Effect of teat skin disinfection on the rate of infection and interval to infection in cows exposed to high levels of *Staphylococcus aureus*

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Summary. All teats of a herd of lactating cows were contaminated after each milking with *Staphylococcus aureus*. Two teats of each cow were subsequently dipped in an iodine solution, while the remaining 2 teats were not dipped. For cows with a single infection the interval to infection in dipped and undipped quarters was 64 and 60 d respectively, while for the first infected quarter of cows with multiple infections the interval to infection in dipped and undipped quarters was 50 and 39 d respectively. For the second quarter infected in cows with multiple infections, the period between the first and second infection was 21 and 12 d for dipped and undipped quarters respectively. It is suggested that while teat skin disinfection was effective in reducing the rate of new infection, it had little effect on the process of infection in those quarters becoming infected. The occurrence of an infection in one quarter doubled the rate of infection in the other quarters.

Post milking teat skin disinfection has been shown to reduce the new intramammary infection rate by about 50% (O'Shea et al. 1975; Philpot & Pankey, 1978; Sheldrake & Hoare, 1980a). It is imperative to conduct new infection trials to evaluate mastitis control procedures as both O'Shea et al. (1975) and Sheldrake & Hoare (1980a) have found that in vivo investigations of bactericidal properties of disinfectants, while being cheap and easy to conduct, may yield results contrary to studies of new infection rates made on lactating cows.

In conducting new infection trials the disinfectant must be soundly appraised and independent factors such as cow susceptibility to mastitis must be minimized. Wesen & Schultz (1970), in recording new infection data, allowed more than one infection per quarter per lactation. However Philpot & Pankey (1978) eliminated any quarter from further analysis during a particular trial once that quarter had become infected, and Sheldrake & Hoare (1980a) eliminated a quarter from the experiment once the quarter had been recorded as having an infection, irrespective of whether the infection had been eliminated, either spontaneously or due to antibiotic therapy during a dry period, while the experiment was being conducted.

Kingwill et al. (1970), Natzke et al. (1972) and Hoare et al. (1977) have shown that the prevalence of mastitis decreases following the adoption of a mastitis control system, and Dodd & Neave (1970) showed that the proportion of cows infected in a herd was influenced both by the number of infections and the duration of infection. However, there is a dearth of detailed epidemiological data available relating the components of a control system to the rate of new infection in a herd, particularly under high levels of contamination.
Table 1. Effect of teat skin disinfection on the occurrence of new intramammary infection for cows with single or multiple quarters infected during lactation

<table>
<thead>
<tr>
<th>Disinfectant treatment</th>
<th>Cows with a single infection or the first infection of cows with multiple infection</th>
<th>Second infection of cows with multiple infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dipped</td>
<td>Undipped</td>
</tr>
<tr>
<td>No. of quarters infected in cows with a single infection</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>No. of quarters infected in cows with multiple infections</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Total no. of quarters infected</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>No. of quarters exposed to infection</td>
<td>244</td>
<td>244</td>
</tr>
<tr>
<td>Proportion of quarters becoming infected, %</td>
<td>74</td>
<td>12±1</td>
</tr>
</tbody>
</table>

This paper studies the occurrence of new infections in a herd of lactating cows and suggests how post milking teat skin disinfection modifies this process.

MATERIALS AND METHODS

Experimental procedures

The data used in this paper were obtained from studies previously reported as expt 2 by Sheldrake & Hoare (1980a, b). Briefly, all teats were challenged, within 1 min of completion of milking, with a solution containing approximately $5 \times 10^6$ colony forming units (cfu)/ml of *Staphylococcus aureus*. Two teats (right fore and left hind) were then dipped in a post-milking teat skin disinfectant containing 5000 mg available iodine/l (mg avI/l), while the remaining 2 teats (left fore and right hind) remained undipped.

A quarter was considered to be infected if *Staph. aureus* was isolated at 2 of 3 consecutive samplings. The quarter was considered to be infected from the initial isolation. Cows found to have an infected quarter during the pre-experimental period were excluded from the project.

Day 0 was taken as the time an uninfected cow was introduced into the herd and exposed to experimental infection with *Staph. aureus*. Cows were introduced to the experiment within 2 weeks of calving and remained in the experiment until removed from the herd or until the experiment was concluded.

Data analysis

The number of days prior to infection for each cow was calculated from d 0 until infection. The period between the first and second quarters of a cow becoming infected was from the initial isolation of organisms of the first quarter infected until the initial isolation of organisms of the second quarter infected. The mean number of d was determined using a log$_{10}$ transformation of the data and comparisons between mean values were made using Student's $t$ test.

The proportion of quarters infected in cows with single infection and the first infection in cows with multiple infection were related to the total number of uninfected quarters exposed to infection throughout the trial in both the dipped and undipped treatments.
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Table 2. Interval between start of the experiment and detection of new intramammary infection. The results are shown for control quarters (undipped) and those treated with a disinfectant after milking (dipped)

<table>
<thead>
<tr>
<th></th>
<th>Undipped</th>
<th></th>
<th></th>
<th>Dipped</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Geometric</td>
<td>No. of</td>
<td>Mean</td>
<td>Geometric</td>
<td>No. of</td>
</tr>
<tr>
<td></td>
<td>(log₁₀)</td>
<td>mean</td>
<td>cows</td>
<td>(log₁₀)</td>
<td>mean</td>
<td>cows</td>
</tr>
<tr>
<td>Days to first infection (s.d. = 0.31, 37 df)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows with one infection</td>
<td>1.78</td>
<td>60</td>
<td>20</td>
<td>1.80</td>
<td>64</td>
<td>9</td>
</tr>
<tr>
<td>Cows with more than one infection</td>
<td>1.59</td>
<td>39</td>
<td>5</td>
<td>1.70</td>
<td>50</td>
<td>7</td>
</tr>
<tr>
<td>Days between first and second infection (s.d. = 0.37, 20 df)</td>
<td>1.06</td>
<td>12</td>
<td>7</td>
<td>1.32</td>
<td>21</td>
<td>5</td>
</tr>
</tbody>
</table>

df, Degrees of freedom.

For the second quarter infected in cows with multiple infections, the number of quarters exposed to infection were those quarters remaining uninfected after the first confirmed infection. Thus, if the first infection was in a dipped quarter, 2 undipped quarters remained exposed for the second infection, and only one dipped quarter; and *vice versa*.

The rate of new infection/1000 quarter milkings was determined for the first quarters infected of all cows, and for the second quarter infected of those cows with more than one infected quarter. The rate was calculated by dividing the number of new infections by the sum of the product of the number of quarters/cow available for infection and the number of milkings the cow was exposed to infection, and multiplying by 1000. For the first quarter infected of each cow, all quarters were eligible. For the second quarter, only quarters of cows with one quarter already infected were eligible.

**RESULTS**

Table 1 shows the proportion of cows exposed to infection which became infected with single or multiple infections. For cows with a single infection or the first infection of cows with multiple infections, 12.1 and 7.4% of undipped and dipped quarters respectively became infected. A similar result was obtained for second quarters infected in cows with multiple infections; 12.3 and 7.6% of undipped and dipped quarters respectively.

The rate of new infection/1000 quarter milkings for the first infection of all cows in undipped quarters was 0.58, while for dipped quarters the rate was 0.37, a reduction of 36%. For the second infection of cows with multiple infections the rate for undipped quarters was 1.16 and for dipped quarters 0.64, a reduction of 44%.

The mean number of organisms being excreted from the first infection at the time of the first isolation of the second infection was approximately $5 \times 10^3$ cfu/ml.

As shown in Table 2, for undipped quarters the interval to infection in cows with a single infected quarter (60 d) and the interval to first infection in cows with multiple quarters infected (39 d), were not significantly different. However, in cows with multiple infections the interval to infection of the second quarter (12 d) was significantly less than the interval to detection of the first infected quarter (39 d).

For dipped quarters the trends were similar. The interval to infection in cows with a single infected quarter was 64 d, and to the first infection of cows with multiple infections 50 d. The interval between the first and second infection for cows with multiple infection was 21 d. This period was not significantly less than the interval to detection of the first infected quarter of these cows (50 d).
DISCUSSION

For the first infection of all cows teat dipping reduced the new infection rate by 36%. However, the number of days prior to infection was similar for both dipped and undipped quarters; but 64 and 60 d respectively for cows with a single infection, and 50 and 39 d for the first infection of cows with multiple infections.

O'Shea & Meaney (1979) found that, for those quarters with a teat duct infection, the period of this infection was the same, irrespective of whether the teats were disinfected or not: 23 and 25 d respectively. They also found that the number of quarter milkings/new infection was decreased by approximately 50% following disinfection.

The results found here and those of O'Shea & Meaney (1979) suggest that, while teat dipping reduces the number and rate of new intramammary infections, once the teat end has been colonized the process of infection is similar for both dipped and undipped quarters. King, Neave & Williams (1979) showed that the majority of teats colonized prior to use of a disinfectant could be de-colonized within 7 d following routine use of a disinfectant. However on 2 occasions teat colonies were not eliminated. Some teat duct infections may be out of reach of the disinfectant. If this occurs then post milking disinfection will have no effect on halting the infection process of that quarter, and machine factors such as described by Thiel (1974) will play the same role in the infection process for dipped and undipped teats.

For second infections in cows with multiple infections the proportion of quarters infected was similar to the proportion of first or single quarters infected; 7-6 and 12-3 % for dipped and undipped quarters respectively. However, the period prior to infection was substantially less for the second infection, suggesting that either the presence of an existing infected quarter increased the rate of infection, or that cows with multiple infection were more susceptible to mastitis.

The first infection of cows with multiple infection had mean pre-infection periods of 39 and 50 d for dipped and undipped quarters respectively. The shorter pre-infection periods, compared with those of single infection of 64 and 60 d respectively, suggests that cows with multiple infection may be more susceptible to infection. However, the mean pre-infection period for cows with multiple infections may under-estimate the length of the pre-infection period of these cows compared with cows with a single quarter infected. If the period of observation had been longer some of the cows with single infections may have become infected in a second quarter and as a consequence the mean pre-infection period of multiple infected cows would be increased. Morris (1975) reported similar difficulties in studying the length of infection periods, and suggested that mean duration was under estimated unless the period of observation was in excess of 3 years. To conclude that cows with multiple infections were more susceptible to mastitis than cows with a single infected quarter is not justified by the data.

If an existing infection increases the rate of new infection in other quarters it is most likely that this is due to increased contamination of the teat orifices. However, the number of organisms being excreted from the infected quarter (5 x 10^8 cfu/ml) was much less than in the contaminating suspension, and it is suggested that the time and method of bacterial contact with the teat orifice may be more important than the concentration of the challenge suspension. Alternatively, the bacteria grown in vivo may be more pathogenic than those cultured in vitro. Thompson et al. (1978) postulated methods by which bacteria may enter the teat cistern. They fall into 2 groups. Either bacteria are forced through the orifice during milking, having
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originated from a colony present at the orifice or from a bacteria-laden droplet being impacted against the orifice. Alternatively, entry occurs during the inter-milking period, as a result of capillary action or colonization.

The results from this study and that of Bramley, Griffin & Grindall (1978) showed that teat skin disinfection was effective in preventing new infection, although Thompson et al. (1978) found it to be ineffective. The latter authors suggested that organisms were penetrating the orifice as a result of impact during milking, concurring with the findings of Thiel et al. (1969). If teat dipping is effective in preventing new infections then it is postulated that teat end colonization occurs prior to infection.

It is concluded that data from cows with multiple infections may be used to assess teat skin disinfectants. However, if the majority of infections occur as a result of faults in the milking machine, causing bacteria-laden droplets to penetrate the orifice during milking, teat dipping may not be effective, and trials to assess disinfectants may give inconclusive results.

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REFERENCES


