SIMULTANEOUS ISOLATION OF ANAEROBIC BACTERIA FROM UDDER ABSCESSES AND MASTITIC MILK IN LACTATING DAIRY COWS

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A variety of non-sporulating anaerobic bacterial species were isolated from udder abscesses in 10 lactating dairy cows. Fifty percent of the abscesses yielded multiple anaerobic species and the other 50% only 1 species. The anaerobic bacteria, however, were always accompanied by classical facultative anaerobic mastitogenic bacteria. In four of the five cows also afflicted with mastitis in the quarters with abscesses, the anaerobic and facultative anaerobic bacteria were identical. Peptococcus indolicus was the most commonly isolated organism followed by Eubacterium and Bacteroides spp.

Bacteroides fragilis was resistant to penicillin, ampicillin and tetracycline.

Key words: Anaerobic bacteria, abscesses, bovine mastitis.

INTRODUCTION

There are numerous reports regarding the importance of obligate anaerobic species of bacteria in clinical infections in humans1 8 9. Human infections in which anaerobes are commonly found or in which they are the predominant pathogens are: abscesses in the breast, lung, liver and brain, puerperal sepsis, appendicitis and various other conditions9. Love14 15 isolated various anaerobes from subcutaneous "fight wound" abscesses in cats. Berkhoff & Redenbarger3 cultured anaerobes from abscesses, joint fluids, foot rot swabs and other specimens from diseased animals. Anaerobic bacteria were also isolated from pneumatic lungs of cattle4 and abscesses in pigs5.

In recent investigations on mastitic milk of South African dairy cattle, Bacteroides fragilis, Bacteroides eggerthii, Peptococcus indolicus, Eubacterium lentum, Eubacterium aerofaciens, Eubacterium combesii, Propionibacterium granulosum, Propionibacterium acnes, Fusobacterium necrophorum, Clostridium sporogenes and a Peptostreptococcus sp. were isolated from sporadic cases of bovine mastitis7. Hirsh et al.10 isolated various anaerobes from non-specified abscesses, joints, genital tracts and various other sites from animals.

We report here our findings on the isolation of a variety of anaerobic and facultative anaerobic bacterial species from sporadic udder abscesses, and normal and mastitic milk samples from clinical and subclinical mastitic cases from the same udder quarters of lactating dairy cows.

MATERIALS AND METHODS

Animals

The animals consisted of 10 lactating Friesian dairy cows each with an udder abscess in one quarter, encountered in 6 dairy herds on the Transvaal Highveld. According to criteria of the International Dairy Federation12 2 of the cows had clinical and 3 subclinical mastitis, respectively, in the quarters in which the abscesses occurred.

Collection of anaerobic milk samples

Milk samples were obtained from each of the 10 quarters which had an abscess. After disinfection of the teats, all milk samples were taken anaerobically via the teat canal from the teat or gland cistern of the udder by means of a 150 mm x 1,0 mm catheter attached to a 10 ml disposable syringe. The syringe and catheter were preflushed with an anaerobic gas mixture consisting of N₂ (80%); CO₂ (10%); H₂ (10%) on a volume basis to remove all traces of atmospheric oxygen from the system. Samples were immediately injected into 100 x 30 mm vaccine bottles, fitted with crimped butyl rubber sealers and containing an atmosphere of oxygen-free CO₂. Samples were transported on ice, and analysis was initiated within 6 hours after collection.

Anaerobic collection of pus from udder abscesses

Pus from udder abscesses was aspirated under similar conditions as the collection of anaerobic milk samples by means of a 19-gauge needle and 10 ml disposable syringe after disinfection of skin that covered the abscess. The pus samples were handled in the same manner as the milk samples.

Bacteriology

The anaerobic and facultative anaerobic bacteria isolated from milk and pus samples were cultivated and identified according to methods previously described7 11.

Antimicrobial sensitivity

The susceptibility of the anaerobic organisms to concentrations of penicillin-G (10 units/ml), ampicillin (4 µg/ml), cephalothin (6 µg/ml), clindamycin (3,2 µg/ml), chloramphenicol (12 µg/ml), tetracycline (10 µg/ml), erythromycin (3 µg/ml) and metronidazole (6 µg/ml) was determined by a broth-disc method according to Wilkins & Thiell18.
RESULTS
Isolation of anaerobic and facultative anaerobic bacteria (Table 1)
Although 6 (60%) cows were treated by their owners before sampling with various types of antibiotics (chloramphenicol, penicillin, streptomycin or tetracycline or combinations thereof) intramuscularly and Cows 6 and 10 also intramammary, a wide range of anaerobic and facultative anaerobic bacteria was isolated from the udder abscesses. The abscesses in all of the 10 lactating dairy cows yielded anaerobic bacteria. In the case of Cows 1, 2 and 6, three different anaerobic bacterial species and in Cow 10, four were isolated from the udder abscesses while the rest revealed only 1 or 2 species. Five abscesses (50%) revealed only 1 anaerobic bacterial species. In the case of Cow 10, three facultative anaerobic bacterial species were isolated in combination with the 4 anaerobic bacterial species to give a total of 7 bacterial species from 1 abscess. The mean number of bacterial species isolated from an abscess lies between 3 and 4. In the cases of Cows 6 and 10 with clinical mastitis and Cows 4 and 7 with subclinical mastitis, in the same quarters where the abscesses occurred, the anaerobic and facultative bacteria isolated from the mastitic milk were identical to those isolated from the udder abscesses. In the case of Cow 1 with SCM only, S. aureus was isolated from the mastic milk from the same quarter in which the abscess occurred. P. indolicus was isolated most frequently from udder abscesses, followed by E. lentum.

Antimicrobial sensitivity
The susceptibility of some anaerobic bacterial isolates isolated from udder abscesses to commonly used antimicrobial agents is recorded in Table 2. Whereas the majority of anaerobic bacteria were sensitive to all the antimicrobials tested, B. fragilis was resistant to penicillin, ampicillin and tetracycline.

DISCUSSION
As in humans, species of obligatory anaerobic bacteria appear to play an important role in clinical veterinary medicine. In humans anaerobes are the bacteria responsible for most brain abscesses. The organisms usually found are Peptostreptococcus, Fusobacterium and Bacteroides spp. Not infrequently, only anaerobes are isolated, or are found in combination with facultative anaerobic bacteria. We consistently isolated anaerobes from udder abscesses and 2 cases of clinical mastitis concurrently with facultative anaerobic bacteria which are known to be associated with bovine mastitis.

This report demonstrates the large number and variety of species of anaerobes that can be recovered from udder abscesses with or without clinical mastitis and

Table 1: Anaerobic and facultative anaerobic bacteria isolated concurrently from udder abscesses in lactating dairy cows

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Herd No.</th>
<th>Udder abscess</th>
<th>Mastitis</th>
<th>Anaerobic bacteria</th>
<th>Facultative anaerobic bacteria</th>
<th>Antibiotic therapy before sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>SCM**</td>
<td>SCM**</td>
<td>Bacteroides eggerthii Eubacterium combesii Propionibacterium acnes</td>
<td>Corynebacterium pyogenes Staphylococcus aureus</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>NP</td>
<td>NP</td>
<td>Eubacterium alactolyticum Megasperaera elsenelii Peptostreptococcus anaerobius</td>
<td>C. pyogenes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>NP</td>
<td>NP</td>
<td>Peptococcus asaccharolyticus P. anaerobius</td>
<td>C. pyogenes</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>SCM*</td>
<td>SCM*</td>
<td>Peptococcus indolicus</td>
<td>Escherichia coli Streptococcus agalactiae</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>NP</td>
<td>NP</td>
<td>Eubacterium lentum</td>
<td>C. pyogenes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>CM*</td>
<td>CM*</td>
<td>Clostridium cadaveris E. lentum P. anaerobius</td>
<td>C. pyogenes</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>SCM*</td>
<td>SCM*</td>
<td>P. indolicus</td>
<td>C. pyogenes</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>NP</td>
<td>NP</td>
<td>P. indolicus</td>
<td>C. pyogenes</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>NP</td>
<td>NP</td>
<td>P. indolicus</td>
<td>C. pyogenes</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>CM*</td>
<td>CM*</td>
<td>Bacteroides fragilis Eubacterium aerofaciens P. indolicus</td>
<td>C. pyogenes S. aureus S. agalactiae</td>
<td>Yes</td>
</tr>
</tbody>
</table>

SCM = subclinical mastitis
NP = not present
* = anaerobic and facultative anaerobic bacteria isolated from mastitic milk are the same as those isolated from udder abscesses
CM = clinical mastitis
** = only S. aureus have been isolated from mastitic milk
subclinical mastitis in the same quarters of lactating dairy cows. Similar findings have been reported for abscesses and this may indicate the reason why it has been given such prominence in the literature on anaerobes which play a role in bovine mastitis. The most frequent combination of anaerobic and facultative anaerobic bacteria in this study involved P. indolicus and C. pyogenes. This corresponds with previous findings in cases of clinical mastitis. The isolation of identical anaerobic and facultative anaerobic bacteria from clinical and subclinical mastitic milk and abscesses from the same quarters (Table 1) suggests at least a secondary role for the anaerobic isolates in bovine udder health.

The propensity of anaerobes to act as primary pathogens in nature is still unclear. In infections where both anaerobes and facultative anaerobic bacteria are present, it is not always possible to ascribe roles to all the organisms present or to state categorically that one or the other organism is the pathogen and that the others are of no consequence. All the organisms of both the anaerobic and facultative anaerobic bacterial species described in this paper may be considered to play a part in the clinical infections from which they were isolated. The similarity in anaerobic flora from 2 cases of clinical mastitis and 2 of subclinical mastitis show that the corresponding abscesses may be indicative of a pathogenic role for anaerobes found in mastitic quarters.

B. fragilis was both penicillin and tetracycline resistant whereas the rest of the isolates were sensitive to all the antimicrobials tested. Although the majority of cows had received antibiotic treatment before sampling a wide range of anaerobes could still be isolated from abscesses. Because of reduced blood supply as well as the penetration barrier created by abcess formation, the efficacy of the antibiotics used may have been reduced.

ACKNOWLEDGEMENTS

Financial assistance by the Department of Agriculture and Water Supply is gratefully acknowledged.

REFERENCES