

THE SPECTRUM OF PATHOLOGY ASSOCIATED WITH NATURAL CHRONIC STAPHYLOCOCCAL MASTITIS IN RABBITS

Viana D.¹, Selva L.¹, Callanan J.J.², Segura P.¹, Corpa J.M.^{1*}

¹Departamento Atención Sanitaria, Salud Pública y Sanidad Animal (Histología y Anatomía Patológica), Facultad de Ciencias Experimentales y de la Salud, CEU-Cardenal Herrera University, Edificio Seminario, s/n, 46113 Moncada (Valencia), Spain

²Conway Institute of Biomolecular and Biomedical Research, Veterinary Pathology, School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Republic of Ireland

*Corresponding author: jmcorma@uch.ceu.es

ABSTRACT

Staphylococcal mastitis is the main cause for culling adult does from rabbitries. However very few scientific reports study this condition in rabbits. The objectives of this work were to improve the mammary pathological knowledge in natural cases of chronic mastitis through a histomorphological classification and to compare the differences between the lesions caused by several *S. aureus* genotypes. In order to define the spectrum of gross pathology changes, the macroscopical characteristics of the mammary glands of 130 does with chronic mastitis were recorded. The number of glands was registered, which varied between 8 and 10 mammary glands per animal. The incidence of affection of the different glands was also studied. Mastitis cases were classified according to defined histopathological criteria (abscess-type, rosette-type, sandwich-type and mixed-type). The abscess-type was the most frequently diagnosed (64.4% of the animals). This type was characterized by the presence of one (unifocal) or several (multifocal) well differentiated abscesses of variable size. The purulent material consisted mainly in heterophils of different grades of degeneration and of debris, together with large clumps of Gram-positive bacteria. The rosette-type was characterized by the presence of large non-encapsulated inflammatory areas, in which there were abundant necrosis and bacterial colonies. Other smaller non-encapsulated secondary areas existed in the periphery as satellites of the previous ones. The sandwich-type lesion was characterized by a broad band of inflammatory tissue in the periphery of the mammary gland that extended to the subcutaneous tissue and sometimes to the abdominal muscles. The mixed-type lesion had two histological characteristics, as described above. The most frequent strain to be isolated from lesions was genotype A1/III/δ. However, there was no clear relationship between different genotypes and the histopathological type of mastitis.

Key words: *Staphylococcus aureus*, Mastitis, Rabbit, Pathology, Natural and experimental infections.

INTRODUCTION

Staphylococcal infections cause substantial economic losses in livestock industry worldwide. In rabbits this bacterium results in dermal lesions and invades subcutaneous tissues, causing different lesions such as mastitis, abscess or pododermatitis (Okerman *et al.*, 1984; Vancraeynest *et al.*, 2004). Staphylococcal mastitis is the main gross lesion for which adult does from rabbitries are culled (Segura *et al.*, 2007). However there are very few specific studies which focus on this condition in industrial rabbits (Adlam *et al.*, 1976). This scarcity of scientific studies is a problem because there is not enough information about this important condition in rabbits. Therefore, the objectives of this study were (1) to improve the mammary pathological knowledge in natural cases of chronic mastitis through a histomorphological classification using a large number of samples from numerous industrial farms; and (2) to compare the differences between lesions caused by several *S. aureus* genotypes.

MATERIALS AND METHODS

Naturally infected rabbits

A total of 185 adult female rabbits (*Oryctolagus cuniculus*), between 6 and 34 months old, were used for bacteriological studies. Of them 130 were studied macroscopically, and histological studies were carried out in 87 of them. They had been discarded from the farms by owners because of chronic mastitis. The animals came from 36 industrial rabbitries with signs of chronic staphylococcosis.

Pathological studies

Rabbits were euthanized by an intravenous injection of barbiturate. After a complete necropsy, mammary glands and any gross lesions were recorded. Tissues were processed histologically and stained by haematoxylin and eosin (HE) and Gram's methods.

Bacteriological procedures

A total of 198 rabbit *S. aureus* samples, obtained from mammary glands, were included. In some animals where more than one mammary gland was affected, several samples were taken to detect different strains. Standard microbiological studies were performed from the mastitis observed in the animals. For each positive gland, several colonies were chosen for further analysis.

Genotyping

Staphylococcal chromosomal DNA was extracted using a Genelute Bacterial Genomic DNA Kit (Sigma) according to the manufacturer's protocol, except that the bacterial cells were lysed by lysostaphin (Sigma; 12.5 µg/ml) at 37°C for 1 hour before DNA purification. Molecular typing, based on the analysis of the polymorphic regions of the *coa*, *spa* and *clfB* genes, was carried out as previously described (Viana *et al.*, 2007).

Statistical analysis

Differences between frequencies of affection of the mammary glands were analyzed by the Chi-square test, and the Yates' correction was considered when only a single degree of freedom was concerned.

RESULTS

All does showed chronic purulent mastitis affecting one gland or more. The affected glands appeared as a thickening or induration of the mammary tissue around or near one teat or more. Sometimes, studied does developed well demarcated abscesses in the mammary tissue of 1 to 12 cm in diameter. In order to define the spectrum of gross pathology changes, the macroscopical characteristics of the mammary glands of 130 does with chronic mastitis were recorded. The number of glands of the 130 animals was registered, and this varied between 8 and 10 mammary glands per animal. Forty-nine, 43 and 38 animals showed 8, 9 and 10 glands, respectively. In 55 animals, only one gland was injured and in 48, 14 and 13 animals, two, three and four mammary glands or more were affected, respectively. Finally, the frequency of affection of the different glands was studied (Table 1).

Table 1: Chronic mastitis affection of mammary glands in different anatomical locations

	Right row	Left row	Total
First couple (cranial)	17 (6.5%)	8 (3.2%)	25 (9.7%)
Second couple	25 (9.9%)	28 (11.1%)	53 (21%)
Third couple	43 (17%)	37 (14.6%)	80 (31.6%)
Fourth couple	28 (11.1%)	34 (13.4%)	62 (24.5%)
Fifth couple (caudal)	22 (8.7%)	11 (4.3%)	33 (13%)
Total	135 (53.4%)	118 (46.6%)	253 (100%)

Note: Data are shown in absolute numbers with percentages in brackets

In 87 rabbit does, histopathological analyses from the affected mammary glands were carried out. Mastitis cases were classified according to defined histopathological criteria (abscess-type, rosetta-type, sandwich-type and mixed-type) (Table 2). All mastitic glands presented chronic purulent inflammation characterized by the presence of large numbers of heterophils within the alveolar spaces. Some alveoli were packed with these cells, while others appeared normal or contained only a few cells. In old established lesions, large areas of the gland had become necrotic, and large clumps of bacteria were visible in both the alveoli and interstitium.

Table 2: Different types of natural mammary lesions and characterized genotypes

Genotype	¹ Abscess		Rosette	Sandwich		² Mix		Total	
	Encapsulated			Complete	Incomplete	EA/R	S/R		
	Compact	Non-compact							
A1/III/δ	14 (M)	9 (M)	15 (10M+5U)	8	2	6	1	3	58
A1/III/ε	-	1 (M)	-	-	-	-	-	-	1
A1/III/η	-	1 (U)	1 (U)	-	-	1	-	-	3
A1/III1/δ	1 (U)	-	2 (M)	1	-	-	-	-	4
A2/III2/δ	-	-	1 (M)	-	-	-	1	-	2
B1/I1/α	-	1 (M)	-	-	1	-	-	-	2
B1/I1/δ	-	-	1 (U)	-	-	-	-	-	1
B1/IV1/α	1 (M)	-	1 (U)	1	-	-	-	1	4
B1/IV2/α	1 (U)	-	-	-	-	-	-	-	1
B1/IV2/β	-	-	1 (M)	-	-	-	-	-	1
B2/IV1/δ	1 (M)	-	-	-	-	-	-	-	1
C1/I1/β	1 (U)	-	-	-	1	3	-	-	5
C1/I1/γ	-	-	-	-	1	-	-	-	1
D1/IV1/α	1 (U)	-	1 (U)	-	-	-	-	-	2
F1/III1/δ	-	-	1 (M)	-	-	-	-	-	1
³ Total	20	12	24	10	5	10	2	4	87
	(5m/15nm)	(5m/7nm)	(6m/18nm)	(6m/4nm)	(4m/1nm)	(6m/4nm)	(1m/1nm)	(4m/0nm)	(37m/50nm)

¹Abscesses: (M): Multifocal, (U): Unifocal; ²Mix: EA/R: Histological characteristics of Encapsulated Abscesses and Rosette; S/R: Histological characteristics of Sandwich and Rosette; ³Presence of milk in alveoli: Milk (m), No milk (nm)

Abscess-type

This was the most frequently diagnosed type observed in 56 out of 87 animals (64.4%). This presentation was characterized by the presence of one (unifocal) (14 cases) or several (multifocal) (42 cases) well differentiated abscesses of variable sizes. The purulent material consisted mainly of heterophils of different grades of degeneration and of debris, together with large clumps of Gram-positive bacteria. In 20 animals, abscesses were well delineated by a thin, mature and compact fibrous capsule (Figure 1a). In 12 cases however, abscesses showed a loose, non-compact fibrous capsule infiltrated by lymphocytes, plasma cells, macrophages, fibroblasts and prominent congested blood vessels (Figure 1b). In 24 cases, abscesses were not encapsulated, but were bordered by a thin band of connective tissue that had undergone acute necrosis.

Rosette-type

Unlike the abscess-type, the rosette-type was characterized by the presence of large non-encapsulated inflammatory areas, in which there were abundant necrosis and bacterial colonies. Other smaller non-encapsulated secondary areas existed in the periphery, like satellites of the previous ones, but they usually involved an alveolus where there was occasional glandular epithelial necrosis. This gave the lesion a rosette-like appearance (Figure 1c). Ten animals (11.5%) presented this type of lesion.

Sandwich-type

This type of lesion was observed in 15 out of 87 animals (17.2%). It was characterized by a broad band of inflammatory tissue in the periphery of the mammary gland (usually between the skin and mammary gland), and extended to the subcutaneous tissue, and sometimes to the abdominal muscles. The inflammation either partially (10 cases) or totally (5) surrounded the gland. When inflammation completely surrounded the gland tissue, extensive coagulative necrosis with numerous Gram-positive bacteria was detected within the gland (Figure 1d). Skin lesions were observed in 3 animals.

Mixed-type

These mammary glands had two histological characteristics, as described above. In two animals a mixture of encapsulated abscesses and rosettes were seen, while a mixture of sandwich- and rosette-type lesions were noted in four does.

Molecular characterization of rabbit staphylococcal strains

A PCR-RFLP analysis using the *coa* and *spa* genes specific primers, as well as a PCR analysis of the *clfB* gene of 198 rabbit isolates from 36 herds, were performed to characterize the different

staphylococcal isolates. Collectively, typing using the *coa*, *spa* and *clfB* genes resulted in 17 genotypes (A1/III1/δ, A1/III1/ε, A1/III1/η, A1/III1/δ, A2/III2/δ, B1/I1/α, B1/I1/δ, B1/III1/δ, B1/IV1/α, B1/IV2/α, B1/IV2/β, B2/IV1/δ, C1/I1/β, C1/I1/γ, C1/II2/γ, D1/IV1/α and F1/III1/δ). Genotype A1/III1/δ was the most frequently isolated (79.8%). Histopathological studies were carried out in 87 of the 198 analyzed mammary glands. Similarly in these samples, Genotype A1/III1/δ was most frequently isolated (66.6%) since it was isolated in 58 out of 87 processed samples (Table 2). Only two genotypes (B1/III1/δ and C1/II2/γ) were not identified in the samples from those animals in which histopathological studies were carried out. Apparently, there was no clear relationship between different genotypes and the histopathological type of mastitis, except in two occasions: (1) Interestingly, 33 out of 38 lesions classified as abscess types produced by genotype A1/III1/δ displayed a multifocal pattern. (2) Similarly, 4 out of 5 lesions where the genotype C1/I1/β was isolated were classified as sandwich (1 complete and 3 incomplete).

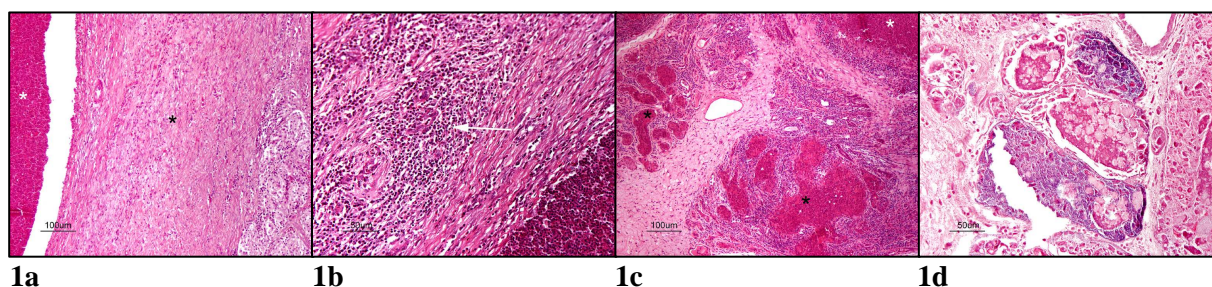


Figure 1: a) A well delineated abscess by a compact fibrous capsule (black asterisk) with abundant purulent material (white asterisk). H-E. Bar=100μm; b) Abscess with a loose non-compact fibrous capsule infiltrated by plasma cells and macrophages (arrow). H-E. Bar=50μm; c) Rosette-type lesion with a large, non-encapsulated area of necrosis (white asterisk) surrounded by numerous non-encapsulated secondary areas in the periphery where glandular epithelial necrosis can be observed (black asterisks). H-E. Bar=100μm; d) Sandwich-type mastitis characterized by an extensive coagulative necrosis with numerous Gram-positive bacteria in the lumen of the alveoli and interstitium. H-E. Bar=50μm

DISCUSSION

The number of teats was not predisposed to suffer mastitis. Both lines of teats were affected with the same frequency, but the cranial and caudal pairs of teats were less frequently inflamed compared with the third pair. This may be due to the fact that these teats are more protected against traumatism and to access of young rabbits by the fore and hind legs. The lactating mammary gland of the rabbit is susceptible to natural infection by staphylococci. Two types of disease occur. The first, which is rarely fatal, is typified by the development of pus-filled abscesses under or near to one teat or more (Adlam *et al.*, 1976). The second, sometimes called “blue-breast”, is a spreading condition in which the mammary tissue becomes oedematous and haemorrhagic, and is rapidly fatal (Adlam *et al.*, 1977). All the rabbit does studied in this work suffered chronic mastitis. Mammary gland infections were grouped into four different types: abscess, rosette, sandwich and mixed. The abscess-type lesion could be surrounded by a compact or loose fibrous capsule, or may not be encapsulated. These lesions show three different stages of the same process: the compact encapsulated abscesses would be the most mature and under control lesions; the non-compact encapsulated abscesses, an intermediate phase; and non-encapsulated abscesses would be the previous and most active stages. The rosette-type lesion would either be a very early stage of the abscess-type lesion or the reflection of the immune system not being able to control and surround the inflammatory process, leading to the bacteria evading it, thus invading new sites of the mammary gland. Although the teat canal is the most common route of infection into the mammary gland in cattle, percutaneous entries of infection into the mammary gland are also possible (Jubb *et al.*, 1993; McGavin *et al.*, 2001). Trauma to teats may cause a breakdown of this natural barrier and render the animal more susceptible to infections (Jones *et al.*, 1997), equally perturbations in any or all of the defence mechanisms (non-immune-mediated or immune-mediated),

and may increase susceptibility to mastitis (Jones *et al.*, 1997; McGavin *et al.*, 2001). It has been also reported that invasion may be percutaneous as a complication of local dermatologic conditions of the skin (Jubb *et al.*, 1993). In this study, the development of the sandwich-type mastitis may be due to a laceration in the skin (via skin wounds) by the kits during lactation, or by a traumatism in the cages with straw or other materials on the floor. Histologically, skin wounds were seen in some animals. The mixed-type was classified into two subtypes. The first has histological characteristics, which are common to the encapsulated abscess and rosette. This lesion could be due to a reactivation of the purulent process (abscess) or to a re-infection. Rabbit does have been reported to often become re-infected during a subsequent lactation (Adlam *et al.*, 1976). The second subtype has histological characteristics of both the sandwich- and rosette-types. These cases could be due to the inflammation extending to adjacent tissues: a sandwich-type that invades more deeply into the mammary gland, or a rosette-type that affects the peripheral mammary connective tissue and extends around the gland, under the skin and near the abdominal muscles. The strain that was most frequently isolated from the lesions was genotype A1/III/ δ . This broad distribution of a limited number of genotypes has been previously reported in rabbits (Hermans *et al.*, 2000; Viana *et al.*, 2007). However, the results do not show a clear relationship between the histopathological type of lesions and genotypes. It is, however, remarkable that 33 out of 38 mastitis cases classified as the abscess-type caused by genotype A1/III/ δ presented a multifocal distribution. This could be due to the fact that either this genotype has some characteristics that confer more virulence, or as is the most widespread in the studied geographical area (Segura *et al.*, 2007), it had more probabilities of re-infections. Equally, 4 out of 5 lesions where the C1/II/ β type was isolated were classified as sandwich (1 complete and 3 incomplete). Although the number of samples is low, this strain might be suspected of being a frequent skin colonizer that could affect the mammary gland after injury. More studies are necessary to confirm this asseveration. It has been reported that one strain may produce gangrenous mastitis on some occasions, and only a mild disease on others (Jubb *et al.*, 1993). Therefore, this pathological spectrum of mammary gland infection is the reflection of the fight between the bacteria and the animal's immune system.

ACKNOWLEDGEMENTS

This study was supported by grants from the CEU-Cardenal Herrera University (PRUCH02/25, PRUCH04/11, PRUCH05/09 and PRCEU-UCH01/08) and Generalitat Valenciana (GV05/202 and ARVIV/2007/007). The fellowship support for David Viana and Laura Selva from the CEU-Cardenal Herrera University is gratefully acknowledged. The authors acknowledge Dr. E. A. Gómez for statistical analysis

REFERENCES

- Adlam C., Thorley C.M., Ward P.D., Collins M., Lucken R.N., Knight P.A. 1976. Natural and experimental staphylococcal mastitis in rabbits. *J. Comp. Pathol.*, 86, 581-593.
- Adlam C., Ward P.D., McCartney A.C., Arbuthnott J.P., Thorley C.M. 1977. Effect immunization with highly purified alpha- and beta-toxins on staphylococcal mastitis in rabbits. *Infect. Immun.*, 17(2), 250-6.
- Hermans K., De Herdt P., Devriese L.A., Godard C., Haesebrouck F. 2000. Colonisation of rabbits with *Staphylococcus aureus* after experimental infection with high and low virulence strains. *Vet. Microbiol.*, 72, 277-284.
- Jones T.C., Hunt R.D., King N.W. 1997. Veterinary Pathology. 6^a ed. Williams & Wilkins.
- Jubb K.V.F., Kennedy P.C., Palmer N. 1993. Pathology of Domestic Animals. 4^a ed. Academic Press, Inc. San Diego, USA.
- McGavin M.D., Carlton W.W., Zachary J.F. 2001. Thomson's Special Veterinary Pathology. 3^a ed. Mosby Press.
- Okerman L., Devriese L.A., Maertens L., Okerman F., Godard C. 1984. Cutaneous staphylococcosis in rabbits. *Vet. Rec.*, 114, 313-315.
- Segura P., Martinez J., Peris B., Selva L., Viana D., Penades J.R., Corpa J.M. 2007. Staphylococcal infections are the main pathological culling causes of rabbit does in two industrial farms. *Vet. Rec.*, 160(25), 869-72.
- Vancraeynest D., Hermans K., Martel A., Vanechoutte M., Devriese L.A., Haesebrouck F. 2004. Antimicrobial resistance and resistance genes in *Staphylococcus aureus* strains from rabbits. *Vet. Microbiol.* 101, 245-251.
- Viana D., Selva L., Segura P., Penades J.R., Corpa J.M. 2007. Genotypic characterization of *Staphylococcus aureus* strains isolated from rabbit lesions. *Vet. Microbiol.*, 121(3-4), 288-98.

