

STRAINS OF *Staphylococcus aureus* AND PATHOLOGY ASSOCIATED WITH NATURAL CHRONIC AND EXPERIMENTAL ACUTE SUPPURATIVE MASTITIS

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ABSTRACT

Staphylococcal mastitis is one of the main causes of culling of adult rabbit does from commercial rabbitries. The aims of this work were: 1) to provide a detailed description of the spectrum of gross and microscopic lesions observed in chronic natural cases of staphylococcal mastitis in adult does, 2) to determine whether there is a correlation between the different *Staphylococcus aureus* genotypes and the pathology observed and 3) to study the host immune response after an experimental infection with this bacterium.

The results showed that the different lesions observed were independent of the bacterial genotype. A broad spectrum of different pathological stages could be established based on the histomorphological characteristics and the cellular composition of the lesions. There were two major types of pathologies, one focused on the mammary gland structures (with variations which could suggest possible stages of infection) and one focused on periglandular tissue. Mammary glands were histologically grouped into four different types: abscesses (66.3%), suppurative mastitis (lobular pattern) (7.9%), cellulitis (19.6%) and mixed (6.2%). In the experimental infection, the important role of the individual immune response was considered after observing that animals inoculated with the same bacterial strain showed histological lesions of different level of severity.

Key words: *Staphylococcus aureus*; Mastitis; Rabbit; Natural infection; Experimental infection

INTRODUCTION

Staphylococcus aureus is an adaptable, opportunistic pathogen able to persist and multiply in a variety of environments causing a wide spectrum of diseases both in humans and animals (Cucarella *et al.*, 2004). In rabbits, this bacterium causes dermal lesions and invades subcutaneous tissues, causing different lesions such as pododermatitis, abscesses or mastitis (Vancraeynest *et al.*, 2004; Corpa *et al.*, 2009). Although staphylococcal mastitis is one of the main reasons for culling adult rabbit does from rabbitries (Segura *et al.*, 2007; Rosell and de la Fuente, 2009), specific studies focused on this pathology in industrial rabbits are scarce. There are many genotypes of *S. aureus* able to produce many different pathologies in rabbits, even though it has not been possible to correlate these pathologies with independent bacterial genotypes (Viana *et al.*, 2007). Hence, it is possible to postulate that the host immune response plays an important role in the development of these pathologies. Therefore, the first objective of this study was to provide a detailed description of the spectrum of gross and microscopic lesions of chronic staphylococcal mastitis in adult rabbit does and to determine if there is a correlation between *S. aureus* different genotypes and the severity of the pathological findings. The second objective was to develop an experimental infection using different genotypes of *S. aureus* in order to characterize the host immune response during early stages of mastitis. To this end, experimental infections were matched for variable factors, such as the duration and the route of the infection.

MATERIALS AND METHODS

Natural mastitis

Animals

One hundred and seventy eight adult rabbit does (*Oryctolagus cuniculus*) with chronic clinical mastitis were studied. Rabbits were euthanized by an intravenous injection of barbiturate (Dolethal. V  toquinol SA).

Histological examination

After post-mortem examination, mammary glands and any other organs or tissues showing gross lesions were routinely processed for histological examination and stained with haematoxylin and eosin and by Gram's Method. Lesions of the mammary gland were classified into abscesses, suppurative mastitis with a lobular pattern, cellulitis and mixed lesions.

Bacteriological procedures

One *S. aureus* strain isolated from one mammary gland from 178 rabbits was inoculated onto blood agar (BioM  rieux) and incubated aerobically at 37   C for 24-48 h. Several colonies from each positive isolate were chosen for genotypic characterisation of *S. aureus* strains (Viana et al. 2007).

Experimental mastitis

Animals

Experimental infection was performed on the mammary gland of 34 multiparous rabbit does from the same farm. The animals were inseminated in the farm and transported to the faculty one week before giving birth.

Infection protocol

The experimental infection protocol was based on protocols of infections previously described (Adlam et al. 1976; Amorena et al.1991). In the experimental procedure, 1000 CFU of different strains of *S. aureus* dissolved in 0.5 ml PBS were inoculated in the mammary gland (Table 1). The rabbits were inoculated two days postpartum and young rabbits were kept with their mothers. Prior to inoculation, milk samples were taken in order to verify the absence of bacteria. The mammary glands were disinfected with chlorhexidine 2% and the inoculation was performed with a hypodermic needle (25G) at the base of the nipple. Three glands were alternatively inoculated (left cranial, right middle and caudal left). The contra lateral mammary glands were used as controls (cranial right, left middle and right caudal) and were inoculated with 0.5 ml of PBS. Inflammation (swelling and induration) for each gland and rectal temperature were reported at 24 and 48 hours post-inoculation. Prior to euthanizing at 48 hours, a sample of milk from each infected gland was again taken in order to confirm the presence of the inoculated strain.

Table 1: Strains used in the experimental mastitis

Genotype	Number rabbits inoculated
A1/III/��	7
A1/III/��	3
B1/I/��	3
B1/IV1/��	3
B1/IV2/��	3
B2/IV1/��	4
C1/I/��	4
C1/II2/��	4
D1/IV1/��	3

Pathological studies

Rabbits were euthanized by intravenous inoculation (marginal ear vein) of an overdose of barbiturate (Dolethal. V  toquinol SA). Then, each mammary gland was evaluated both macroscopically and microscopically. The pathological findings were classified as severe, moderate or mild injuries, considering the degree of inflammation.

RESULTS AND DISCUSSION

Histological lesions in mammary glands with chronic mastitis were classified into four main types: **Abscesses** were observed in 66.3% does and consisted of one or several well-demarcated abscesses of variable sizes. The abscesses were delineated by a thick, compact fibrous capsule (*Compact abscesses*, $n = 39$ cases) (Fig. 1); a loose fibrovascular capsule infiltrated by heterophils, lymphocytes, plasma cells and macrophages (*Non-compact abscesses*, $n = 41$) (Fig. 2) or a thin band of connective tissue (*Non-encapsulated abscesses*, $n = 38$) (Fig. 3). These lesions appeared as three different stages of the same process: compact encapsulated abscesses were the most mature and controlled lesions; non-compact encapsulated abscesses were an intermediate phase and non-encapsulated abscesses were the previous and most active stages. **Suppurative mastitis** was observed in 7.9% does and consisted of non-encapsulated areas of inflammation, with necrosis and bacterial colonies, related to lobules of glandular tissue (Fig. 4). This lesion may either be a very early stage of the non-encapsulated abscess lesion type or the evidence of the immune system incompetence that is not able to surround and control the inflammatory process; therefore, bacteria evade it and invade new locations of the mammary gland. **Cellulitis** was observed in 19.7% does and consisted of a broad band of inflammatory tissue surrounding the mammary gland, often between the skin and the mammary tissue, extending into the subcutaneous tissue and sometimes to the abdominal musculature (Fig. 5). Skin lesions were observed grossly in 16 does with cellulitis type mastitis. The development of cellulitis type mastitis may be due to detected lacerations in the skin since percutaneous entries of infection into the mammary gland have been previously reported (Foster, 2007). The mammary glands with two or more of the histological features described above were classified as **mixed lesions** (Fig. 6). Four rabbit does showed a lobular pattern of suppurative mastitis with abscessation. Seven rabbit does showed a lobular pattern of suppurative mastitis accompanied by cellulitis in the surrounding tissue.

On the basis of molecular characterisation of *S. aureus*, 19 genotypes were identified. The most frequent genotype, A1/III1/δ, was isolated from 56.7% of the animals. There was no clear relation between lesions and strains at mammary gland, indicating that different lesions are not necessarily related to the virulence of strains. This fact reveals the important role of the host immune response for the development of a severe, moderate or mild lesion.

With the aim of characterizing the rabbit does immune response, an experimental infection was carried out using nine different genotypes of *S. aureus*. Four genotypes produced severe injury: A1/III1/δ, B2/IV1/δ, C1/II2/γ and D1/IV1/α. Generally, rabbits infected with these strains had fever and the infected glands appeared as a thickening or induration of the mammary tissue. Two to eight centimeters in diameter nodules were reported in the mammary glands of the rabbit does, as well as darkened skin, edema and necrosis (Fig. 7). Histopathological affected mammary glands showed hyperemia, edema and necrosis. Strains B1/II1/α and A1/III1/δ caused macroscopic lesions in 4 out of 6 animals that were infected, ranging from mild to moderate mastitis. Mild thickening of the mammary tissue and induration of the gland was observed, although none of them showed nodules. These macroscopic alterations were reflected microscopically in an increased number of heterophils, especially near the nipple, with a small area of hyperemia and necrosis.

Strains B1/IV1/α, B1/IV2/α and C1/II1/β produced no macroscopic and microscopic alterations or were very mild. Moreover, not all rabbits infected with these strains showed the same level of severity in their lesions. One third of animals infected with strains A1/III1/δ, B2/IV1/δ, C1/II2/γ and D1/IV1/α showed mild lesions (Fig. 8). Therefore, there were strains that produced severe injuries, while others did not produce any alteration. Moreover, there was variability between rabbits infected with the same strain. These results demonstrate the importance of the characteristics of the strain and the host immune response.

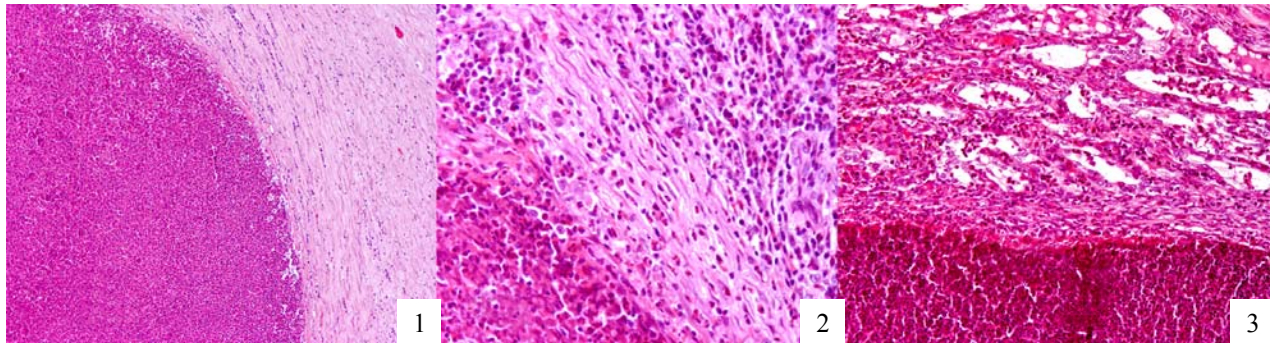


Figure 1-3. Abscesses type mastitis. **1.** Compact abscess. **2.** Non-compact abscess. **3.** Non-encapsulated abscess.

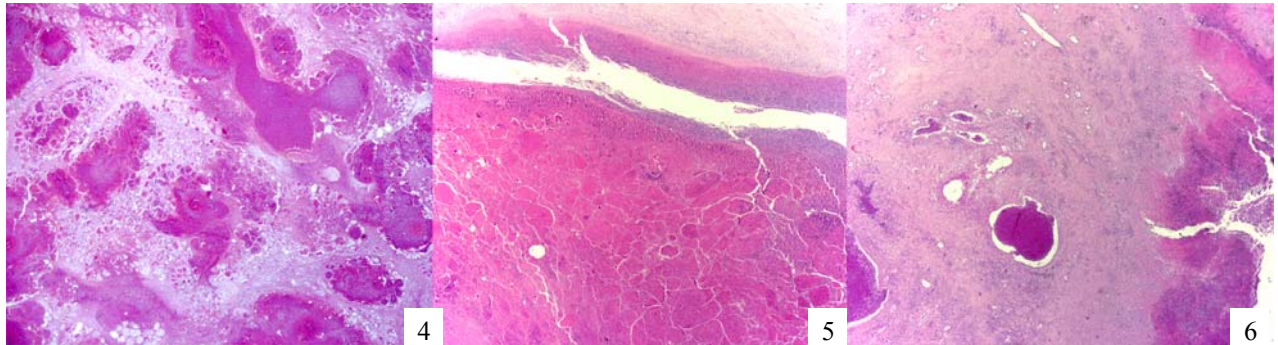


Figure 4. Suppurative mastitis (lobular pattern).

Figure 5. Cellulitis and necrotising mastitis.

Figure 6. Mixed lesion, with a necrotic band surrounding the mammary gland and abscesses.

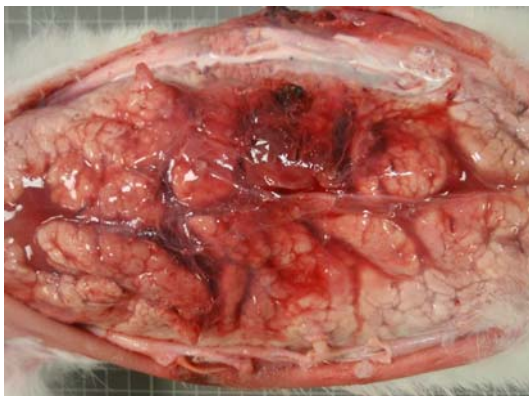


Figure 7: Mastitis showing severe injury with hyperemia, edema and necrosis 48 hs post inoculation of strain A1/III/δ



Figure 8: Right mammary gland showing mild inflammation 48 hs post inoculation of strain A1/III/δ, next to a non-infected mammary gland

CONCLUSIONS

Chronic mastitis caused by *S. aureus* in rabbits could be classified according to pathological findings into abscesses, suppurative mastitis with a lobular pattern, cellulitis and mixed lesions. Different types of lesions were not related to genotypic differences among strains suggesting that these patterns may be related to time and route of infection and/or to host immune response. In experimental infection, the results showed that infection resulted in acute mastitis. There was variability between strain virulence and the inflammatory response of animals infected with the same strain, which demonstrates the importance of the characteristics of the strain as well as probably of the host immune response.

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