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ANTIBIOTIC TREATMENTS AT DRYING-OFF OF INDUCED AND NATURAL INFECTIONS OF THE BOVINE MAMMARY GLAND

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Introduction

The advantage of the systematic use of an antibiotic such as cloxacillin at drying off in all four quarters of a cow has been demonstrated by the English research workers (Kingwill et al., 1967). Such a treatment is both a therapy and a prophylactic measure as it is aimed at clearing existing infectious and preventing new infections during the dry period. The antibiotic of choice in such a treatment must therefore be active against the principal bacteria causing mastitis (Staphylococci, Streptococci and enterobacteria) and persist as long as possible at a sufficient concentration in the udder (Ziv, 1975).

The demonstration of the efficiency of an antibiotic preparation must be based on rigorous scientific criteria which are often
difficult to set up and control in field trials. Induced infections, carried out under standard conditions allows the analysis of the different parameters capable of influencing the efficiency of a treatment, as Newbould (1974) has demonstrated for the treatments during the lactation period.

In this study we have planned to measure and analyse the efficiency of two long acting antibiotic preparations, cloxacillin and an association of penicillin and streptomycin, used at drying-off against experimentally induced infections of *Staphylococcus aureus* and the naturally occurring infections of other bacteria.

### Material and methods

#### COWS AND MANAGEMENT

34 Frisian cows of the experimental herd of the Research Center were used. The number of lactation varied from 2 to 9. Mechanical milking was carried out in the cow shed. Before milking, each quarter was washed with warm water using an individual cloth for each cow. Between each milking these clothes were washed in hot water (80 °C). Teat dipping was carried out systematically with an iodophore (Lanodip, Roger Bellon). Drying off was carried out abruptly two months before calving for all cows and sometimes before for those cows whose lactation had dropped below 5 kg per day. At the time of drying off the average daily lactation was 7 kg with a variation of between 3.1 and 11.4 kg.

#### INFECTIONS

The mammary infections due to *Staphylococcus* were either naturally acquired (3 cases) or experimentally induced (48 cases). The infections were induced during the lactation period using the technique previously described (Postle, Roguinsky and Poutrel, 1977) with 10 strains of *Staph. aureus*. The staphylococcal infections remaining at drying-off were either infections having resisted one or more treatments during lactation (39) or infections not having been treated (12). (Roguinsky, Postle and Poutrel, submitted for publication.)

Infections due to bacteria other than *Staph. aureus* were all naturally acquired.

#### SAMPLES

At the evening milking, approximately 20 ml of milk per quarter, constituting the first part of the milk were sterilely collected after washing the quarters and disinfecting the teat extremities with a 70 % solution of alcohol. These samples were taken routinely every fortnight from all cows in lactation. Samples were also taken at the time of treating and on the day of calving. Cell counts and bacteriological examinations were carried out on each sample but only the bacteriological results were taken into consideration in this experiment.

#### BACTERIOLOGICAL EXAMINATION AND STRAIN CHARACTERISTICS

Each milk sample plated on sheep blood agar plates within 24 hours of collection. The bacteria isolated were identified after 24 or 48 hours incubation using classical bacteriological techniques (Plommet, 1962).

In order to analyse the results, the infections due to the major pathogens, *Staph. aureus*, *Streptococci* species, Enterobacteria and those due to the minor pathogens, *Corynebacterium bovis*, *Micrococi* and *Staph. epidermidis* were grouped together (Griffin et al., 1977).

Serological typing was carried out on all strains of *Staph. aureus* used for the induced infections and on all strains isolated at the time of drying off and calving (Plommet and Plommet, 1970).

An antibiotic sensitivity test was carried out on the strains of *Staph. aureus* isolated from the different quarters at drying off and at calving using the corresponding antibiotic.

#### TREATMENTS

The antibiotics used were:
- Cloxacillin benzathin, 500 mg per dose (Orbenin, Roger Bellon);
- procaine penicillin G, 1 million units and dihydrostreptomycin, 1 g per dose (Combisec, Pfizer-Clin).

The two preparations contained the same long acting base : peanut oil gel with 3 % aluminium monostearate.

The choice of preparation to be used was carried out at random at the time of treatment. The four quarters of the same cow
received the same antibiotic using one dose per quarter.

The infusion of the product by the teat canal was followed by an extensive massage of the udder.

62 quarters per preparation were treated in this way. It should be noted that despite the fact that the choice of the preparation to be used was carried out at random, the number of quarters treated by each preparation was the same.

STATISTICAL ANALYSIS

The statistical significance of the results were determined using the $\chi^2$ test.

DEFINITIONS

Infection: all quarters from which the same bacteria was isolated from two separate samples, or 3 samples in the case of conflict, taken at the time of drying off and in the month preceding, were considered to be infected.

Clearance: all quarters from which it was not possible to isolate the bacteria initially present when two samples were taken. The first on the day of calving and the second during the 15 following days, were considered cleared. In the case of any doubt the result of a third sample taken a fortnight after the second was used.

New infection: all quarters from which a bacteria, not initially present at drying off, was isolated from a sample taken at calving or during the fortnight following were considered to be newly infected. In the case of any doubt a third sample was taken.

Results and discussion

1. THERAPEUTIC EFFECTS

Results (table 1)

For the mammary infections caused by the major pathogens, the clearing rates observed after treatment at drying off, 75 % (21 infections cleared from 28 treated) with cloxacillin and the combination of penicillin and streptomycin, 81 % (26 infections cleared from 32 treated) were not significantly different.

For the group of mammary infections caused by the minor pathogens the clearance rates observed, 58 % (15/26) and 100 % (12/12) respectively were significantly different ($P < 0.001$).

For the infections caused by Staph. aureus the clearance rate observed after treatment with the association penicillin-streptomycin 85 % (23/27) was significantly greater than that observed after treatment with cloxacillin 71 % (17/24) ($P < 0.02$).

These results correspond with those already published by other authors using the same antibiotics at the same dose rates (Kingwill et al., 1967; Brookbanks, 1968; Loosmore et al., 1968; Rosenzuaig and Mayer, 1970; Langley et al., 1971; Hoare et al., 1973; Christie et al., 1974; Hill and Keefe, 1974; Postle and Natzke, 1974; Clegg et al., 1975; Meaney, 1976).

The infections caused by the Micrococci were all cured regardless of the antibiotic used.

For the infections caused by Corynebacterium bovis, the difference observed between the clearance rates for two treatments is statistically significant ($P < 0.001$). The association Penicillin-Streptomycin cleared twice as many cases as the cloxacillin (10/10 against 12/23). However the absence of any method for characterising the strains does not allow one to differentiate the failures of the new infections. The observed difference could have resulted from the fact that the preparation of Cloxacillin does not last as long as the association Penicillin-Streptomycin.

Analysis of the treatment failures of the infections due to Staph. aureus.

— Sensitivity of the strains to the antibiotics used.

In the case of a treatment failure all the strains isolated from the infected quarters at drying off and at calving were sensitive «in vitro» to the antibiotics used in the treatment of these quarters. Under these conditions, the failure of a treatment might be attributed to the nature of the lesions caused by Staph. aureus, these lesions being inaccessible to the antibiotic (Schalm et al., 1971).

— Influence of previous treatments during lactation.

In the case of a treatment failure all the strains isolated from the infected quarters at drying off and at calving were sensitive to the antibiotics used in the treatment of these quarters. Under these conditions, the failure of a treatment might be attributed to the nature of the lesions caused by Staph. aureus, these lesions being inaccessible to the antibiotic (Schalm et al., 1971).

No significant difference was observed between the clearance rates of the Staphylococcal infections treated during the lactation preceding the treatment at drying off.
Table 1: Therapeutic and prophylactic effects of two treatments, related to the micro-organisms involved

<table>
<thead>
<tr>
<th>Quarters infected</th>
<th>Organisms (^a)</th>
<th>Quarters infected</th>
<th>Organisms (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At drying-off</td>
<td>47</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>p. 100</td>
<td>76</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>At calving</td>
<td>25</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>p. 100</td>
<td>40</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Infections eliminated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p. 100</td>
<td>17</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>New infections</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>p. 100</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) S.a.: Staphylococcus aureus; Str.: Streptococci; Ent.: Enterobacteria; Mic.: Micrococii + Staphylococcus epidermidis (coagulase $-$); C.b.: Corynebacterium bovis.

b) the 51 staphylococcal infections are distributed in 48 induced infections and 3 natural infections.

c) the 8 streptococcal infections are distributed in 5 Streptococcus uberis, 1 Streptococcus agalactiae, 1 Streptococcus dysgalactiae and 1 Streptococcus faecium.

d) the 11 new infections are distributed in 10 Streptococcus uberis and 1 Streptococcus faecium.
and the infections not treated, no matter which antibiotic was used (table 2). This result shows that treating during the lactation does not necessarily select the most difficult cases to clear. The nature of the lesion is probably thus not the only reason of a treatment failure.

Of the 39 infections no cleared by one or more treatments during the lactation preceding the drying off, 30 infections were eliminated by calving time after treatment with either cloxacillin or the association penicillin-streptomycin (table 2). This observation, superiority of the treatment at drying off over the treatment during the lactation is in agreement with other results already published (Dodd and Griffin, 1975).

— Influence of the length of infection.

For the staphylococcal infections experimentally induced less than eight months before the treatment, the length of the infection has no influence on clearance rates observed after treatment with cloxacillin 81 % (13/16) nor with the association penicillin-streptomycin 86 % (19/22). The quantity of data for infections established for longer than eight months is not sufficient to draw any conclusions (table 2). The clearance rate calculated for the totality of data is significantly less 70 % far the infections established for more than 8 months than for the infections established for less than 8 months, 84 % (P < 0.05), (7/10 against 32/38). These results confirm those already published by Griffin (1971) for naturally acquired infections. They show that the infections which are established during the first 2 months of the lactation should be treated as soon as they are detected as they are more difficult to eliminate during the dry period.

— Influence of the serotype of the strain (table 3).
In the case of all staphylococcal infections, the strain isolated at drying off and at calving in the case of a treatment failure, for a given quarter, belonged to the same serotype as that inoculated during the lactation. The restricted number of serotypes treated by each preparation does not allow a study of the influence of the serotype on the efficiency of the treatments. The clearance rates observed for the serotype 51 was 56 %, when cloxacillin (8/13) and the association Penicillin-Streptomycin (1/3) were used. These clearance rates were significantly less (P < 0.001) than for the total of the other serotypes, 89 % (9/11 and 23/24 respectively). When cloxacillin has been used as a drying off treatment, large variations between herds in its efficiency against staphylococcal infections have been observed («herd effect») although no precise reason or reasons for these variations has been determined (Dodd and Griffin, 1975). Our results suggest that these variations could be linked to the serotype of the infecting strain. It should be noted however that the serotype 51 was only represented in our experiment by two strains.

2. PROPHYLACTIC EFFECTS (Table 1)

When the totality of the mammary infections caused by the major pathogens are considered, there is no significant difference between the rate of new infections observed after treatment at drying off with cloxacillin or with the association Penicillin-Streptomycin. The rate of new infections was respectively 8 % (5 new infections from 62 quarters treated) and 13 % (8 new infections from 62 quarters treated). All results considered the rate of new infections for this group of bacteria was 10 %. This value corresponds with that already published (Postle and Natzke, 1974; Dodd and Griffin, 1975; Meaney, 1976) using identical treatments. The two strains of Staph. aureus isolated from the cases of new infections had the same serotype (52 and 54) as that of the strains induced experimentally into the other quarter or quarters of the same cow. The rate of new infections caused by streptococci is higher after the treatment with cloxacillin than that observed when cloxacillin was employed (6 %). This difference is however not statistically significant. Streptococcus uberis was responsible for 10 of the 13 new infections due to the major pathogens. This observation has already been reported (Dodd and Griffin, 1975). In the future, it would be advisable to study the preparations capable of preventing the establishment of this type of infection during the dry period.

For the mammary infections caused by the minor pathogens, no significant difference occurred between the rate of new infections after treatment with cloxacillin or with the association Penicillin-Streptomycin 6 % (4/62) and 3 % (2/62) respectively.

3. OVERALL CONCLUSIONS (Table 1)

For the major pathogens, the number of infections observed at calving compared to the number of infections at drying off was reduced by the same proportion by cloxacillin and the association Penicillin-Streptomycin. 28 infections (45 %) were present at drying off and 12 (19 %) at calving for the cloxacillin treatment. 31 infections (52 %) were present at drying off and 14 (23 %) present at calving for the association Penicillin-Streptomycin. The efficiency of the treatments is thus globally identical. The higher clearance rate of staphylococcal infections observed for the association Penicillin-Streptomycin was counteracted by a higher number of new infections observed after this treatment. For the major pathogens the level of infection dropped from 48 % at drying off to 21 % at calving, confirming the interest of the systematic treatment at drying off.

Of the numerous commercial preparations of antibiotics only a few of them have undergone a rigorous study of their efficacy. Cloxacillin is the antibiotic for which there exists the most information of quality. It thus constitutes the treatment of reference at drying off.

A certain number of other works have reported the use of other antibiotics. Their results can not easily be employed as they lack precision concerning the dosage used, the composition of the antibiotic preparation, the regularity of the observation and the criteria observed. There is also an absence of comparable groups with insufficient numbers of cases treated.

Using experimentally induced infections it is possible to analyse certain factors capable of influencing the efficacy of a treatment. Results obtained from these experimental infections allow one to envisage the
establishment of a standard model for appreciating accurately the efficacy of an antibiotic preparation either by comparing it with a non treated group or by comparing it with a reference group.

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Summary

Two long acting antibiotic preparations, Cloxacillin (0.5 g) and an association of Penicillin G (1 million units) and Streptomycin (1 g) in an oily base, associated with 3 % aluminium monostearate, were used for the treatment at drying off of 32 cows (124 quarters) either infected spontaneously or induced. For the group of infections due to the major pathogens the therapeutic efficiency of the two preparations was the same. 17 of 24 (71 %) staphylococcal infections were cleared by cloxacillin against 23 of 27 (85 %) for the combination Penicillin-Streptomycin. This difference was significant.

The treatment failures of the 48 induced infections by Staphylococcus aureus could not be linked to the antibiotic sensitivity of the strains used nor to a previous treatment during the lactation. On the other hand the length of infection and the serotype of the strains have an influence on the clearance rate obtained.

The rate of new infections observed after the cloxacillin treatment (8 %) is not significantly different to that observed when the association Penicillin-Streptomycin is used (13 %). Of 13 new infections due to the major pathogens, 10 were infections due to Streptococcus uberis.

The therapeutic and prophylactic efficacy of cloxacillin and an association Penicillin-Streptomycin, when considered globally is the same.

References


