

RESEARCH COMMUNICATION

SCANNING ELECTRON MICROSCOPY-AIDED OBSERVATIONS ON AND THERAPY OF TEAT CANAL INFECTIONS

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ABSTRACT

DU PREEZ, J. H., 1979. Scanning electron microscopy-aided observations on and therapy of teat canal infections. *Onderstepoort Journal of Veterinary Research* 46, 231-234 (1979).

An examination of teat canal swabs established that 51 teat canals out of 68 quarters of machine-milked cows were colonized by *Staphylococcus aureus*. Only 31 of these quarters yielded milk from which *S. aureus* could be cultured, and 6 out of the 31 produced milk containing somatic cell counts in excess of 500 000/mL.

No inhibitory substances could be detected in milk samples 12 h after 10 mg of sodium cloxacillin had been deposited in the teat canal on 1-4 successive occasions. Teat canal swabs and milk sample cultures of the same quarters became and remained bacteriologically negative for at least a week after the last treatment. Six quarters, which according to the International Dairy Federation criteria were suffering from subclinical mastitis, became negative after local teat canal therapy.

Scanning electron micrographs of one infected teat canal revealed the presence of cocci in depressions and crevices on the epithelial surface, suggesting that such cocci are not always flushed out into milk samples.

Teat canal therapy should make a marked contribution to the control of bovine mastitis.

Résumé

OBSERVATIONS SUR LA MICROSCOPIE ÉLECTRONIQUE À BALAYAGE ET LA THÉRAPIE DES INFECTIONS DU CANAL MAMELLAIRE

Un examen des sécrétions des canaux mamellaires a établi que 51 de ces canaux sur 68 quartiers de vaches traitées à la machine étaient colonisés par le *Staphylococcus aureus*. Seulement 31 de ces quartiers produisirent un lait à partir duquel *S. aureus* a pu être isolé et 6 de ces 31 quartiers produisirent un lait contenant des comptes de cellules somatiques en excès de 500 000/mL.

Aucune substance inhibitrice ne put être détectée dans les échantillons 12 heures après avoir déposé dans le canal mamellaire 10 mg de cloxacilline de sodium à raison de 1-4 fois successivement. Les sécrétions du canal mamellaire et les cultures d'échantillons de lait des mêmes quartiers devinrent et demeurèrent bactériologiquement négatifs pour au moins une semaine après le dernier traitement. Six quartiers qui, suivant les critères de la Fédération Laitière Internationale, souffraient de mastitis subclinique, devinrent négatifs après une thérapie locale du canal mamellaire.

Les micrographies électroniques à balayage d'un canal mamellaire infecté révélèrent la présence de cocci dans les dépressions et les crevasses sur la surface épithéliale, ce qui suggère que de tels cocci ne sont pas toujours évacués dans les échantillons de lait.

La thérapie du canal mamellaire devrait fournir une contribution considérable dans le traitement de la mammite bovine.

INTRODUCTION

Studies by Giesecke & Van den Heever (1974), Giesecke & Viljoen (1974) and Van den Heever & Turner (1976) revealed that teat canal infections are far more common than is generally realised. A lack of appreciation of the situation could be responsible for incorrect diagnosis of subclinical mastitis and consequent unnecessary treatment. Teat canal infections may also lead to infection of the udder. That this is feasible is implicit in the work of Philpot & Pankey (1975; 1978), who showed that the incidence of mastitis was markedly reduced if teat canal infections were controlled by regular teat dipping.

This investigation was undertaken to study the aetiology and location of teat canal infections, and to determine whether they could be eliminated by local treatment with appropriate antibiotics.

MATERIALS AND METHODS

Detection of teat canal infections

Seventeen lactating Friesian type cows were selected for this experiment.

For several weeks prior to the investigation the cows were milked by machine and thereafter all the teats were regularly immersed in a registered (Act 36/1947) iodophore teat dip (containing 2 000 mg/dl iodine). The 68 quarters of the 17 cows were washed with clean, running water and dried with disposable paper towels. The tips of the teats were then vigorously

swabbed with cotton-wool pledgets moistened with 70% ethanol, and after the first 2 jets of milk were discarded into a strip cup, a separate foremilk sample was collected aseptically from each quarter.

The milk thus collected was directly plated onto a blood-tryptose-agar (BTA) in 0,01 mL quantities and, if no growth was observed after incubation for 24 h at 37 °C, 0,5 mL was transferred into 10 mL of 10% serum broth for enrichment at 37 °C for 24 h before plating of 0,01 mL of broth onto BTA.

After the milk samples had been collected, the teat canals were each swabbed by means of a small cotton-wool swab introduced to a depth of 8-10 mm, rotated once and returned to its container. Cultures were prepared from the swabs on BTA within 30 minutes of collection.

Therapy of teat canal infections

The relevant quarters were treated by depositing 0,25 mL of an intramammary formulation containing 200 mg of sodium cloxacillin/5 mL* into the teat canal.

Localization of teat canal infections by scanning electron microscopy

For the ultrastructural investigation, a cow outside the series of 17 was slaughtered and a small portion from the wall of one teat canal was removed and processed for scanning. Before the cow was slaughtered, a swab from the teat canal concerned had

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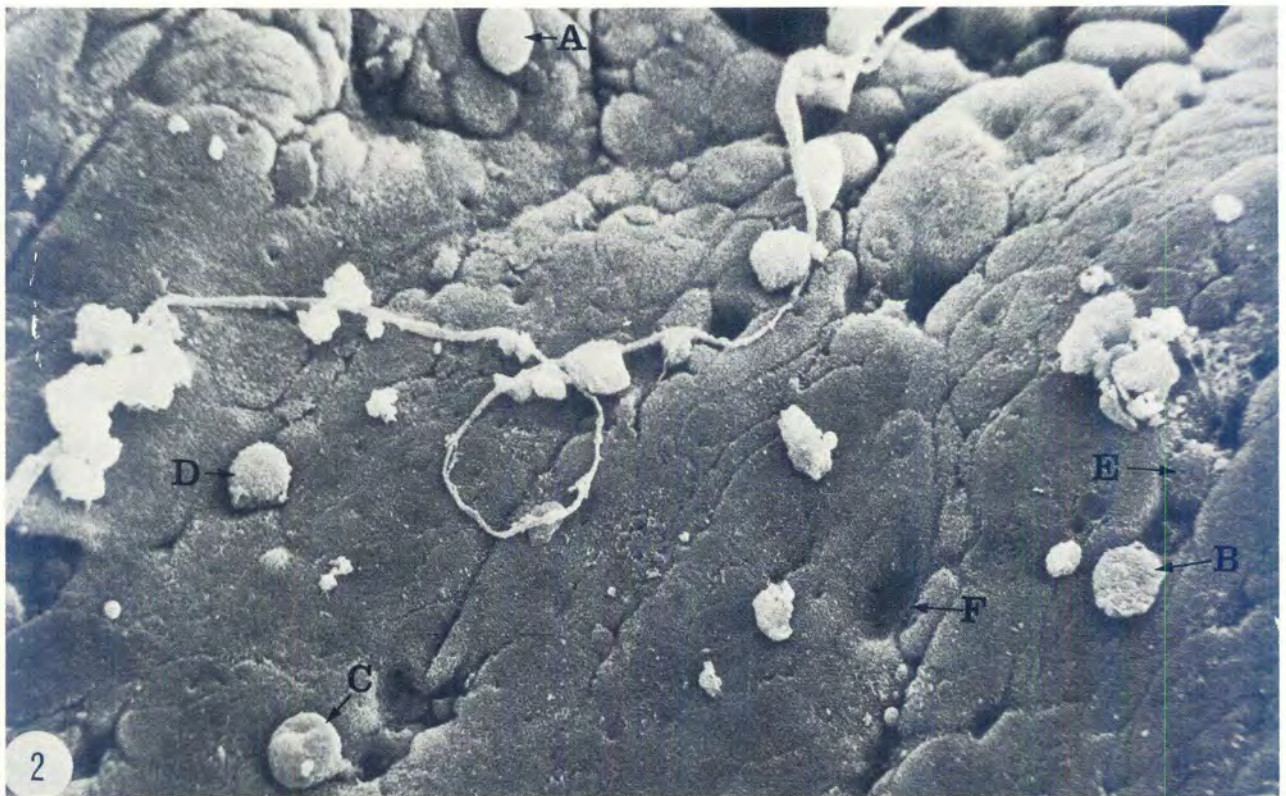


FIG. 1 General view of a section of the epithelial surface removed from the central area of a bovine teat canal colonized by *S. aureus*. $\times 300$

FIG. 2 Relatively smooth area of the same surface at $2100\times$ magnification with coccoid bacterial cells lying on the surface (A) and respectively sunken slightly (B), distinctly (C), markedly (D) and completely (E) into the epithelial layer. (F)=epithelial depressions left by coccus

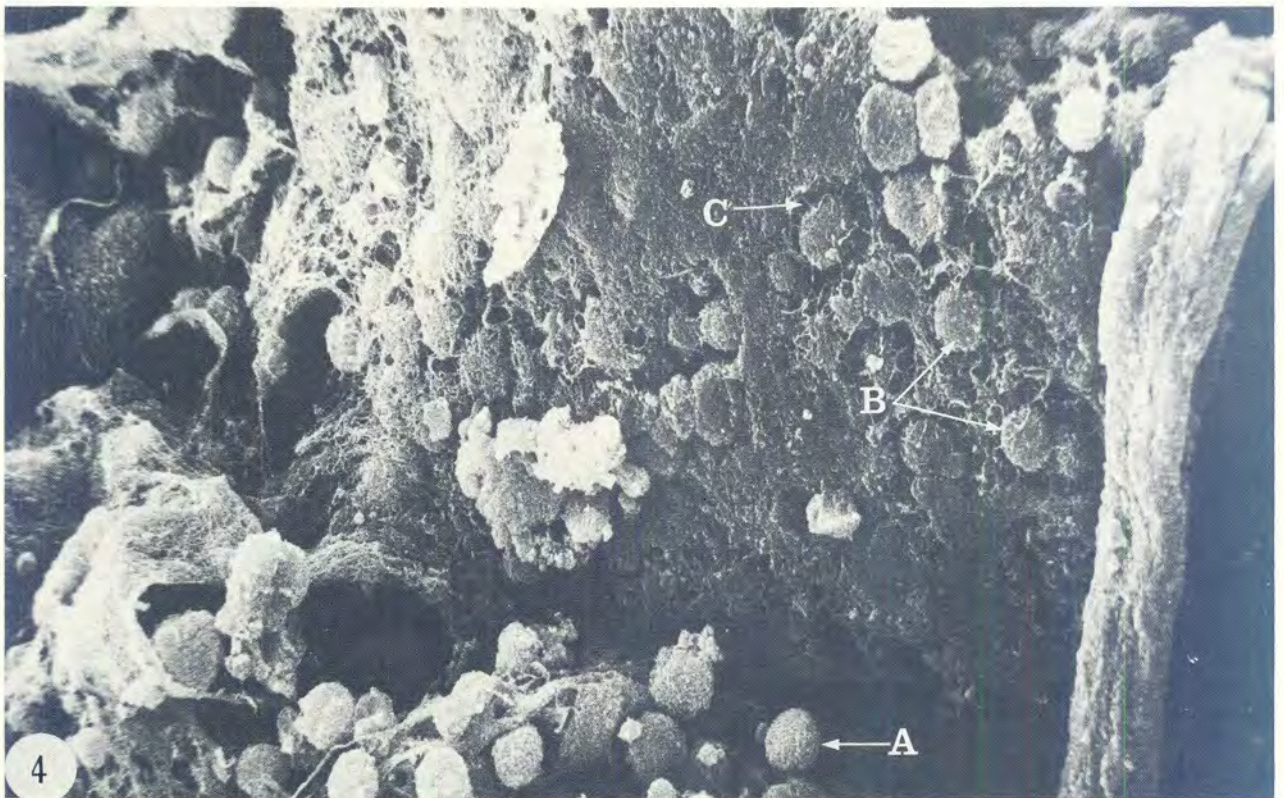


FIG. 3 Eroded area of the same surface at 960 \times magnification with superficial portions of stratum corneum (A) presumably surrounding a deeper lying layer (B) of the epithelium. (C)=cocci
 FIG. 4 View into an epithelial crevice at 2 250 \times magnification with cocci on surface (A) and in deeper regions (B) of the epithelium. Adhesions between coccus (C) and epithelial surrounding

yielded growth of *S. aureus*, but aseptically drawn foremilk proved negative. The specimen was fixed in 3% phosphate buffered glutaraldehyde, washed twice in buffer and post-fixed with 1% OsO₄ in Millonig's buffer for 1 h. The material was then dehydrated in acetone and critical point dried (CPD) with CO₂. After CPD the specimen was mounted on 15 mm aluminium stubs and coated with a thin layer of gold in a sputtering device (Milling, 1961). The specimen was then viewed in a ISI-100 scanning electron microscope operating at 25 kv. Micrographs were taken at 30° tilt at various magnifications.

RESULTS

Detection of teat canal infections

From the 68 quarters examined, 51 (75%) yielded *S. aureus* on culture of teat canal swabs, whereas only 31 (45%) of them also yielded *S. aureus* on culturing the milk samples. Six out of 31 quarters were affected with subclinical septic mastitis according to International Dairy Federation criteria (Kästli, 1967). Of the 68 quarters, 17 were not bacteriologically infected at any stage of the experiment.

Therapy of teat canal infections

Foremilk samples, drawn at the following routine milking from treated teats and examined at 12-hourly intervals on 1-4 successive occasions by means of the Thermocult plate method*, did not reveal the presence of any thermo-resistant inhibitory substances. Of the 51 positive quarters 7,8%, 25,4%, 13,7% and 59,9% became bacteriologically negative after 1, 2, 3 and 4 treatments respectively, and were still negative on examination 1 week after the last treatment. Moreover, local teat canal treatment given 4 times at 12-hourly intervals was effective in curing all the quarters showing subclinical septic mastitis. This recovery persisted for at least 1 week after treatment was discontinued.

The results of the treatment suggest that, despite the very limited amount of antibiotic which was administered into all the 51 teat canals, *S. aureus* infections were successfully eliminated from every teat canal after 1-4 treatments.

Localization of teat canal infections by scanning electron microscopy

The scanning electron micrographs (Fig. 1-4) show irregular teat canal surfaces with many crevices and apparent erosions of the epithelial surface. Where the surface appears smooth (Fig. 2), the staphylococci can be seen lying in distinct depressions. These depressions might protect the bacteria from being flushed out when milk is drawn through the canal and explain why the quartermilk samples failed to yield *S. aureus*, whereas the teat canal swabs yielded a heavy growth of the organism.

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CONCLUSIONS

From these data it seems reasonable to conclude that:

1. Teat canal infections with *S. aureus* may persist despite regular teat dipping.
2. The infections may be eliminated by suitable antibiotic administration into and limited to the teat canal.
3. Such treatment may be of major significance in controlling and preventing new cases of mastitis.
4. In some instances colonization of the teat canal resulted in *S. aureus* being isolated from teat canal swabs from quarters with milk containing more than 500 000 somatic cells per ml. Such a quarter would be considered to be affected with subclinical mastitis in terms of IDF criteria. After the local teat canal treatment described above, the somatic cell content decreased to below 500 000 somatic cells/ml and no organisms could be cultured from the milk, i.e. the quarters could be reclassified as mastitis negative, according to IDF criteria. This response to local teat canal therapy suggests that the quarters concerned were not truly affected with subclinical mastitis but were, in fact, teat canal infections.
5. The fact that no antibiotic residues were found in milk drawn at the first milking 12 h after treatment suggests that such local teat canal treatment may be applied without the necessity of withholding milk of treated quarters from market supplies.

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