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THE INVASIVENESS OF STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS EPIDERMIDIS FOR THE MAMMARY GLAND OF THE MOUSE

By

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ANDERSON, J. C. and O. HOLMBERG: *The invasiveness of Staphylococcus aureus and Staphylococcus epidermidis for the mammary gland of the mouse.* Acta vet. scand. 1977, *18*, 129—137. — The invasiveness of Staphylococcus aureus and Staphylococcus epidermidis for the mammary gland of the mouse was assessed by contaminating the damaged teats of suckling mice and sampling the mammary glands for the contaminating organism 48 hours later. Using this test system Staphylococcus aureus strain BB invaded 17 of 40 glands (42.5 %) and Staphylococcus epidermidis strain 279 invaded 2 of 40 glands (5.0 %). The histopathological changes in glands infected with Staphylococcus aureus were more severe than in those infected with Staphylococcus epidermidis.

bacterial invasiveness; mastitis; Staphylococcus; mouse.

In a previous study (*Anderson* 1972) the mouse model for mastitis was used to study the effect of teat damage on the incidence of experimental staphylococcal mastitis. It was found that the combination of teat damage, suckling and the presence of a pathogenic staphylococcus on the damaged teat was necessary to establish infection and inflammation. The combination of teat damage and suckling in the absence of pathogenic organisms resulted in a sterile inflammation. Mastitis was not induced by any of the remaining 6 combinations of the 3 variables.

In that study the same strain of Staphylococcus aureus was used throughout, and the results were interpreted in terms of the contribution of teat damage to the establishment of mastitis.

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However, by making teat damage and suckling constant factors and varying only the contaminating microorganism, it seemed possible that the invasiveness of potential pathogens could be assessed. At the same time the histopathological changes could be observed following an infection process which resembled that in natural cases of mastitis.

The invasiveness of a strain of Staphylococcus aureus and of Staphylococcus epidermidis was compared and the results are reported in this paper.

MATERIALS AND METHODS

Mice

Lactating NMRI mice bred at the National Veterinary Institute were used 4—7 days after parturition. Each mouse and her offspring was individually boxed.

Strains of staphylococcus

Strain BB of Staphylococcus aureus (Sa BB) is coagulase positive (tube test using rabbit plasma) and produces alpha and beta haemolysins on 5 % ox blood agar. Strain 279 of Staphylococcus epidermidis (Se 279) is coagulase negative (tube test using rabbit plasma), non-haemolytic and produces phosphatase but does not ferment mannitol. Further properties of Se 279 are described by *Holmberg* (1973). Sa BB was recovered from a cow with acute mastitis, and Se 279 was recovered from subclinical bovine mastitis.

Preparation of staphylococci

An 18-hr. growth of each strain on blood agar was suspended in sterile saline (0.15 M-NaCl) and washed 3 times in sterile saline. The organisms were finally suspended in 25 % (v/v) glycerol in sterile isotonic saline and standardized by the use of Opacity tubes (Burroughs Wellcome & Co) to yield $2—3 \times 10^{10}$ organisms per ml (*Miles et al.* 1938).

Experimental procedures

The experiments performed are outlined in Table 1. Teat damage was accomplished by removal of the tip of teats R4 and L4 (fourth glands on right and left sides) with scissors under ether anaesthesia, above the level of the teat canal. Bacterial

contamination was achieved by placing a 25 µl drop (Oxford Sampler, Boehringer Corporation (London) Ltd., Uxbridge Road, London) of the appropriate bacterial suspension on the teat and spreading it over the teat with a sterile wire loop. Each mouse was then returned to its offspring.

All mice were killed after 48 hrs. and R4 and L4 were treated similarly and separately. The glands were exposed by making a midline abdominal incision and reflecting the skin. Incisions were made with a sterile scalpel at 2 levels of each gland, and the incised tissue was sampled with a sterile wire loop. The samples were plated-out on blood agar and incubated for 24 hrs. at 37°C.

The organisms isolated from mammary glands were identified with the contaminating organisms by colonial morphology and haemolytic pattern in the case of Sa BB, and by morphology, Gram staining, coagulase test and the reaction in 17 "sugars" in the case of Se 279. A specimen of each gland was fixed in 12 % neutral buffered formalin for histological processing. Selected glands were embedded in paraffin wax, and sections (5 µm) were stained with Giemsa's stain and by Gram's method.

RESULTS

Contamination with Sa BB

Forty-eight hrs. after contamination of damaged teats with Sa BB 18 of 20 mice were clinically normal and 2 had rough coats and were not as alert as the other mice. Sa BB was recovered from 17 of 40 mammary glands (Table 1). In 10 of the 17 positive iso-

Table 1. Number of infected mammary glands from suckling mice 48 hrs. after contamination of damaged teats by Staphylococcus aureus strain BB or Staphylococcus epidermidis strain 279, and control groups.

Contaminating organism	Teat damage	Number of glands (Number of mice)	Number from which Sa BB was recovered	Number from which Se 279 was recovered	% glands infected
Sa BB	+	40 (20)	17	—	42.5
Se 279	+	40 (20)	—	2	5.0
none	+	20 (10)	0	0	
Sa BB	—	10 (5)	0	—	
Se 279	—	20 (10)	—	0	

lations the number of colonies per gland exceeded 50, and in all but 2 glands Sa BB was recovered in pure culture.

Histological examination was made of those glands from which staphylococci were recovered. The histopathological change common to all the infected glands was the presence of neutrophils in the alveolar lumen. Most of these neutrophils were morphologically normal, and occasionally staphylococci were seen within a neutrophil; staphylococci were not seen free in the alveolar lumen. In these areas of neutrophil infiltration excessive vacuolation was the only distinctive change in the epithelium. There were granulocytes and mononuclear cells in the interalveolar tissue (Fig. 1).

In 2 of the mammary glands, 1 from each of the clinically affected mice, there were areas of necrosis. The epithelial cytoplasm was eosinophilic and there was nuclear karryorhexis. In some alveoli the necrotic cells were fixed in situ as in coagulative necrosis, and in other areas much of the cytoplasm was lost and only the degenerate nuclei remained on the basement membrane

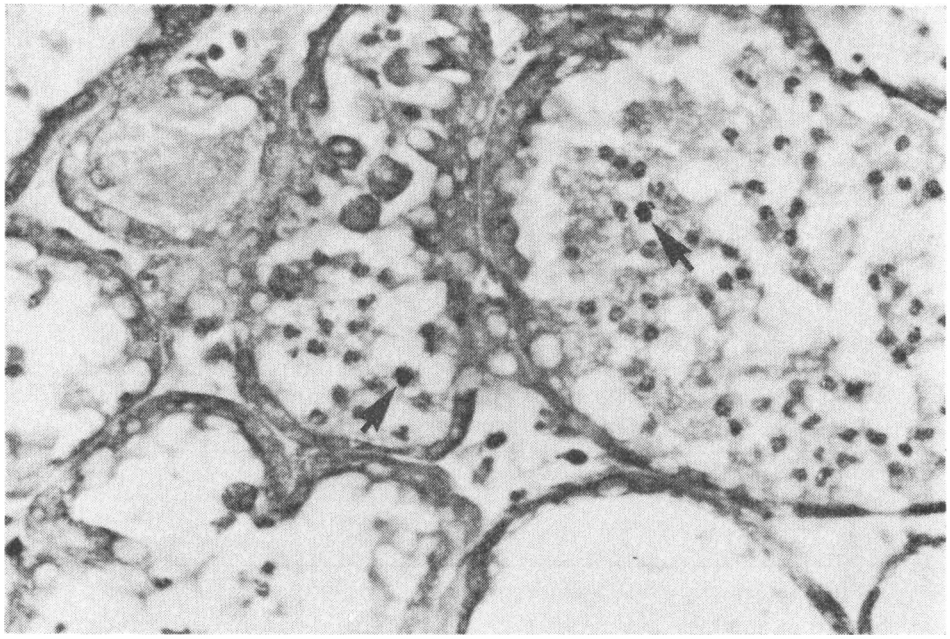

Figure 1. Mastitis following teat contamination with Sa BB. There are neutrophils and epithelial cells in the alveolar lumen. The arrowed neutrophils contain staphylococci. Gram × 438.

as in liquefactive necrosis. Irrespective of the type of necrosis the alveolar lumen was devoid of morphologically normal neutrophils but contained many staphylococci and cell debris which were randomly scattered throughout the lumen (Fig. 2).

In a further 3 of the infected glands early abscesses were found in 2 sites. Parenchymal abscesses consisted in a small focus of alveolar necrosis which was surrounded by alveoli in which there was an intense neutrophil response and which formed the wall of the abscess (Fig. 3). In ductal abscesses the focus of necrosis was found in the centre of a lactiferous duct which was tightly packed with neutrophils and cell debris; the duct wall formed part of the abscess wall.

Contamination with Se 279

Se 279 was recovered in pure culture from 2 of 40 mammary glands, and in each case the number of organisms isolated per gland exceeded 50. Neither of the mice showed any signs of illness.

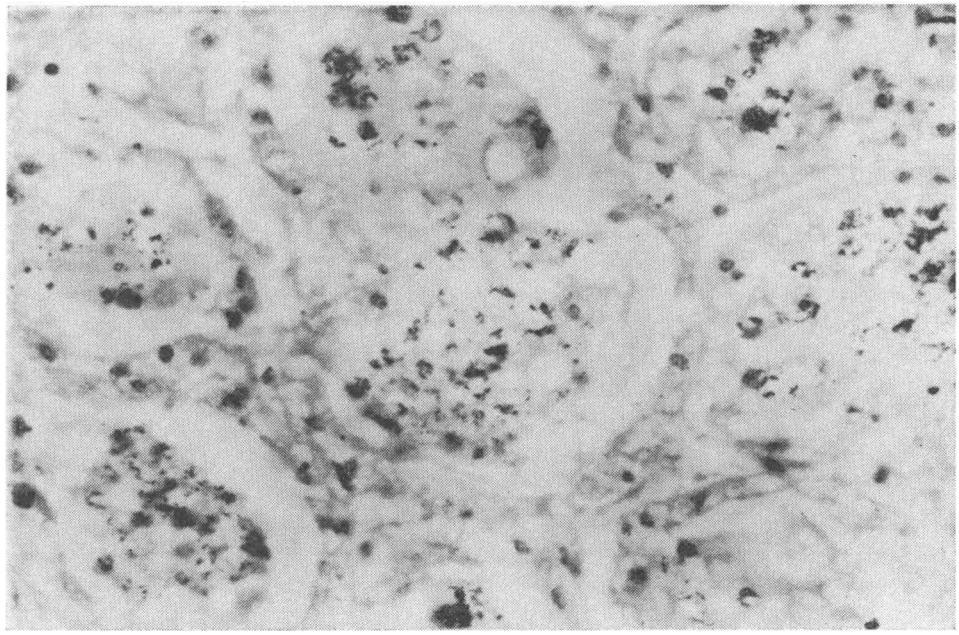

Figure 2. Mastitis following teat contamination with Sa BB. There is necrosis of the epithelium and numerous staphylococci but no neutrophils in the lumen of the alveoli. Giemsa × 438.

Histological examination of the infected glands revealed a neutrophil response in the alveoli, and occasionally staphylococci could be found within neutrophils (as in Fig. 1). The epithelium was normal and there was a mild influx of granulocytes and mononuclear cells into the interalveolar tissue.

Control experiments

The bacteriological results of control experiments are indicated in Table 1. Neither Sa BB nor Se 279 was recovered from mammary glands following teat contamination when there was no teat damage. Histological examination of randomly selected mammary glands from each control group showed that there was no histopathological change in the mammary gland in the absence of teat damage despite contamination by Sa BB or Se 279. Teat damage in the absence of contamination resulted in a mild neutrophil response in the alveoli.

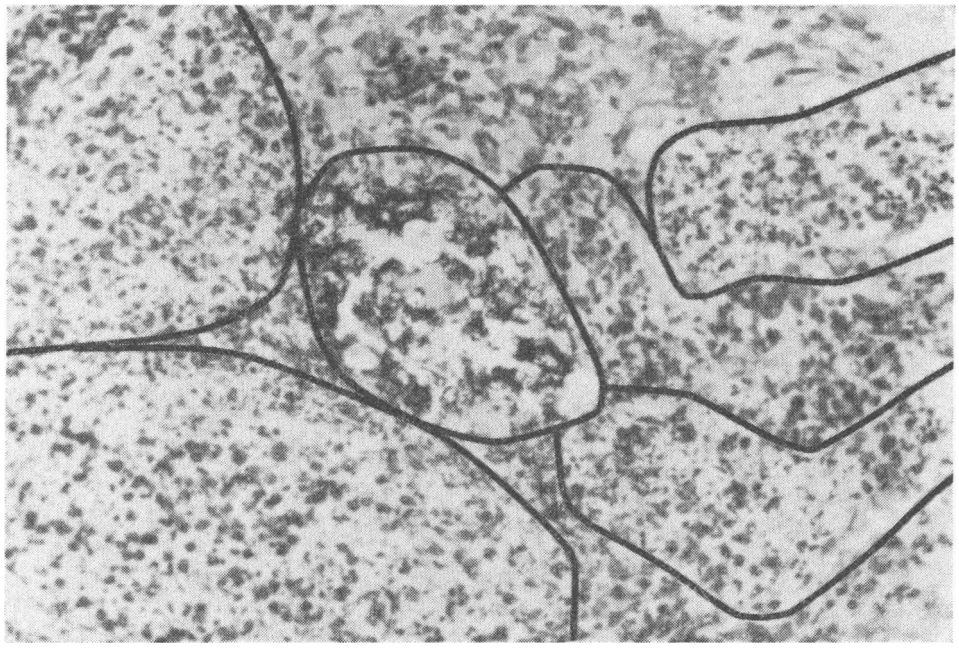

Figure 3. Mastitis and parenchymal abscess formation following teat contamination with Sa BB. The central alveolus contains masses of staphylococci and there are many cells in the adjacent alveoli (outlined) and interalveolar tissue. Giemsa × 656.

DISCUSSION

It is generally accepted that the normal route of infection in mastitis is through the teat and that infection comes about when the teat duct barrier is breached. It is possible under experimental conditions to by-pass the teat duct and place organisms in the teat cistern where they may multiply and induce mastitis. While this approach may yield information on the virulence of an organism, by by-passing the teat duct the experimentor may confer an advantage on an organism that it does not possess under natural conditions, and thus give a false impression of the invasiveness of the organism and the frequency with which it causes mastitis. For example, several strains of ureaplasma if inoculated into the teat cistern of the mammary gland of the mouse (*Howard et al.* 1975) or of the cow (*Gourlay et al.* 1972), will cause mastitis yet there is no evidence that ureaplasmas are responsible for natural cases of mastitis. In these circumstances a test of the invasiveness of a potential pathogen would be of value; the system described in this paper was developed in an attempt to provide such a test. If an organism which is placed on the surface of a damaged teat is isolated 48 hrs. later from the substance of the gland, then it may reasonably be said to have invaded and become established in the gland even though the mechanism of invasion is unclear. The invasiveness of an organism can then be expressed numerically according to the number of times invasion occurs within the conditions of the test system.

In this study the invasiveness of Sa and Se was assessed and compared. The histopathological changes associated with the presence of Se in the bovine udder have been described (*Stabenfeldt & Spencer* 1966, *Lee & Frost* 1970), mastitis has been induced in the cow by intramammary inoculation of Se (*Holmberg* 1973) and Se has been recovered from milk of cows said to have mastitis (*Sandvik & Brown* 1965) or to be free of mastitis (*Edwards & Jones* 1966), but there is no information on the invasiveness of Se for the mammary gland. Our results in the mouse show that Se 279 invaded and became established in 2 of 40 mammary glands (5.0 %). This indicates that Se is capable of successfully invading the mammary gland and although it may not do so frequently it must be seriously considered as a mastitis causing organism. On the other hand Sa is an undisputed cause of bovine mastitis, and in this mouse model Sa BB invaded and

became established in 17 of 40 mammary glands (42.5 %); Sa BB invaded the mammary gland 8.5 times more frequently than did Se 279.

The interpretation of the histopathological changes is complicated by the mild neutrophil response induced by teat damage alone and which the organisms must have overcome in those glands in which infection was established. However, the neutrophil response was more intense in the infected glands, and in some glands there were additional more advanced histopathological changes similar to those observed following intramammary inoculation of Sa BB in mice (*Anderson & Chandler* 1975). Among the infected glands necrosis and localized necrosis (abscesses) were found only in mammary glands in which Sa BB had been established, and only the relatively less severe neutrophil infiltration was found in glands infected with Se 279. This agrees with observations in the cow that histopathological changes associated with Sa are generally more severe than those associated with Se (*Stabenfeldt & Spencer* 1965, 1966, *Lee & Frost*).

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SAMMANFATTNING

Benägenheten hos Staphylococcus aureus och Staphylococcus epidermidis att invadera mjölkkörtlarna hos möss.

Benägenheten hos Staphylococcus aureus och Staphylococcus epidermidis att invadera mjölkkörtlarna hos möss studerades genom att skadade spenar hos diande djur kontaminerades, varefter körtlarna undersöktes bakteriologiskt 48 timmar senare. Staphylococcus aureus BB invaderade 17 av 40 körtlar (42,5 %) och Staphylococcus epidermidis 279 invaderade 2 av 40 (5,0 %). De histopatologiska förändringarna i körtlarna infekterade med Staphylococcus aureus var kraftigare än de som infekterats med Staphylococcus epidermidis.

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