A Study of the Incidence and Significance of Intramammary Enterobacterial Infections Acquired During the Dry Period

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ABSTRACT

We assessed the incidence of enterobacterial infection of the mammary glands of 629 cows, from six commercial herds in Somerset, during the nonlactating period; samples were collected from all clinical quarters of these cows during the subsequent lactation. A rise in the incidence of intramammary enterobacterial infection was detected between drying off and before calving. Quarters infected with an enterobacterial organism during the dry period were more likely to develop mastitis due to that pathogen than were uninfected quarters. Of all enterobacterial mastitis occurring in the first 100 d of lactation, 52.6% arose in quarters previously infected, during the dry period, with the same strain of bacteria, as identified by DNA fingerprinting using enterobacterial repetitive intergenic consensus primers. When compared with unsampled controls, quarters sampled during the dry period did not show a higher incidence of infection at calving or of subsequent clinical mastitis. These findings suggest that chronic infections are important in the epidemiology of enterobacterial mastitis and that environmental management during the dry period may greatly impact the incidence of enterobacterial mastitis in the subsequent lactation.

INTRODUCTION

Since its inception in the 1960s, implementation of the five-point plan has resulted in a marked decline in the incidence of contagious mastitis (1). This decline has not been accompanied by a comparable fall in the incidence of environmental mastitis (7). Classically, the nonlactating mammary gland has been considered refractory to enterobacterial infection (9). However, research in the United States from as early as 1943 has implicated the dry period as the time of greatest risk for the acquisition of new gram-negative IMI (5, 13, 14, 16, 17), with 61% of new IMI reported to occur at this time (17). Further studies have illustrated the ability of such infections to remain quiescent within the udder until calving, subsequently causing clinical mastitis in early lactation (12).

To date, similar studies to validate these findings have not been carried out in the United Kingdom. As a consequence, the importance of the dry period in the control of environmental mastitis in the United Kingdom remains equivocal.

The aim of the research outlined in this paper was to investigate the prevalence of IMI during the nonlactating period of dairy cattle, under UK field conditions and the subsequent impact of these infections on clinical mastitis incidence in the ensuing lactation. The effects of parity and season on incidence of infection were also investigated.

MATERIALS AND METHODS

Herd Selection

Six herds were selected on the basis of location (Somerset), low bulk milk SCC (3-mo geometric mean <250,000 cells/ml), nonseasonal calving pattern, and likelihood of owner compliance with the study protocol. The herds were not selected on the basis of a previous history of enterobacterial or environmental mastitis.

Cow Selection

All cows, from all six herds, dried off between February 10, 1997, and January 26, 1998, and calving between March 22, 1997, and April 4, 1998, were recruited to the study. No specific criteria (e.g., pedigree) were used in the allocation of line numbers to cows before the first lactation.

Housing

The dry cows in all six herds were managed at grass during the summer months and in cubicle or straw yard.
systems during the housing period. Periparturient cows were managed at grass or in loose boxes and lactating cows at grass or in cubicle housing systems.

**Sampling Strategy**

We collected duplicate quarter lacteal secretion samples from all four quarters at drying off and during the week following calving. During the dry period, duplicate samples were taken from two ipsilateral quarters (left front and left hind, odd line numbered cows or right front and right hind, even line numbered cows) once weekly during the 2 wk before the anticipated calving date. Two quarters remained unsampled as controls to assess whether the sampling procedure was a cause of IMI. Any cow not calving by its 'expected' date was sampled weekly until parturition. During the subsequent lactation, milk samples were collected from all clinically mastitic quarters as identified by the herdspersons who had been previously trained in sample collection (see below). Clinical mastitis was identified in quarters on the basis of the presence of abnormal secretion or udder changes (e.g., pain, heat, swelling).

**Sampling Procedure**

Teats were initially wiped to remove gross contamination and dipped in a solution containing 2800 mg/kg of available chlorine (Agrisept, Pharmacia and UpJohn, UK). Following a minimum 30-s contact time, the teats were wiped dry. Each teat was subsequently scrubbed with a cotton wool swab soaked in 70% ethanol and allowed to dry. Before the first sample was collected, the teat ends were scrubbed for a second time with 70% ethanol, and foremilk was discarded (except from udders assessed as having little secretion present during the dry period, when foremilk was collected). Following a third scrub of the teat ends, duplicate samples were collected. After sampling, teats were dipped in a solution containing 2800 mg/kg of available chlorine and cows were confined to a loafing yard for at least 30 min. Milk samples were immediately stored in a cool box and maintained at or below 4°C. Bacteriology assessment was usually performed within 24 h. Disposable gloves were worn throughout the sampling process and were changed between cows as well as between the first and duplicate samples.

During the first 100 d of lactation, mastitis cases were identified and sampled by the herdspersons using the same sampling protocol as outlined above. These were frozen and submitted once each week to the laboratory. Aliquots of all samples were stored for future use.

**Dry Cow Therapy**

No attempt was made to alter existing farm policy for dry cow antibiotic selection and usage. Dry cow products containing either cloxacillin (Orbenin Extra, Pfizer, UK), cephalonium (Cepravin, Mallinckrodt, UK) or procaine penicillin G (Mylipen, Mallinckrodt, UK) were used. Only one of these products was used on any one farm.

We administered dry cow therapy following collection of the 'drying off' samples. The teat ends were scrubbed with 70% ethanol for a fourth time before partial insertion of the tube canula. Following treatment, teats were dipped with a solution containing 2800 mg/kg of available chlorine, and cows were confined to a loafing yard for at least 30 min.

**Bacteriology**

Samples were submitted to the Langford Veterinary Investigation Centre for bacteriology. Ten microliters of secretion was inoculated onto sheep blood agar and Edward’s agar; 100 μl of secretion was inoculated onto MacConkey agar to enhance the detection of Enterobacteriaceae. Plates were incubated at 37°C and read at 24 and 48 h. Organisms were identified and quantified using standard laboratory techniques (15). *Escherichia coli* was identified by colony morphology, oxidase, and indole tests, and other *Enterobacteriaceae* were identified by a microtube identification system (RapiD 20 E, bioMérieux, UK). All pathogens were subject to two rounds of colony purification before storage by a commercial microorganism storage system (Cryopreservation Beads, Prolab, UK).

**Definition of Terms used for Analysis**

**Dry period.** The time between the infusion of dry cow antibiotic and calving.

**Intramammary infection: screening samples.** Isolation of an organism was considered to be an IMI. If a screening sample was obviously contaminated (e.g., contained *Bacillus* spp and fecal Streptococci) or contained more than one enterobacterial isolate, the duplicate sample was submitted for bacteriological examination, and IMI were diagnosed on the basis of reisolation of the organism.

**Cause of mastitis.** If an organism was isolated in pure growth, or was the predominant growth, then the organism was considered to be the cause of mastitis. A sample was called a 'mixed growth' if there was growth of two known mastitis pathogens. A sample was labeled 'contaminated' if more than three organisms were isolated.
Seasonality. The effect of season on infection rates was assessed. Seasons were ascribed as: Winter (Dec, Jan, Feb); Spring (Mar, Apr, May); Summer (Jun, Jul, Aug), and Autumn (Sep, Oct, Nov).

Parity. The effect of parity on infection rate was assessed. Parities two to five were assessed separately. Parities greater than five were assessed as a group.

Persistence and acquisition of infections. If an organism was present at drying off and was subsequently identified in the same quarter during the dry period or in the post calving sample, then the organism was considered to have persisted. A quarter was considered to have acquired a new infection if the organism was not present at drying off and was subsequently found to be present during the dry period.

Results Collation and Statistical Analysis

Results were collated and analyzed with Microsoft Excel, Microsoft Access, and Epi-Info (6). Drying off, calving, and sampling dates and the dates of mastitic episodes were recorded. Cow identities and parities were also recorded. Cows with a dry period of less than 30 d were excluded from the analysis. The $\chi^2$ test was used to determine whether infected quarters were more likely to get mastitis than uninfected quarters and to detect seasonal and parity variations. A Layered Bonferroni correction was used to compensate for multiple comparisons (3). A significance probability was set at $P \leq 0.05$ for a two-tailed test. No allowance was made for cows culled during the first 100 d of lactation since each cow had paired sampled and unsampled quarters.

DNA Fingerprinting

When an enterobacterial isolate was cultured from a clinical mastitis case occurring in a quarter previously identified as infected with the same species of enterobacterial organism, DNA fingerprinting was used to determine whether the same enterobacterial strain was present on each occasion.

Enterobacterial isolates of interest were retrieved from the microorganism storage system and grown overnight in LB broth (10 g of enzymic caesein digest/L, 5 g of yeast extract/L, 5 g of NaCl/L) at 37°C. Chromosomal DNA was extracted with a commercially available kit (QIAamp Tissue Kit, Qiagen, Valencia, CA). DNA sequences were then amplified by PCR with 50 ng of template DNA, enterobacterial repetitive intergenic consensus sequence primers, Taq polymerase (Qiagen), and a Hybaid Touchdown Thermalcycler. Following an initial denaturation step of 2 min at 94°C, the reaction was cycled 35 times under the following conditions: 30 s at 94°C (denaturation), 15 s at 40°C (annealing), 5 min at 72°C (extension), followed by a final extension step of 10 min at 72°C. After PCR, the enterobacterial strains were discriminated by their DNA polymorphism patterns with agarose gel electrophoresis and visualization under UV light (11, 18, 19). Enterobacterial strains exhibiting an identical DNA fingerprint were considered to be the same.

RESULTS

Sample Numbers

Quarter samples ($n = 2565$) were collected from 642 cows at drying off, and 2503 quarter samples were collected from 628 cows at calving. Calving samples were not obtained from 14 cows that were dried off and subsequently either calved after the termination of the study or were culled. Quarter samples ($n = 423$) were collected from 213 cows in the third week before calving because the calving date was later than expected. Quarter samples ($n = 1003$) from 502 cows and 1197 quarter samples from 599 cows were collected in the second and last weeks before calving, respectively, as a result of cows calving before their estimated date. A small number of cows had blind or nonfunctioning quarters, thus accounting for the disparity in quarter and cow numbers.

Prevalence of Infection

There was a significant increase in the proportion of quarters infected with Enterobacteriaceae between drying off (2.7%) (69 of 2565 quarters) and 1 wk before calving (7.8%) (93 of 1197 quarters, $P < 0.001$). There was a similar significant increase in E. coli infections, 55 of 2565 quarters at drying off (2.1%) and 62 of 1197 quarters at 1 wk before calving (5.2%), $P < 0.01$. There was no significant difference in the proportion of quarters infected with E. coli or Enterobacteriaceae at 3, 2, or 1 wk precalving or in the week after calving ($P > 0.2$). The percentage of quarters infected with each of the Enterobacteriaceae, at each sampling time point, is shown in Table 1.

Parity

The prevalence of infection by parity for E. coli and all Enterobacteriaceae is shown in Table 2. There was a trend toward a higher prevalence of infection with all Enterobacteriaceae in older cows at 2 and 1 wk precalving, although this was not reflected in the postcalving sample.
Table 1. Percentage of quarters infected with *Enterobacteriaceae* at each of the sampling time points.

<table>
<thead>
<tr>
<th></th>
<th>Drying off (n = 2565)</th>
<th>3 wk precalving (n = 423)</th>
<th>2 wk precalving (n = 1003)</th>
<th>1 wk precalving (n = 1197)</th>
<th>Postcalving (n = 2503)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.14a</td>
<td>4.73b</td>
<td>3.69b</td>
<td>5.18b</td>
<td>5.27b</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>0.27</td>
<td>0.71</td>
<td>0.80</td>
<td>1.00</td>
<td>0.32</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</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>Serratia</em> spp.</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>Citrobacter</em> spp.</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>Morganella</em> spp.</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>Proteus</em> spp.</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>Salmonella</em> spp.</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 <em>Enterobacteriaceae</em></td>
<td>2.69a</td>
<td>7.09b</td>
<td>6.58b</td>
<td>7.77b</td>
<td>6.39b</td>
</tr>
</tbody>
</table>

*a,b*Subcolumn means within row, time points with different superscripts differ (*P* < 0.05).

Seasonality

The prevalence of infection by season for *E. coli* and all *Enterobacteriaceae* is shown in Table 3. The prevalence of infection with all *Enterobacteriaceae* at drying off was significantly higher in the summer than in the spring and winter (*P* < 0.05). There were no significant differences between the seasons at any of the other sampling time points.

Persistence and Acquisition of New Infections in the Dry Period

The calculation of persistence and acquisition of new infections during the dry period is based on 1197 quarters sampled at least once during the dry period (Table 4). Of 27 quarters infected with *E. coli* at drying off, only three (11.1%) were detected at a later sampling point. New IMI with *E. coli* were detected in 8.6% of quarters (101 of 1170) during the dry period. New IMI accounted for 97.0% (*n* = 98) of all *E. coli* infections detected during the dry period. New IMI with *Enterobacteriaceae* at drying off, only five (14.3%) were detected at a later sampling point. New enterobacterial IMI were detected in 12.8% of quarters (149 of 1162) during the dry period. New IMI accounted for 96.6% (*n* = 144) of all enterobacterial infections detected during the dry period.

Mastitis

One hundred and fifty-three cases of clinical mastitis occurred in cows in the study within 100 d of calving; 85 of these occurred in quarters sampled during the dry period. In 40.5% of cases, a diagnosis of enterobacterial origin was made. *E. coli* was isolated in 50 cases, *Klebsiella* spp. in four cases, *Serratia* spp. in six cases, and *Citrobacter* spp. in two cases.

Control Quarters

There was not a significantly increased incidence of *E. coli* infections in the routine postcalving milk samples of quarters that were sampled during the dry period (67 out of 1194 quarters) compared with those which were not (60 out of 1194 quarters) (*P* = 0.58). Similarly, there was no significant increase in enterobacterial infections in the postcalving milk samples of quarters that were sampled during the dry period (82 out of 1194 quarters) compared with those which were not (71 out of 1194 quarters) (*P* = 0.40). There was also no significant increase in incidence of *E. coli* mastitis in quarters sampled during the dry period (22 out of 1194 quarters) than those not (19 out of 1194 quarters) (*P* = 0.75), nor of enterobacterial mastitis in quarters sampled during the dry period (28 out of 1194 quarters) compared with those not (21 out of 1194) (*P* = 0.39).

Table 2. Percentage of quarters infected with *Enterobacteriaceae*, by parity at each of the sampling time points.

<table>
<thead>
<tr>
<th></th>
<th>Drying off</th>
<th>2 wk precalving</th>
<th>1 wk precalving</th>
<th>Postcalving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 3 4 5 6 5</td>
<td>2 3 4 5 6 5</td>
<td>2 3 4 5 6 5</td>
<td>2 3 4 5 6 5</td>
</tr>
<tr>
<td></td>
<td>707 452 504 395 465</td>
<td>284 190 187 168 172</td>
<td>342 226 240 180 207</td>
<td>707 469 484 379 460</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.26 1.04 1.59 2.78 3.23</td>
<td>2.82 4.21 3.21 5.95 2.91</td>
<td>4.68 3.98 5.83 5.56 6.28</td>
<td>5.09 3.84 5.37 6.86 5.65</td>
</tr>
<tr>
<td>All <em>Enterobacteriaceae</em></td>
<td>2.55 1.66 2.18 3.04 4.30</td>
<td>4.58 5.26 4.81 11.31 8.72</td>
<td>5.56 4.87 7.50 10.56 12.56</td>
<td>5.37 4.90 5.99 8.44 8.26</td>
</tr>
</tbody>
</table>
Table 3. Percentage of quarters infected with *Enterobacteriaceae*, by season at each of the sampling time points.

<table>
<thead>
<tr>
<th>Season</th>
<th>Drying off</th>
<th>2 wk precalving</th>
<th>1 wk precalving</th>
<th>Postcalving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Summer</td>
<td>Autumn</td>
<td>Winter</td>
</tr>
<tr>
<td>n</td>
<td>656</td>
<td>637</td>
<td>707</td>
<td>565</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1.52a</td>
<td>3.45</td>
<td>2.26</td>
<td>1.24</td>
</tr>
<tr>
<td>All <em>Enterobacteriaceae</em></td>
<td>1.52a</td>
<td>4.55b</td>
<td>2.92ab</td>
<td>1.42a</td>
</tr>
</tbody>
</table>

Subcolumn means within row and time points, seasons with different superscripts differ (*P* < 0.05).

Table 4. Apparent persistence and acquisition of enterobacterial infections during the dry period.

<table>
<thead>
<tr>
<th></th>
<th>Quarters infected at drying off (no.)</th>
<th>Quarters infected at drying off (%)</th>
<th>Infections persisting through the dry period (no.)</th>
<th>Infections persisting through the dry period (%)</th>
<th>Quarters uninfected at drying off (no.)</th>
<th>Quarters acquiring an infection during the dry period (no.)</th>
<th>Quarters acquiring an infection during the dry period (%)</th>
<th>New infections as a proportion of infections detected in the dry period (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1197</td>
<td>27</td>
<td>2.26</td>
<td>3</td>
<td>11.1</td>
<td>1170</td>
<td>101</td>
<td>8.6</td>
</tr>
<tr>
<td>All <em>Enterobacteriaceae</em></td>
<td>1197</td>
<td>35</td>
<td>2.92</td>
<td>5</td>
<td>14.3</td>
<td>1162</td>
<td>149</td>
<td>12.8</td>
</tr>
</tbody>
</table>

*Based on quarters sampled at least once during the dry period.*
Outcome of Infections Acquired During the Dry Period

The outcome of infections acquired during the dry period is summarized in Table 5. Of the 1197 quarters, 104 (8.68%) were infected with *E. coli* at one or more of the sampling points during the dry period. Seven (6.73%) of these quarters later developed clinical mastitis due to *E. coli*; these quarters had 10 cases in total. Conversely, of the 1093 quarters that were not infected with *E. coli* during the dry period, 15 (1.37%) developed clinical *E. coli* mastitis, accounting for 18 cases in total. There was a significantly greater risk of a quarter previously infected (at any time during the dry period) later developing clinical mastitis than an uninfected quarter (*P* < 0.001).

One hundred and fifty-four (12.87%) quarters of the 1197 sampled during the dry period were infected with an enterobacterial organism during the dry period. Thirteen (8.44%) of these infected quarters later developed clinical mastitis due to the same species of bacteria, and these quarters had 20 cases in total. Conversely, 1043 quarters were not infected with an enterobacterial organism during the dry period and 15 (1.44%) developed clinical enterobacterial mastitis, accounting for 18 cases in total. There was a significantly greater risk of an infected quarter later developing clinical mastitis than an uninfected quarter (*P* < 0.001).

In quarters that were sampled during the dry period, a total of 38 cases of clinical enterobacterial mastitis occurred in 28 quarters. Of these 38 cases, 20 (52.63%) occurred in quarters that had previously been found to be infected with the same species of bacteria during the dry period. This pattern can be broken down by species to 35.71% of the *E. coli* mastitis (n = 28), 100% of the *Klebsiella* spp. mastitis (n = 4), 100% of the *Serratia* spp. mastitis (n = 5) and 100% of the *Citrobacter* spp. mastitis (n = 1). Clinical enterobacterial mastitis arising from quarters that became infected during the dry period occurred in all seasons and on five of the six farms included in the study. The time between first detection of an organism and subsequent cases of clinical mastitis was very variable (2 to 109 d). For quarters identified as infected during the dry period, the mean time elapsed between calving and subsequent cases of clinical *E. coli* mastitis was 27 d (1 to 83 d) and for all *Enterobacteriaceae* was 31 d (1 to 83 d).

In 71% of the 38 clinical enterobacterial mastitis cases occurring in the first 100 d of lactation, the same species of pathogen had been detected in the same quarter in an earlier screening sample (i.e., at drying off, pre- or postcalving).

### Table 5. Outcome of infection detected during the dry period.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Infected Quarters</th>
<th>Uninfected Quarters</th>
<th>Proportion of quarters uninfected</th>
<th>Proportion of quarters mastitis arising from infections detected during the dry period (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1197</td>
<td>104</td>
<td>8.68</td>
<td>15.00</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td>1197</td>
<td>8</td>
<td>0.67</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>1197</td>
<td>4</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.</td>
<td>1197</td>
<td>9</td>
<td>0.75</td>
<td>0.00</td>
</tr>
<tr>
<td>All <em>Enterobacteriaceae</em></td>
<td>1197</td>
<td>154</td>
<td>12.87</td>
<td>10.43</td>
</tr>
</tbody>
</table>

| a,bSubcolumn means within row, risk of developing mastitis with different superscripts differ (*P* < 0.05).
of entering the udder, causing a transient infection and mastitis, occasionally accompanied by severe systemic disease. Subclinical infections, although recognized (4, 8, 10) are not thought to be significant in the epidemiology of the disease. The dry period has been implicated as a crucial period for acquisition of new coliform IMI in the United States, with more than 60% of all new IMI occurring at this time (17). The data from our study support that finding, since a significant rise in the level of infection was detected during the dry period. Previous studies have been unable to satisfactorily implicate these new infections, acquired during the dry period, in subsequent mastitis (17). Also, the absence of unsampled control quarters means that the role of iatrogenic introduction of infection was a possibility (17). The design of this investigation controlled for the effect of sampling quarters during the dry period and has, by the use of DNA fingerprinting, more conclusively implicated naturally occurring dry period infections in subsequent mastitic episodes.

Studies in the United States suggest that the early and late dry periods are the times of maximal risk, correlating with involution and colostrogenesis, respectively (16), and that infections acquired in the early dry period are unlikely to persist until calving. The data from this study support this finding because few entero- bacterial infections (14.3%) appeared to persist through the dry period. However, the data from this study suggest that the dry udder is at risk before the onset of colostrogenesis because no further significant rise was found in the prevalence of infection between 3 and 1 wk precalving. The percentage of quarters and cows infected with the Enterobacteriaceae at any stage in the dry period is very similar in this study to the study carried out by Smith et al. (16) in the United States who reported infection in 7.7% (cf 6.7%) of quarters and 20.1% (cf 20.2%) of cows. It has been suggested (1) that the dry gland is resistant to entero- bacterial infection as a result of the high levels of lactoferrin, but this study suggested that the dry gland may be resistant to clinical entero- bacterial mastitis but that it is not resistant to the acquisition of new entero- bacterial IMI.

It is interesting to note the lack of seasonal variation in the percentage of quarters infected with entero- bacterial organisms. There were no significant differences between the numbers of quarters infected during the dry period in any of the seasons. One would perhaps have expected infection rates to be lower when the stock was at pasture in UK conditions. A possible cause of the lack of variation could have been that the summer of 1997 was comparatively wet, possibly leading to increased challenge. There was, however, a significant difference in the levels of infection at drying off, with more quarters being infected during the summer.

Figure 1. The DNA fingerprints of entero- bacterial isolates from quarters experiencing mastitis following acquisition of infection during the dry period. [M is the DNA standard in base pairs; (Lane 1 contains molecular weight markers (1-kb ladder, Promega)]. Lanes 1 to 7 contain Escherichia coli isolates from the right hind (RH) quarter of cow 744B at 26, 12, and 5 d precalving and from cases of mastitis on d 2, 41, 62, and 83 of lactation. Lanes 8 to 11 contain E. coli isolates from the left hind (LH) quarter of cow 167S at 15, 8, and 3 d precalving and from a case of mastitis on d 2 of lactation. Lanes 12 to 14 and 15 to 17 contain E. coli isolates from the left front (LF) and LH quarters of cow 55H, respectively, at 1 d precalving, calving, and from a case of mastitis on d 1 of lactation. Lanes 18 and 19 contain E. coli isolates from the LH quarter of cow 63S at 1 d precalving and from a case of mastitis on d 77 of lactation. Lanes 20 and 21 contain E. coli isolates from the LF quarter of cow D23F at 6 d precalving and from a case of mastitis on d 3 of lactation. Lanes 22 to 24 contain E. coli isolates from the RH quarter of cow B7F at 2 d precalving, calving, and from a case of mastitis on d 1 of lactation. Lanes 25 to 29 contain Klebsiella isolates from the RH quarter of cow 312B at 10 d precalving, calving, and from cases of mastitis on d 56, 62 and 68 of lactation. Lanes 30 to 32 contain Klebsiella isolates from the RH quarter of cow 232B at 2 d precalving, at calving, and from a case of mastitis on d 72 of lactation. Lanes 33 and 34 contain Serratia isolates from the LF quarter of cow B7F at 13 d precalving and from a case of mastitis on d 14 of lactation. Lanes 35 to 38 contain Serratia isolates from the LH quarter of cow B7F at 13 d precalving and from cases of mastitis on d 14, 20, and 30 of lactation. Lanes 39 and 40 contain Serratia isolates from the right front (RF) quarter of cow 52N at 6 d precalving and from a case of mastitis on d 1 of lactation. Lanes 41 and 42 contain Citrobacter isolates from the RF quarter of cow 18N at 2 d precalving and from a case of mastitis on d 2 of lactation.

DNA Fingerprinting

Results of the DNA fingerprinting of the entero- bacterial isolates apparently persisting from the dry period and subsequently causing clinical mastitis are illustrated in Figure 1. Fingerprinting confirmed in all cases that the mastitis arose as a result of persistence of the organism from the dry period rather than from acquisition of a new infection during lactation.

DISCUSSION

The Enterobacteriaceae have classically been classified as opportunistic environmental pathogens capable
autumn. These findings are difficult to explain, but the cause could be that these cows would have been in early lactation during the autumn and winter in their previous lactations and were challenged with high numbers of organisms, some of which may have persisted until drying off.

Perhaps the most compelling figures generated from this study are that more than 50% of all clinical mastitis due to enterobacterial organisms in the first 100 d of lactation arose from quarters that had previously become infected with the same strain of organism during the dry period (i.e., at 3, 2, or 1 wk precalving) and that 71% of enterobacterial mastitis occurred in quarters that had previously been found to be subclinically infected with the same species of pathogen (i.e., at drying off, 3, 2, and 1 wk precalving or at 1 wk postcalving). These findings question our current understanding of the epidemiology of enterobacterial mastitis. It would appear that enterobacterial organisms are able to persist in the udder for prolonged periods of time and subsequently recrudesce to cause disease. It may be that improved techniques, such as DNA fingerprinting, have allowed this phenomenon (which may have always existed) to be identified. Alternatively, it could be that this phenomenon represents a change in pathogenic behavior of enterobacterial organisms. It is possible that these organisms have become adapted for long-term survival in the bovine mammary gland rather than purely acting as opportunistic pathogens. The mechanisms for this adaptation have not yet been identified; this would be a useful area of further research.

One potential problem in detecting IMI with environmental pathogens is that samples collected could reflect contamination and not the true status of the gland. However, the sampling technique was fastidious, and the principal investigators collected all screening samples. The use of fingerprinting to trace isolates added power to the study by confirming that identical organisms were isolated from repeat cultures.

One constraint of this type of study is the need to use bacteriology as the means of identifying IMI. The bacteriological status of secretion expressed from the teat may bear little resemblance to what might be happening deep within the gland. Also, subclinical infections with the Enterobacteriaceae can exist with very low numbers of bacteria in milk (18). In an attempt to overcome this problem, a variety of solutions have been proposed, including an increased volume of inoculum, preincubation, or duplicate sample culture. In this study, 100 µl of secretion was plated onto the MacConkey agar plate to enhance the detection of the Enterobacteriaceae (16). Duplicate samples were collected at each of the individual time points, and these were utilized if the primary sample was identified as contaminated.

Another potential problem of this investigation was the reliability of identification of clinical cases of mastitis by the herdspersons. However, mastitis was probably well detected, as the herdspersons were trained, motivated, and had a heightened awareness of mastitis throughout the study.

Another difficulty experienced was in the interpretation of the bacteriology results from the screening samples. Attributing significance to bacterial isolates is not always straightforward; the concentration of bacteria that should be classified as an IMI is uncertain. Because of these difficulties, we decided to define an IMI on the basis of isolation of an organism. This may have slightly overestimated the prevalence of infection, but it would have led to an underestimate of the significance of those infections as a cause of mastitis in the subsequent lactation.

Many outstanding questions that warrant further investigation remain following this research. Why do only some quarters acquire IMI in the dry period and why do only some of these subsequently develop mastitis? Why is there a delay between acquisition of infection and subsequent mastitis, and are some or all tribes and strains of Enterobacteriaceae capable of colonizing the bovine udder, persisting and subsequently causing 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 required includes intervention studies to reduce dry period infection rates, such as the efficacy of dry cow therapy with Gram (−) activity and teat sealants.

CONCLUSIONS

The findings of this study suggest that significant numbers of intramammary enterobacterial infections are acquired during the dry period under UK field conditions and that quarters that acquire an infection are more likely to develop mastitis in the subsequent lactation. DNA fingerprinting has demonstrated persistence of enterobacterial organisms over long periods of time, and it may be that greater than 70% of enterobacterial mastitis occurs following a period of subclinical infection. These findings must alter our understanding of the epidemiology of enterobacterial mastitis and, therefore, our approaches to its control.

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REFERENCES