

## Pathology of Acute Necrotizing Mastitis Caused by *Staphylococcus aureus* in a Dairy Cow

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### Abstract

Acute necrotizing mastitis in a cow was investigated pathologically. The udder was firmly enlarged to twice the size of a basketball. Histologically, the epithelium of the interlobular duct of the mammary glands was displaced by necrotic debris and bacterial clumps (Gram-positive cocci). The intralobular ducts were also necrotic. The alveoli showed scattered necrotic foci varying in size and containing bacterial clumps. The vascular walls of the interlobular stroma exhibited fibrinous necrosis with fibrinous thrombi. The liver showed centrilobular fatty and droplet degeneration of the hepatocytes. Extramedullary hematopoiesis was observed in the liver, spleen, adrenal glands, lungs and kidneys. The bacteria observed in the lesions of the mammary glands were stained positively with immunoperoxidase using an antibody against *Staphylococcus aureus*. Ultrastructurally, the bacteria were round or oval in shape and had thick cellular walls. Capsule-like fibrous material was present around the surface of the bacteria. Bacteriologically, *Staph. aureus* was isolated in pure culture from the mammary gland. These findings suggest that *Staph. aureus* caused the acute necrotizing mastitis. The pathogenesis of the disease was examined.

**Discipline:** Animal health

**Additional key words:** bovine mastitis

### Introduction

Bovine mastitis is caused by various bacteria (*Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Pasteurella*, *Brucella* and *Escherichia coli*), *Mycoplasma* and fungi (*Candida*)<sup>2,3,7</sup>. Gangrenous mastitis is caused mainly by *Staph. aureus*<sup>2,3</sup> and *E. coli*<sup>1-3</sup>. *Staph. aureus* and *Clostridium perfringens* were isolated from gangrenous mastitis<sup>6</sup>. *Staph. aureus* and *E. coli* are often present in the cow's skin and in the environment around the cow. Therefore the localization of these bacteria in the mammary lesions should be investigated in order to determine the relationship between the bacteria and the lesions. Although there are some reports concerning the bacteriology<sup>4,6</sup> or histology of the disease<sup>1,5</sup>, there are no reports on histological, immunohistochemical and ultrastructural features of gangrenous (necrotizing) mastitis.

This paper describes the histological, immunohistochemical and ultrastructural features of acute necrotizing mastitis caused by *Staph. aureus* in a cow.

### Materials and methods

An adult Holstein cow delivered a calf uneventfully. A few days after the delivery, the cow developed swelling of the udder, bloody diarrhea, pyrexia, difficulty in standing, and a positive reaction for the complement-fixation test of Johne's disease. Ten days after the delivery, the cow was sent to our laboratory for pathological examination.

The cow was euthanized with anesthesia for necropsy. The udder, liver, spleen, kidneys, lungs, heart, adrenal glands, stomach, small intestine, large intestine, and other tissues were collected, and fixed in 10% neutral-buffered formalin. The tissues were processed routinely and stained with hematoxylin and eosin (HE). Selected sections were stained with Azan

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stain, Gram stain and phosphotungstic acid hematoxylin (PTAH). The paraffin sections were stained using an avidin-biotin-peroxidase complex (ABC)-immunostaining kit (Vector Lab., U.S.A.). A rabbit antibody against *Staph. aureus* was used at 1:2,000 dilution. Mammary gland tissues were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmic acid, dehydrated with alcohol, and embedded in epoxy resin. Thin sections were stained with 1% uranyl acetate and Reynold's lead citrate, and examined with a transmission electron microscope (TEM). Attempts to isolate bacteria from the mammary glands, the granuloma in the liver and the colon of the cow were made by aerobically culturing the samples on sheep blood agar plates and desoxycholate-hydrogen sulfide-lactose (DHL) agar plates.

## Results

The udder (all 4 quarters of the udder) was firmly enlarged to twice the size of a basketball. Cheese-like material oozed from the lactiferous ducts of the teat papilla and the cut surface of the parenchyma. Encapsulated abscesses (about 2 cm in diameter) were

observed in the omentum and in the peritoneum in addition to pathological findings such as, nutmeg liver, stagnation of the bile in the gall bladder, subcutaneous gelatinous infiltration and jaundice, and congestion and hemorrhages in the small and large intestines. *Staph. aureus* was isolated in pure culture from the mammary glands of the cow.

In the mammary gland, interlobular ducts were severely affected. The epithelium of the interlobular duct was displaced by necrotic debris and bacterial clumps (Gram-positive cocci) (Fig. 1). The intralobular ducts of most of the lobules of the mammary glands were also necrotic. The alveoli showed scattered necrotic foci varying in size and containing bacterial clumps. In the small necrotic foci, bacterial clumps were surrounded by the alveolar epithelial cells showing necrosis. Fusion of such necrotic foci led to the formation of larger foci. The intact area of the parenchyma revealed hyperemia. The interlobular stroma showed edematous loosening with some neutrophils and mononuclear cells. The vascular walls of the interlobular stroma showed fibrinous necrosis with fibrinous thrombi (PTAH-positive) (Fig. 2).

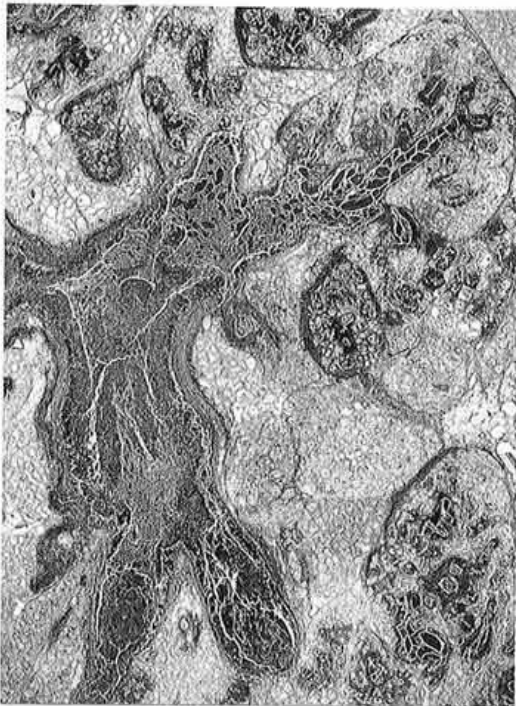


Fig. 1. Severe necrosis of interlobular and intralobular ducts

The lesions affected the interlobular duct, intralobular ducts and alveoli (Azan  $\times 30$ ).

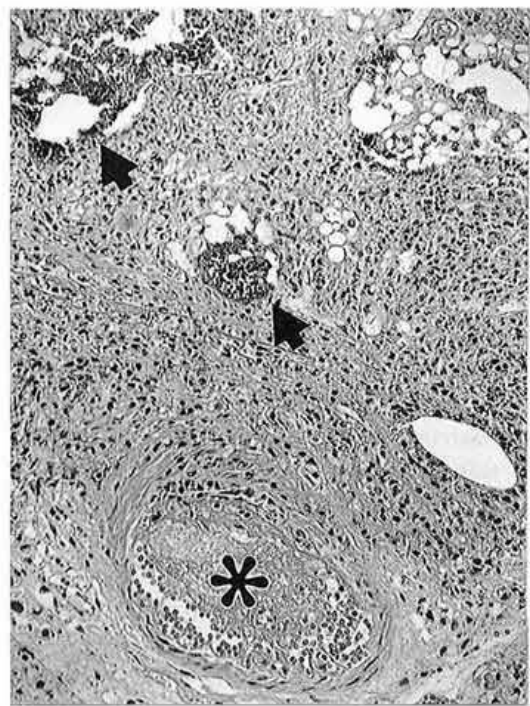


Fig. 2. Bacterial clumps (arrows) surrounded by alveolar epithelial cells undergoing necrosis

Thrombus(\*) is seen in the blood vessel (HE  $\times 100$ ).

In the liver, the hepatocytes underwent fatty and droplet (Fig. 3) degeneration. Occasionally, megakaryocytes, erythroblasts and granulocytes migrated into the sinusoids (Fig. 4). The spleen showed a marked congestion with megakaryocytes and granulocytes migrating into the red pulp. Severe migration of megakaryocytes, erythroblasts and granulocytes was observed into the blood capillaries of the cortex of the adrenal gland. Occasionally, megakaryocytes were seen in the alveolar walls of the lung and in the blood capillaries of the kidney. Granulomatous nodules, consisting of eosinophilic debris surrounded by multinucleated giant cells and macrophages with proliferation of the fibrous connective tissue, were noted in the pleura on the rib. Gram-positive, small, rod-shaped bacteria were seen in the debris which were present in the granuloma. The cocci observed in the lesions of the mammary glands were stained positively with ABC stain using an antibody against *Staph. aureus* (Fig. 5), unlike the rods in the granuloma of the pleura. In the mammary glands, the bacteria which were round or oval in shape and had thick cellular walls showed the characteristics of Gram-positive bacteria (Fig. 6-a). The size of the bacteria ranged from 0.8 to 1.0  $\mu\text{m}$  in diameter. Ruthenium-red-positive fibrous material

(capsule) was present around the surface of the bacteria (Fig. 6-b).

## Discussion

In the present case, the lesions of the mammary glands consisted of vascular necrosis with fibrinous thrombosis. These changes had been reported previously in staphylococcal mastitis<sup>3)</sup>. This vascular damage may have played a role in the acute coagulative necrosis of the mammary gland parenchyma observed in our case also. Acute staphylococcal mastitis occurs shortly after parturition<sup>3)</sup>. Extramedullary hematopoiesis in the liver, spleen, adrenal glands, lungs, and kidneys observed in the present case suggests that the cow may have been anemic, presumably due to parturition. The cow showed a positive reaction to the complement-fixation test of Johne's disease and had small nodules of encapsulated abscesses in the omentum and in the peritoneum. The stresses of parturition, anemia, and infection with another bacterium may have led to a higher sensitivity to staphylococcal infection.

Previously, researchers had examined histologically 47 cows from an abattoir, and mastitis was recorded in 36 cows<sup>5)</sup>, of which, 2 showed necrotizing

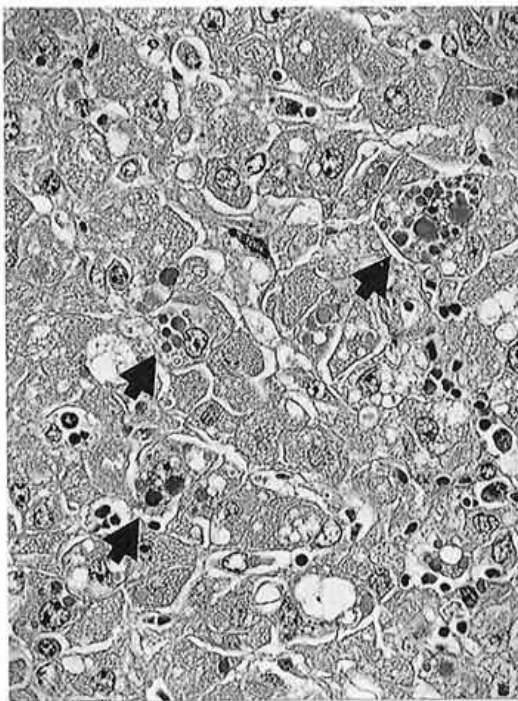


Fig. 3. Droplet (arrows) and fatty degeneration of hepatocytes (HE  $\times$  400)

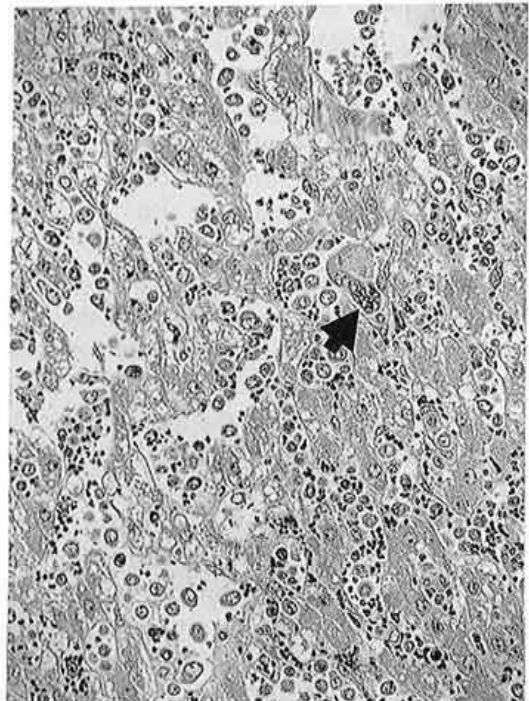


Fig. 4. Extramedullary haematopoiesis in the liver. Migration of megakaryocytes (arrow), erythroblasts and granulocytes into hepatic sinusoids (HE  $\times$  200).



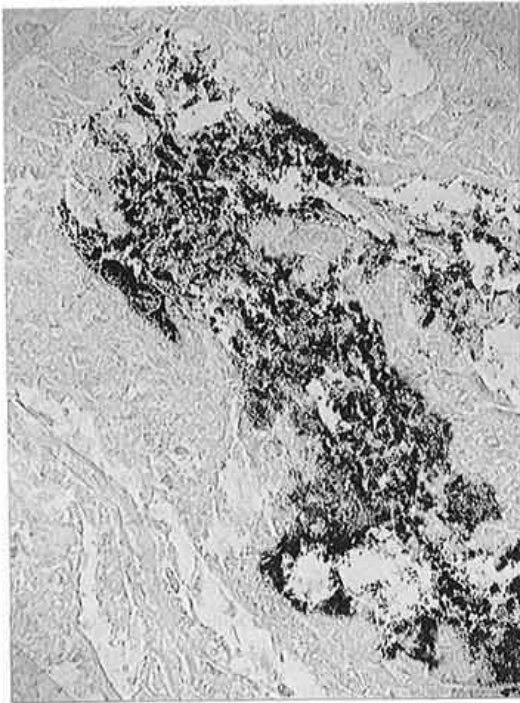


Fig. 5. The cocci in the lesions of the mammary glands show a positive reaction to antibody against *Staph. aureus* (ABC  $\times 200$ )

mastitis. Bacteriological investigation was not performed, but cocci were observed in the alveoli in that report<sup>5)</sup>. Necrotizing mastitis in cows from which *E. coli* were isolated has been reported<sup>1)</sup>. Although the etiological bacteria of mastitis have been usually identified by bacterial isolation from mastitis, there have been no immunohistochemical demonstrations of bacteria in mastitis. Etiological agents of mastitis are usually present in the skin of cattle and in the environment in which the cattle are housed. Immunohistochemical demonstration of the bacteria in the mastitis lesions is important to evaluate the pathogenicity of the bacteria isolated from the lesions. In our present study, the immunohistochemical detection of *Staphylococcus* in the necrotic foci of the mammary gland indicates that necrotizing mastitis may be caused by *Staph. aureus* infection.

We observed capsular materials (ruthenium-red-positive) in *Staph. aureus* within the necrotic lesions of the mammary glands. Encapsulated strains of *Staph. aureus* from bovine milk were isolated by Yokomizo & Isayama<sup>8)</sup>. Their report suggests that encapsulated *Staph. aureus* may play a role in the pathogenesis of bovine mastitis. Further studies on the relationship between necrotizing mastitis and encapsulated *Staph. aureus* should be carried out.

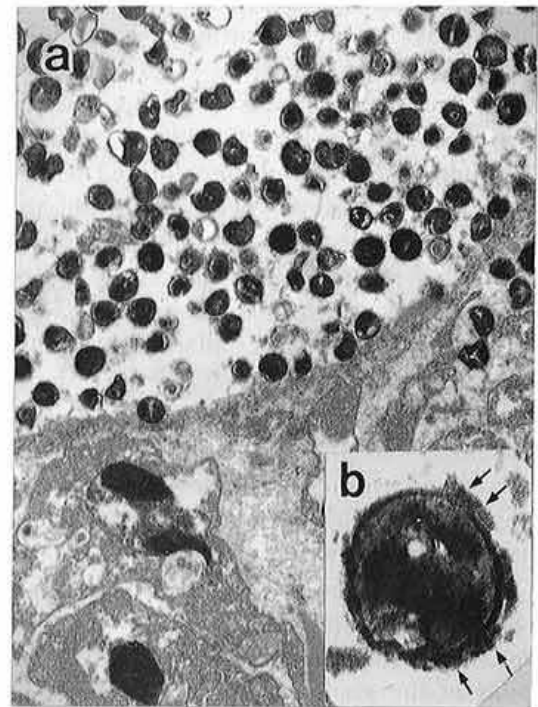


Fig. 6-a. The bacteria were round or oval in shape, showing a thick cell wall, characteristic of gram-positive bacteria (TEM  $\times 40,000$ )

6-b. Fibrous material (arrows) stained by ruthenium-red, around the bacterial cell wall, which forms a capsule (TEM  $\times 250,000$ )

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