agents and the morphological diagnoses observed during the histological evaluation of the mammary glands conducted in this study.

The primary bacterial isolated from suppurative, mixed, and lymphoplasmacytic mastitides were *Streptococcus* spp., CNS, *S. aureus*, *S. agalactiae*, *S. uberis*, and *C. bovis*. The transmission of these bacteria is associated with the habitat of these agents and occurs mainly from cow to cow during milking. *S. agalactiae* and some strains of *S. aureus* are pathogens that must reside in the mammary gland and do not survive in the environment. *S. uberis* can survive in both environments; however, it is mainly found in the feces and bedding (Markey et al. 2013b; Foster 2017). CNS and *C. bovis* are commonly isolated from milk samples and mainly associated with cases of subclinical mastitis in dairy cows. CNS occurs as commensal on the skin of udder and occasionally causes opportunistic infections. However, some strains isolated from mastitis cases have invasive and toxin-producing ability (Anaya-López et al. 2006; Markey et al. 2013b). *C. bovis* is considered a commensal of the mammary gland, mainly in the teat canal, and can prevent infections by other agents (Markey et al. 2013b). This may indicate that not all the isolations of these microorganisms in this research may be associated with the inflammatory process identified.

Suppurative and mixed mastitis showed similar gross pattern, but they differed from lymphoplasmacytic mastitis, mainly in

Table 2 Bacterial agents more frequently identified in bovine mastitis and the patterns of associated lesions

Bacteria	Frequency	Associated lesions in descending order
Streptococcus spp.	18.3% (79/432)	Mixed, lymphoplasmacytic, and suppurative mastitides
Coagulase-negative *Staphylococcus*	13.4% (58/432)	Lymphoplasmacytic, mixed, and suppurative mastitides
Staphylococcus aureus	10.6% (46/432)	Mixed, pyogranulomatous, and lymphoplasmacytic mastitides
Corynebacterium bovis	10.2% (44/432)	Lymphoplasmacytic and mixed mastitides
Trueperella pyogenes	4.9% (21/432)	Abscedative mastitis
Streptococcus uberis	3.0% (13/432)	Lymphoplasmacytic mastitis
Streptococcus agalactiae	2.8% (12/432)	Mixed mastitis
Nocardia sp.	2.5% (11/432)	Pyogranulomatous and necrosuppurative mastitides
Escherichia coli	1.6% (7/432)	Necrosuppurative mastitis

Bacterial agents isolated from mammary quarters without histopathological lesions were disregarded

relation to the aspect of mammary lobulation. Histologically, there was variation in the inflammatory cell population involved among the patterns, as well as the intensity of the repair process (fibrosis), which was scarce in the suppurative, mild in the mixed, and moderate in the lymphoplasmacytic mastitis. Respectively, these pathological patterns indicate a probable acute, subacute, and chronic evolution of the lesions and are in agreement with what is described by other authors (Benites et al. 2002; Schlafer and Foster 2016; Foster 2017). Hyperplasia and degeneration of epithelial cells have been frequently observed in these categories and are mainly associated with the inflammatory process induced by *Streptococcus* spp. and *Staphylococcus* spp. (Schlafer and Foster 2016; Foster 2017). Squamous metaplasia of the glandular epithelium, occasionally observed in this study, is considered an evolution of the hyperplastic lesion and is related to the greater severity of the infectious process (Foster 2017). Alveolar dilation, occasionally associated with the formation of fibrous polyps in the lumen of the lactiferous ducts and sinuses, as well as in the gland cisternae, is also related to the chronicity of the inflammatory process. The pathogenesis involves progressive periductal fibrosis that causes obstruction of milk flow and consequent alveolar dilation (Benites et al. 2002; Schlafer and Foster 2016; Foster 2017).

Piogranulomatous mastitis was associated with different agents, as well as distinct lesions related to these pathogens. *S. aureus* and *P. aeruginosa* produced a botryomycotic lesion pattern (Heyndrickx et al. 2012; Tessele et al. 2014; Vinay et al. 2016), histologically characterized by the Splendore-Hoeppli phenomenon. This reaction is characterized by immunoglobulin aggregates which in cattle are observed primarily in chronic infections caused by *S. aureus*, and in cases of actinobacillosis and actinomycosis (Tessele et al. 2014; Schlafer and Foster 2016). Moreover, it can be observed in infections caused by *Nocardia* spp., *Mannheimia granulomatis*, agents associated with mycetomas (as observed here in a case of fungal mastitis) and in some parasitic pyogranulomas (Tessele et al. 2014).

Mastitis caused by *P. aeruginosa* in dairy cows is related to environmental contamination (Thompson et al. 2001; Schlafer and Foster 2016). Botryomycosis associated with *P. aeruginosa* is commonly described in humans (Heyndrickx et al. 2012; Vinay et al. 2016), but rarely reported in animals. In some known cases of botryomycosis in cattle, infections were localized in the udder skin (Donovan and Gross 1984) and nasopharynx (Thompson et al. 2001). However, the presence of the bacteria in lesions with the appearance of botryomycosis in the mammary parenchyma, as observed in two cases of this study, had never been reported.

Nocardia is a microorganism often found in the soil, and it is transmitted through environmental contamination or by the infusion of contaminated intramammary preparations (Pisoni et al. 2008; Schlafer and Foster 2016). The infection usually occurs as outbreaks on farms with poor hygiene and handling conditions (Pisoni et al. 2008). The agent mainly induces pyogranulomatous lesions (Pisoni et al. 2008; Schlafer and Foster 2016), as observed in five cases described in this study. However, it can cause necrosuppurative lesions in acute infections (Pisoni et al. 2008; Markey et al. 2013b), as observed in three cases.

Abscedative mastitis was characterized by single or multiple abscesses within the mammary parenchyma, similar to previous reports (Benites et al. 2002; Schlafer and Foster 2016). It was predominantly related to the infection caused by *T. pyogenes*, a bacteria present on the skin and in the mucous membranes of several animals (Markey et al. 2013b). Commonly, this type of mastitis was described in cows during the dry period and in heifers, but currently, it is also an important pathogen affecting lactating cows (Markey et al. 2013b; Ishiyama et al. 2017). Often, cows with mastitis caused by *T. pyogenes* tend to be culled as these lesions are associated with low rate of recovery of the mammary quarters, with extensive destruction of the parenchyma (Ishiyama et al. 2017). In agreement with this, we observed extensive lesions with a severe impairment of the mammary parenchyma that justified the removal of the animals from the production system and sending them for slaughter.

The necrosuppurative pattern was characterized by acute lesions mainly associated with coliforms (Hazlett et al. 1984; Markey et al. 2013b). *E. coli* and *Klebsiella* sp. produce endotoxins that cause tissue changes through vascular damage, edema, hemorrhage, and thrombosis, similarly to those observed in this study. Such agents may cause the death of the animal in some cases of environmental mastitis (Schiefer et al. 1976; Hazlett et al. 1984; Schlafer and Foster 2016; Foster 2017).

No bacterial growth in the microbiological culture was observed in all cases of granulomatous mastitis. Only three of these cases, the association of macroscopic, histological, and histochemical findings allowed the identification of mycobacteria, probably *Mycobacterium bovis*, since fast-growing mycobacteria such as *M. smegmatis* and *M. goodii* are not detected by lactoculture (Markey et al. 2013b). Mammary tuberculosis develops slowly with progressive enlargement of the gland which becomes firm. However, most of the time, there is no formation of classic miliary lesions as in other organs (Schlafer and Foster 2016), and this is consistent with the findings of this study.

The high number of mammary quarters with granulomatous lesion without identifiable etiology is similar to idiopathic granulomatous mastitis described in humans, where pathogenesis is not fully elucidated. Hypotheses put forward to explain the etiology of idiopathic granulomatous mastitis include trauma, similar to that observed in the cases of testicular sperm granulomas and granulomatous thyroiditis, as well as hormonal imbalances. Hyperprolactinemia and imbalance in the estrogen-progesterone ratio may lead to increase in protein secretion causing ectasia and rupture of the alveoli and ducts with extravasation of this secretion and consequent development of granulomatous inflammation (Altintoprak et al. 2014).

The results allow us to conclude that mixed, lymphoplasmacytic, and suppurative mastitides were the main histopathological patterns observed with involvement of *Streptococcus* spp., CNS, *S. aureus*, *S. agalactiae*, *S. uberis*, and *C. bovis*. The pyogranulomatous pattern presented different forms depending on the agent involved and was primarily associated with *S. aureus* and *Nocardia* sp. The cases of abscedative mastitis were characterized by extensive destruction of the mammary parenchyma predominantly caused by *T. pyogenes*. The necrosuppurative pattern was characterized by acute lesions predominantly associated with environmental bacteria producing endotoxins, such as *E. coli*. Granulomatous mastitis had the lowest frequency of cases and was occasionally associated with *Mycobacterium* sp.

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Compliance with ethical standards

Statement of animal rights The project that gave rise to the present data was approved to the Research Committee (COMPESQ) of the Universidade Federal do Rio Grande do Sul (UFRGS) (Project number 33527). The manuscript does not contain clinical studies or patient data.

Conflict of interest The authors declare that they have no conflicts of interest.

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