COLIFORM MASTITIS - THE IMPORTANCE OF THE DRY PERIOD

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SUMMARY

Despite significant progress in the control of contagious pathogens, environmental mastitis continues to be a major cause of financial loss to the UK dairy industry. This paper presents some findings from a field study designed to investigate the incidence and significance of coliform infections acquired during the non-lactating period. The bacteriological status of two quarters from each of 629 cows was assessed through the non-lactating period (two quarters were left as unsampled controls); samples were collected from all mastitic quarters of these cows during the subsequent lactation. A significant rise in the incidence of intramammary infection was detected between drying off and prior to calving. When compared to unsampled controls, quarters sampled during the dry period did not show a significantly different incidence of infection at calving, or subsequent clinical mastitis. Quarters infected with a coliform during the dry period were significantly more likely than uninfected quarters to develop mastitis caused by that pathogen. Of all coliform mastitis occurring in the first 100 days of lactation, 52% arose in quarters previously infected, during the dry period, with the same species/strain of bacteria (as identified by DNA fingerprinting). These findings suggest that management during the dry period may influence the incidence of coliform mastitis in the subsequent lactation.

INTRODUCTION

In recent years implementation of the five-point mastitis control plan has resulted in a marked reduction in the incidence of contagious mastitis (1). This decline has not been accompanied by a comparable fall in the incidence of environmental mastitis; which has therefore become of relatively greater importance (2).

Classically, the non-lactating mammary gland has been considered refractory to ‘coliform’ infection (3). However research in the US, from as early as 1943, has implicated the dry period as being the time of greatest risk for the acquisition of new Gram-negative intramammary infections (IMI) (4,5,6,7,8), with some 61% of new IMI occurring at this time. Further studies have illustrated the ability of such infections to remain quiescent within the udder until calving, subsequently causing clinical mastitis in early lactation (9).

To date, despite some anecdotal evidence, similar studies, to validate these findings, have not been carried out in the UK. As a consequence the importance of the dry period in the control of Gram-negative mastitis remains equivocal.

The aim of the research outlined here was to investigate the incidence of intra-mammary infections acquired during the non-lactating period of dairy cattle, under UK field conditions and their subsequent importance in the ensuing lactation.
MATERIALS AND METHODS

Salient aspects of the study methodology are outlined below. Full details have been described elsewhere (10)

**Herd Selection:** Herds were selected on the basis of location (Somerset), low bulk milk somatic cell count (generally <200,000 cells/ml), calving pattern and likelihood of owner compliance with the study protocol. All herds were milk recorded (NMR/DAISY). Herds were not selected on the basis of a previous history of coliform mastitis.

**Sampling Strategy:** Duplicate quarter samples were collected by the authors at ‘drying off’ and during the week following calving. During the dry period, duplicate, samples were taken from two ipsilateral quarters (LF and LH (odd numbered cows) or RF and RH (even numbered cows)) once in each of the two weeks prior to the anticipated calving date. The other two quarters remained as unsampled controls to eliminate the sampling procedure as a cause of new intra mammary infections. Any cow not calving by her ‘due date’ was sampled weekly until parturition. During the subsequent lactation milk samples were collected from all mastitic quarters identified by the herdsmen and the disease graded for severity.

**Sampling Procedure:** Prior to sampling, teats were cleansed of gross contamination and dipped in a solution containing 2800 ppm available chlorine (Agrisept, Pharmacia-UpJohn). Following a minimum 30-second contact time the teats were wiped dry. Each teat was subsequently scrubbed with surgical spirit and allowed to dry. Immediately before sampling the teat ends were scrubbed for a second time using surgical spirit and foremilk was discarded (strict foremilk was collected from udders assessed as having little secretion present). Duplicate samples were then collected, following a third scrub of the teat ends. Following sampling, teats were again dipped with the dame disinfectant and the cows were confined to a loafing yard for at least 30 minutes. Samples were immediately stored in a cool box and maintained at or below 4°C. Bacteriology was performed within 24 hours. The herdsmen, using the sampling procedure outlined above collected a single quarter sample from all mastitic cows. These samples were frozen and batched each week for submission to the laboratory. Gloves were worn throughout the sampling procedure, and were changed both between cows and between duplicate sample sets.

**Dry Cow Therapy:** Dry cow therapy was administered by the authors following collection of the ‘drying off’ samples. The teat ends were scrubbed with surgical spirit for a fourth time prior to partial insertion of the tube canula. Following treatment teats were dipped with disinfectant and the cows confined to a loafing yard for at least 30 minutes. Dry cow antibiotics used were cloxacillin (Orbenin Extra, Pfizer), cephalonium (Cepravin, Schering-Plough) or procaine penicillin G (Mylipen, Schering-Plough).

**Bacteriology:** Samples were submitted to Langford Veterinary Investigation Centre for bacteriology. Approximately 10 μl of secretion was inoculated onto sheep blood agar and Edward’s agar; 100 μl of secretion was inoculated onto MacConkey agar to enhance coliform detection (7). Plates were incubated at 37°C and bacteria colonies observed after 24 and 48 hours. Organisms were identified and quantified using standard laboratory techniques. *Escherichia coli* was identified by colony morphology, oxidase and indole tests, other coliforms were identified using a microtube identification system (RapiD 20 E, bioMérieux).
Definition of an Intra mammary Infection: Isolation of a recognised pathogen, in pure growth was considered an intra mammary infection. If a screening sample was obviously contaminated or contained >1 enterobacterial isolate the duplicate sample was submitted for bacteriological examination and intra mammary infections diagnosed on the basis of re-isolation of the organism.

Statistical Analysis: Results were collated and analysed using Access (Microsoft Corporation), Excel (Microsoft Corporation) and Epi-Info (CDC, Atlanta, GA). Statistical analysis was performed using the \( \chi^2 \) test; results were considered significant when \( p \leq 0.05 \).

RESULTS

Clinical mastitis incidence and aetiology

The incidence and aetiology of clinical mastitis during the study period has been described elsewhere (2). In summary 337 cases of clinical mastitis occurred during the study period. The mean annual incidence was 41.6 cases/100 cows/year (Range 13-75). *Escherichia coli* was the commonest cause of clinical mastitis accounting for 34.7% of all isolates.

Dry period infections

These results are based on 629 cows calving between 22 March 1997 and 4 April 1998. Infection data were collated for the first 100 days of the subsequent lactation. A more detailed presentation of these results has been presented elsewhere (10).

Major pathogens

The percentage of quarters infected at each sampling point with coliform organisms is outlined in Table 1 and illustrated in Figure 1.

### Table 1: Percentage of quarters infected with coliforms at each sampling time point

<table>
<thead>
<tr>
<th></th>
<th>Drying Off</th>
<th>3 Weeks Pre-calving</th>
<th>2 Weeks Pre-calving</th>
<th>1 Week Pre-calving</th>
<th>Post Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2565</td>
<td>423</td>
<td>1003</td>
<td>1197</td>
<td>2503</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2.14</td>
<td>0.71</td>
<td>0.80</td>
<td>1.00</td>
<td>0.32</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>0.04</td>
<td>0.00</td>
<td>0.30</td>
<td>0.08</td>
<td>0.28</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>0.08</td>
<td>0.24</td>
<td>0.60</td>
<td>0.25</td>
<td>0.12</td>
</tr>
<tr>
<td><em>Serratia spp.</em></td>
<td>0.08</td>
<td>0.24</td>
<td>0.40</td>
<td>0.50</td>
<td>0.20</td>
</tr>
<tr>
<td><em>Proteus spp.</em></td>
<td>0.08</td>
<td>0.95</td>
<td>1.30</td>
<td>1.25</td>
<td>0.52</td>
</tr>
<tr>
<td><em>Morganella spp.</em></td>
<td>0.08</td>
<td>0.24</td>
<td>0.10</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>All coliforms</td>
<td>2.69</td>
<td>7.09</td>
<td>6.58</td>
<td>7.77</td>
<td>6.39</td>
</tr>
</tbody>
</table>
There was a significant increase in the proportion of quarters infected with coliforms between drying off (69/2565 quarters) and one week prior to calving (93/1197 quarters, p<0.001). There was a similar significant increase in *E. coli* infections (55/2565 quarters at drying off and 62/1197 quarters at one week prior to calving, p<0.01). There was no significant difference in the proportion of quarters infected with coliforms between three and two weeks pre-calving (p>0.2). There was a trend towards a higher prevalence of infection with the coliforms, in the late dry period, in higher parity cows. No seasonal effects were observed other than a significantly higher prevalence of infection at drying off in the summer than in the winter and spring.

Of 41 quarters infected with *E. coli* at drying off only 3 apparently persisted until calving, though subsequent fingerprinting demonstrated that in all cases this was due to re-infection with a different strain. New intra-mammary infections with *E. coli* were detected in 8.6% of quarters during the dry period. New quarter infections accounted for 97% of all *E. coli* infections detected during the dry period. New coliform infections were detected in 12.8% of quarters during the dry period. New quarter infections accounted for 96.6% of all coliform infections detected during the dry period.

**Mastitis in cows on the study**

153 cases of clinical mastitis occurred in cows on the study, within the first 100 days of lactation. In 40.5% cases a coliform was identified as the causal pathogen. *E. coli* was isolated on 50 occasions, *Klebsiella spp.* on 4, *Serratia spp.* on 6 and *Citrobacter spp.* on 2.

Of 1197 quarters sampled during the dry period, 154 (12.87%) quarters were found to be infected with a coliform at one or more of the sampling points. Some 13 (8.44%) of these infected quarters later developed mastitis due to the same species/strain of bacteria; these quarters had 20 cases in total. Conversely of 1043 quarters not infected with a coliform
during the dry period, 15 (1.44%) which then developed coliform mastitis, some 18 cases in total, there was a significantly greater risk of a previously infected quarter later developing mastitis than an uninfected quarter (p<0.001).

In quarters sampled during the dry period, a total of 38 cases of coliform mastitis occurred in 28 quarters. Of these 38 cases, 20 (52.6%) occurred in quarters previously infected, with the same species/strain of bacteria, during the dry period. In 71% of coliform mastitis cases, occurring in sampled quarters, the same species of pathogen had been detected previously (i.e. at drying off, pre- or post calving).

**Sampled and control quarters**

There was not a significantly increased incidence of coliform infections in the routine post-calving milk samples of quarters sampled during the dry period (82 of 1194 quarters) compared to those which were not sampled (71 of 1194 quarters) (p=0.40). There was also no significant increase in incidence of coliform mastitis in quarters sampled during the dry period (28 of 1194 quarters) than those not (21 of 1194) (p=0.39).

**DISCUSSION**

The data from this study on a small, though arguably typical, cohort of dairy herds would tend to support the anecdotal evidence, which has suggested an increase in the incidence of mastitis due to coliform organisms over the past few decades. The coliforms have classically been classified as opportunistic environmental pathogens capable of colonising the udder, causing a transient infection and mastitis, not uncommonly accompanied by severe systemic disease. Sub-clinical infections, although recognised (11,12) have not been implicated as a significant cause of subsequent disease.

The dry period has been implicated as a crucial period for acquisition of new Gram -ve intra mammary infections (IMIs), with >60% of all new IMIs occurring at this time (8). The data from this study would also support this finding as a significant rise in the level of infection was detected during the dry period.

Previous studies have been unable to implicate conclusively these new infections, acquired during the dry period, in subsequent mastitis (8). Also, the absence of unsampled control quarters means that the role of iatrogenic introduction of infection was unknown (8). The design of this investigation controlled for the effect of sampling quarters during the dry period and has more conclusively implicated dry period infections in subsequent mastitic episodes.

Perhaps the most compelling results generated from this study are that more than 50% of all clinical mastitis due to coliform organisms arose from quarters previously infected during the dry period; and that 71% of coliform mastitis occurred in quarters that had previously been found to be infected at any one time point. These phenomena can be ascribed to two possible scenarios; either bacteria reside within the udder awaiting conditions conducive to multiplication and subsequent disease, or certain quarters show an increased susceptibility to re-infection with the same species of pathogen. DNA fingerprinting studies of the coliform isolates from cases of mastitis apparently arising from dry period infections confirmed that this phenomenon was as a result of persistent infection and not re-infection as may previously have been assumed (10).
Clinical implications and dry period management options

An in-depth review of strategies for minimizing the risk of intra mammary coliform infections and subsequent clinical coliform mastitis is beyond the scope of this paper, however a few management options and issues, affecting the dry cow, are briefly outlined and discussed below

Method of drying off: The act of administering antibiotics to a potentially sterile quarter is the first potential area of hazard and the need for cleanliness in the procedure cannot be over-emphasised. Cleaning, disinfecting and drying the teat, followed by stripping the quarter and at least two teat-end scrubs with spirit is the minimum recommended prior to administration of an antibiotic tube and post dipping. The impact of infections introduced at this stage is difficult to quantify; they are unlikely to persist until the next lactation but can result in acute coliform mastitis prior to involution of the gland.

Dry cow therapy: The selection of antibiotic dry cow therapy may be an important element of a coliform mastitis control scheme. Studies currently being undertaken by the authors are investigating the potential impact of different dry cow formulations on subsequent coliform mastitis incidence.

Teat sealants: ‘External’ teat sealants are already available in the UK and though their efficacy under UK field conditions has yet to be established, they may aid in the control of new intra-mammary infections in the late dry period, by protecting the teat end from environmental contamination. An internal sealant ‘Teatseal’ (Bimeda) has shown promise in New Zealand as an alternative to antibiotic dry cow in preventing new *Streptococcus uberis* intra mammary infections during the dry period (13).

Environmental management: ‘Clean, dry, cool and comfortable’. It is common for dry cows to be housed in the oldest, poorest maintained buildings or kept on pasture with little attention, a foolhardy strategy, when the dry period is probably critical not only in terms of udder health, but also in other aspects of health and production. As a general rule, it is useful to ask the question ‘is the dry cow environment at least as good as for the milking cows’. It is rare that any new accommodation is built for dry cows but it is usually possible to make the best of existing facilities. Cubicles can be adapted and if used inorganic bedding materials such as sand will inevitably support less bacterial growth than straw.

Straw yards provide a better type of system than cubicles for foot health but are almost always worse for environmental mastitis pathogens. Recent work (14) has indicated that below the surface of even a recently bedded straw yard, warmth and moisture provide the ideal environment for sustaining environmental bacteria. Even using recommendations such as bedding up daily, cleaning out every 3-4 weeks and maintaining low stocking densities (10m² per cow bedded area and a cubic capacity of 15m³), infections will still occur. Even in well managed straw yards, intra mammary infections in the dry period are inevitable.

Mixing of groups: It has been reported that housing pregnant heifers and dry cows together increases the risk of *E. coli* mastitis (15). This is something that can often be avoided through the use of simple partitions, without huge building costs.
Leaking milk: Leaking of milk may be a risk factor for mastitis (Peeler, 1999). Problems with leaking milk may be associated with dry cows which are over-fed before calving (rare), are in sight/sound of the parlour which makes milk let down more likely, or are in the presence of calves (i.e. with recently calved cows). These should be avoided if milk leakage is a problem on the unit.

Nutrition: Negative energy balance (16) and number of feed spaces per cow (15) have been related to the severity or incidence rate of E coli mastitis respectively. Dry matter intakes, energy balance and mineral supplementation (particularly vitamin E and selenium (17)) in the late dry (transition) period, around calving and in early lactation are probably important in reducing clinical episodes of coliform mastitis.

Vaccination: Recently a vaccine for coliform mastitis, based on a rough (R) mutant E coli (strain J5) has become available in the UK. Use of the vaccine is not associated with a reduction in the number of new dry period coliform intra mammary infections (18), but has been shown to decrease the incidence and severity of clinical coliform cases. This vaccine will have a role, alongside other preventive measures, in the control of Gram-negative mastitis. It should be stressed that the vaccine is not a substitute for correct management but should be used as an aid to further reduce the incidence and severity of coliform mastitis.

FUTURE/ONGOING WORK

Many outstanding questions raised by this research which warrant further investigation. Why do only some quarters develop intra mammary infections and why do only some of these subsequently develop mastitis? Are quarters denuded of ‘commensal’ minor pathogens, by dry cow therapy more susceptible to infection? Also, why is there a delay between acquisition of infection and subsequent mastitis, and are there strains of E. coli adapted to colonising the bovine udder, which can persist and subsequently cause disease? It is hoped that some of these questions can be answered in further studies, drawing upon the vast stock of pathogens and samples acquired during this study. Additional work, currently underway, include intervention studies to attempt to decrease the incidence of new enterobacterial IMI in the dry period and mastitis in the subsequent lactation. Another interesting ongoing area of study is the feasibility of seasonal, selective use of dry cow therapy in low somatic cell count cows.

CONCLUSIONS

Preliminary findings from this study support those of previous studies and have far reaching implications for our understanding of ‘environmental’ mastitis and our approach to control of outbreaks of disease. In the past, control has centred on the management of the lactating and peri-parturient cow; these findings would suggest that significant effort also needs to be directed towards management of the dry cow. The apparently high ‘new infection’ rate from 3 weeks pre-calving would imply that the whole of the dry period is critical in preventing new infections and as such needs to be addressed by minimising bacterial challenge and enhancing pre-existing host defences.
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