

## The prophylactic effect of a dry-cow antibiotic against *Streptococcus uberis*

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### Abstract

The prophylactic use of a dry-cow antibiotic for reducing the incidence of mastitis due to *Streptococcus uberis* was studied in four seasonally calving dairy herds involving 378 cows. The treatment was a long-acting dry-cow antibiotic preparation administered immediately after the last milking of lactation. New intramammary infections were identified by comparing the bacteriological status of quarters at drying off with that after calving, or through manual udder palpation during the dry period. The administration of dry-cow antibiotic to uninfected quarters at drying off reduced the overall incidence of new infections with *Streptococcus uberis* from 12.3% for untreated quarters to 1.2% of quarters ( $p < 0.01$ ). The reduction was significant ( $p < 0.01$ ) for both dry-period and post-calving infections. The susceptibility of uninfected quarters to new infection by *Streptococcus uberis* appeared to be unrelated to the infection status of a cow at drying off. Clinical infections during the dry period were most prevalent (97%) in quarters identified as having open teat canals. Fewer open teat canals ( $p < 0.05$ ) were observed among antibiotic treated quarters over the first 4 weeks of the dry period. Treated quarters had a lower ( $p < 0.05$ ) incidence of new clinical infection during the ensuing lactation and lower somatic cell counts. This did not affect production levels of milk, milk fat or protein. The results clearly indicated a prophylactic benefit for the dry cow antibiotic treatment against new *Streptococcus uberis* infections during the dry period. (New Zealand Veterinary Journal 43, 228–234, 1995.)

### Introduction

Dry-cow therapy (DCT) has generally been used in New Zealand herds at the end of lactation to cure existing intramammary infections (IMIs), rather than for the prophylactic protection of uninfected quarters during the dry period<sup>(1)</sup>. A survey in 1986 of 2460 seasonally calving New Zealand herds<sup>(2)</sup> found that 81% of herd owners used "selective cow" DCT based on individual cow records of clinical infection and somatic cell count (SCC) levels, but for purposes of therapy rather than prophylaxis. Treatment of all quarters of all cows was reported to be used in only 7% of herds.

Cows are particularly susceptible to new IMIs with the environmental pathogen *Streptococcus uberis* during the early dry period<sup>(3)</sup> and around calving<sup>(4)</sup>. This pathogen is generally considered to be the most common cause of dry period mastitis in New Zealand (pers. comm; data on seasonality of submitted samples made available by Ruakura Animal Health Laboratory) although there are few recent data on prevalence from herd studies. *Streptococcus uberis* is also known to contribute to the early season incidence of milk quality downgrades (pers. comm.; data made available by the South Auckland Independent Testing Laboratory, Hamilton).

The administration of persistent antibiotic formulations at drying off has been demonstrated repeatedly to reduce new dry period IMIs<sup>(5)</sup>. However, the prophylactic role of DCT against *Streptococcus uberis* over this period has not been closely studied in New Zealand herds.

Pankey *et al.*<sup>(1)</sup> used cows in seven seasonal supply

New Zealand herds to demonstrate a reduction of 60% in new dry-period infections due to *Staphylococcus aureus* when an antibiotic, claimed to be of high persistency, was administered at drying off. No apparent effect on the incidence of new *Streptococcus uberis* infections was observed, although cows used in the study had been pre-selected on the basis of being infected with *Staphylococcus aureus* prior to drying off. A recent Australian study<sup>(6)</sup> compared four DCT selection strategies involving 1044 cows in 12 Victorian dairy herds. Reductions in new dry-period IMIs for *Streptococcus uberis* (21 v. 6,  $p < 0.01$ ) were associated with DCT treatment of quarters uninfected at drying off.

Prophylactic administration of dry-cow antibiotics to uninfected quarters could, however, predispose to infection by disruption of the teat canal epithelium, by the accidental introduction of pathogens resistant to the antibiotic, or through disruption of the normal intramammary micro-flora. Browning *et al.*<sup>(6)</sup> found the incidence of clinical mastitis in the season following the administration of DCT to uninfected quarters increased by almost 50% ( $0.05 < p < 0.1$ ). Similarly, Macmillan *et al.*<sup>(7)</sup> found the incidence of clinical mastitis to be higher in the following lactation for uninfected cows which had been DCT-treated in a study using ten New Zealand dairy herds. Cagienard<sup>(8)</sup> indirectly studied the prevalence of clinical mastitis in 142 seasonal-supply herds in New Zealand by dividing the herds into three ranges of mastitis prevalence based on the purchase of lactating cow antibiotics. For herds in the high prevalence range, clinical cases inferred from antibiotic use were less frequent in the season following the use of DCT. However, in low infection herds, the use of DCT was followed by an increase in the number of inferred clinical cases during the following season.

Control of early lactation mastitis in New Zealand has become a key issue following the recent imposition of penalties for bulk tank SCC levels above 400 000/ml.

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**Table I. General details of the experimental herds**

Farm no.	No. of cows <sup>a</sup>	Relationship	Breed <sup>b</sup>	Milkfat <sup>c</sup> (kg/cow)	SCC <sup>c</sup> (cells/ml)	Drying-off date
1	66	Twins	F × J	162	82 000	23.04.92
2	108	Unrelated	F	—	—	16.04.92
3	104	Unrelated	F	191	190 000	01.05.92
4	100	Unrelated	F	171	376 000	24.04.92

a Number of experimental cows within the herd.

b Classified as Friesian-Jersey cross (F × J) or Friesian (F).

c Herd mean values from herd test data for the previous lactation.

General field experience indicates that early lactation mastitis is usually attributable to the pathogen *Streptococcus uberis*, although few data are available. The objective of the present study was to determine whether DCT was of prophylactic value in reducing the incidence of mastitis due to *Streptococcus uberis* in the dry period and during early lactation under New Zealand conditions.

## Materials and Methods

### Herds

Two research and two commercial dairy herds were included in this study (Table I). They were seasonally calved during July-August 1992 and were located in the Central Waikato within 30 km of Hamilton. The research herds comprised cow subgroups within the Dairying Research Corporation (DRC) herds No. 1 and No. 5 (designated Farms No. 1 and No. 2 respectively in this paper). Cows within the DRC No. 1 Herd comprised identical twins. Although 378 cows were initially allocated to the study, attrition reduced this to 371. All cows had previously completed at least one lactation.

### Drying off management

Cows in each herd were milked once daily for at least 1 week before the date of drying off (April-May 1992). Milk production levels were in the range 4-8 l/cow/day during this time. In every case, the drying off strategy was abrupt cessation of once daily milking at the drying off date. The DCT antibiotic was administered to the treated quarters immediately following the removal of teat cups at the end of the last milking.

### Bacteriological sampling

Duplicate foremilk samples were taken from each quarter using aseptic technique within the last 7 days of lactation, then again during the following lactation at 1-4 days after calving. Each quarter identified by palpation as likely to be infected during the dry period was also sampled aseptically for bacterial culture. Teat ends were first scrubbed with cotton wool swabs soaked in 70% alcohol, the first two to three squirts of foremilk discarded and then a sample of about 20 ml of milk was drawn into each of two sterile containers. The samples were collected before the attachment of teat cups at a morning milking and transferred to the laboratory for bacteriological culturing within 2 hours.

### Microbiological procedures

A sub-sample (0.01 ml loop) of foremilk was streaked on to a quarter-plate of Columbia blood agar containing 5% whole bovine blood and 0.1% aesculin. Plates were incubated at 37 °C and examined at 24 and 48 h. Identification of isolates was from colony morphology, haemolysis, aesculin reaction, Gram staining and tube coagulase reaction. Where appropriate, colonies were subcultured on to Edward's medium (modified) containing 5% bovine blood, which is a selective medium for the identification of aesculin-positive strepto-

cocci, and incubated at 37 °C for 48 hours to identify *Streptococcus* spp. As all samples were collected using aseptic methods, aesculin splitting species were considered to be *Streptococcus uberis*. There was a slight possibility of misclassification between *Streptococcus uberis* and *Streptococcus dysgalactiae*, but experience has shown the latter pathogen to be uncommon in New Zealand herds. Unusual or atypical isolates were sub-cultured for more specific identification. Both coagulase-negative staphylococci (CNS) and *Corynebacterium bovis* were classified as minor pathogens in summarising the bacteriological results.

A quarter was classified as infected if the same organism was cultured from both of the foremilk duplicate samples. It was classified as uninfected if pathogenic organisms were not cultured, or if they were isolated in only one of the duplicate samples.

### Treatment groups

Having determined the udder health status prior to drying off, cows were then grouped (Figure 1) on the basis that those with one or more infected quarters were assigned to one of two groups for infected animals (I-TQ and I-TC) while those with all four quarters bacteriologically negative were assigned to one of another two groups (NI-NT and NI-AT). It is possible that cows classified as uninfected on the basis of the bacteriological results had previously been infected and subsequently cured.

The experimental groups were matched as far as possible for cow age, milk production and breed. The infected groups had a mean age of 6.3 years with 29% of these cows being 3 or 4 years of age. The corresponding data for cows in the uninfected cow group were 5.3 years and 43%, respectively.

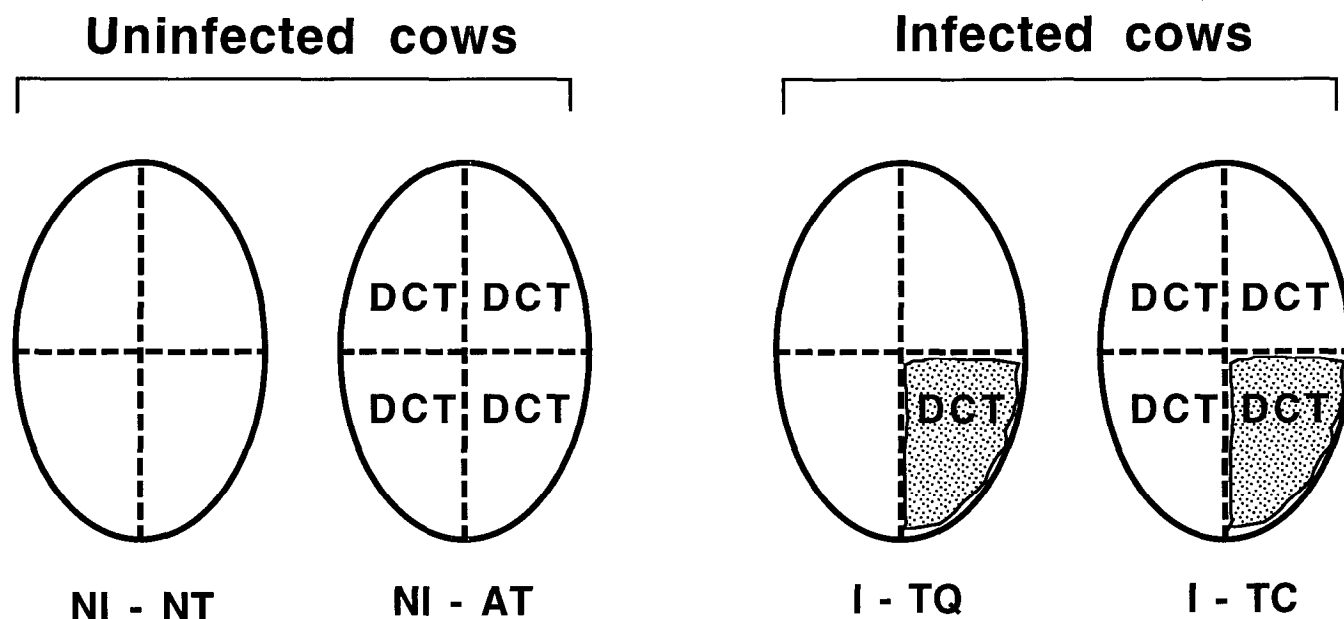
### DCT treatments

All quarters of cows in the NI-AT and I-TC groups, and infected quarters in the I-TQ group, were treated with dry-cow antibiotic. These quarters were infused once with 250 mg of cephalonium antibiotic (Cepravin Drycow; Pitman Moore (NZ) Ltd, Upper Hutt) by research technicians immediately after the last milking of the lactation. Teat ends were first sanitised with cotton wool soaked in alcohol and the nozzle of the antibiotic tube inserted typically 10 mm into the teat canal. The antibiotic was massaged upwards into the gland cistern after infusion and all teats were then sprayed with an iodine-based sanitiser (typically 0.2% iodine) containing an emollient.

### Dry period examinations

Udder examinations to detect the presence of IMIs were carried out on all cows starting at 7 days after drying off and continuing at intervals of 14 days throughout the dry period until the majority of cows in each herd had calved. All of these examinations were carried out by one author (AMD), being blind observations without knowledge of any quarter treatments or previous results.

Each udder examination involved palpation of individual quarters to detect any difference in tissue firmness between adjacent quarters and hence the presence of clinical IMIs. In



**Figure 1.** Definition of cow and quarter groups and associated DCT treatments. NI - NT = cow not infected, no quarters treated with DCT; NI - AT = cow not infected, all quarters treated with DCT; I - TQ = cow infected, treat infected quarters only with DCT; I - TC = cow infected, treat cow with DCT. [stippled] = infection.

addition the status of the teat canal keratin seal was assessed. This involved applying light pressure to the teat sinus by using a gentle milking action with the thumb and index finger, allowing the contents to slip upwards within the teat. If the pressure applied to the teat sinus by this action resulted in a drop of secretion appearing at the orifice, that teat was classified as being open; if not, it was classified as closed. While the technique clearly involved operator variables, all observations were carried out on a blind basis.

Surgical gloves were worn for all examinations and sanitised in an iodine solution after the examination of each udder. Every teat was sprayed with iodine and an emollient following each examination.

Quarters determined to be clinical by palpation were sampled aseptically in duplicate for bacterial culture and then treated with three tubes of lactating cow antibiotic (Orbenin LA, 200 mg cloxacillin/tube, Beecham (NZ) Ltd, Auckland) at intervals of 48 h and finally with one tube of dry-cow antibiotic (Cepravin Drycow, 250 mg cephalonium). After the fourth week of the dry period, new IMIs were treated only with the lactating cow antibiotic, so as not to risk the presence of inhibitory substances in the milk supply after calving.

#### Post-calving measurements

Somatic cell counts, milk, milkfat and protein production were measured periodically for all cows over the ensuing lactation. In the case of the field herds, these data were obtained from the Livestock Improvement Corporation (LIC) herd testing system. The data for the research herds were from routine weekly sampling and milk analyses, but the LIC service was used for SCC data. Three sets of production and SCC data were obtained during the period from August to February, with the timing for individual farms depending on herd testing dates.

All four farms maintained records of clinical mastitis cases identified during milking.

Clinical infections were identified from clots or discolouration in milk samples collected or abnormal quarters diagnosed by individual milkers.

#### Somatic cell counting

Concentrations of somatic cells were measured using a

Fossomatic (Foss Electric, Denmark) system on the second of the duplicate foremilk samples obtained at drying off and after calving.

Routine somatic cell counting was completed for all cows during the ensuing lactation using samples drawn from LIC herd testing meters. Data from three of these tests (the timing of which varied between farms) were analysed within each herd. In the case of Herd No. 2, the somatic cell counting was completed only on morning milk samples, whereas a composite morning-afternoon sample was used for cows in the other three herds.

#### Statistical procedures

Analysis of variance was used to compare the production and SCC data for treated and control groups within the infected and uninfected cow subgroups. In the case of data for milk, milk fat and protein yields, days in milk were used as a covariate. Somatic cell counts were log transformed for analysis.

The incidence of IMIs and proportional comparisons were analysed by chi-square testing from contingency tables or logit models.

## Results

#### New infections

Incidences of new infections were pooled across all four herds for the four experimental quarter groups, into those occurring during the dry-period and those identified at the post-calving aseptic sampling (Table II).

#### General incidence of infection

Overall the incidence of new IMIs was 3.9% (clinical) of quarters over the dry period and 7.1% (clinical + subclinical) after calving. The 52 quarters diagnosed as new clinical infections by udder palpation during the dry period gave bacterial isolations comprising 90% *Streptococcus uberis*, 8% *Staphylococcus aureus* and 2% minor pathogens.

Following calving, a total of 94 (7.1%) quarters was

**Table II. Numbers of new IMIs confirmed during the dry period and after calving in cows classified as not infected (NI) or with at least one infected quarter at drying off (I); uninfected quarters at drying off were either treated with dry cow therapy (AT and TC) or were untreated controls (NT and TQ). Levels of significance are interpolated between the pairs of values which were compared<sup>a</sup>**

Experimental group	NI-NT		NI-AT		I-TQ		I-TC		Total
Total uninfected quarters at drying off	516		556		141		120		1333
New dry-period clinical IMIs									
<i>Staphylococcus aureus</i>	2	ns	2		0	ns	0		4
<i>Streptococcus uberis</i>	32	**	5		10	**	0		47
Minor pathogens	1	ns	0		0	ns	0		1
Total new IMIs	35	**	7		10	**	0		52
Overall new IMIs rate (%)	6.8		1.3		7.1		0		3.9
New post-calving IMIs									
<i>Staphylococcus aureus</i>	4	ns	4		5	ns	1		14
<i>Streptococcus uberis</i>	33	**	2		6	ns	1		42
minor pathogens	16	ns	18		4	ns	0		38
Total new IMIs	53	**	24		15	**	2		94
Overall new IMIs rate (%)	10.3		4.3		10.6		1.7		7.1
Total <i>Streptococcus uberis</i> IMIs	65	**	7		16	**	1		89
Overall <i>Streptococcus uberis</i> IMI rate (%)	12.6		1.3		11.3		0.8		6.8

a ns =  $p > 0.05$ ; \*\* =  $p < 0.01$ .

identified from bacteriological culture as having new IMIs, with 45% being *Streptococcus uberis*, 15% *Staphylococcus aureus* and 40% minor pathogens. The overall incidence of new *Staphylococcus aureus* infections in this experiment was 0.3% of quarters in the dry period and 1.1% of quarters after calving. Minor pathogens were cultured from 2.9% of post-calving samples with about 90% of isolates being CNS.

### ***Streptococcus uberis***

Dry cow therapy treatment of uninfected quarters was associated with a major reduction in the incidence of clinical IMIs due to *Streptococcus uberis* during the dry period. Pooled across infected and uninfected cow groups, the incidences were 0.7% for DCT-treated quarters and 6.4% for untreated quarters ( $p < 0.01$ ). The differences were significant ( $p < 0.01$ ) within both infected and uninfected cow classifications. The DCT treatment was also associated with a significantly lower incidence of new *Streptococcus uberis* infections, 6.4% v. 0.4% ( $p < 0.01$ ) for the uninfected (NI) cow classification after calving.

Pooling the dry period and post-calving data for the two uninfected cow groups (NI-NT v. NI-AT), there was a reduction (12.6% v. 1.3%,  $p < 0.01$ ) in the overall incidence of quarters infected with *Streptococcus uberis* for the DCT treatment.

Uninfected quarters of cows classified as infected at drying off (I-TQ and I-TC groups) also showed a significant ( $p < 0.01$ ) overall reduction in the incidence of new *Streptococcus uberis* infections associated with the DCT treatment when pooled across the two experimental periods (11.3% v. 0.8%).

### ***Staphylococcus aureus***

New IMIs due to *Staphylococcus aureus* were infrequent. Only four (0.3% of quarters) new *Staphylo-*

*coccus aureus* infections were identified during the dry period and 14 (1% of quarters) during the post-calving period. No significant differences were observed between DCT-treated and untreated quarter groups.

### **Minor pathogens**

New IMIs due to coagulase-negative staphylococci and *Corynebacterium bovis* were negligible during the dry period. After calving there were no significant differences in the incidence of IMIs due to minor pathogens between DCT-treated and untreated quarter groups.

### **Susceptibility to infection for infected and uninfected cows**

For quarters classified as uninfected at drying off and which were not treated with DCT (NI-NT and I-TQ groups), there was no significant difference in the overall incidence of new IMIs due to *Streptococcus uberis* between infected and uninfected cow groups (12.6% v. 11.3% respectively). This was the case both during the dry period and at the post-calving sampling.

### **Cure rates for infected quarters**

A total of 152 quarters were identified as infected by bacteriology at drying off, an average of 1.5 infected quarters/cow for the I-TQ and I-TC groups combined. The prevalence of *Streptococcus uberis*, *Staphylococcus aureus* and minor pathogens pooled across these groups at drying off was 3.8%, 2.2% and 5.3% of total quarters ( $n = 1333$ ) respectively.

The cure rates achieved by the DCT treatment administered to these quarters were similar in both I-TQ and I-TC cow groups, pooled values being 88% for minor pathogens, 79% for *Staphylococcus aureus* and 78% for *Streptococcus uberis*.

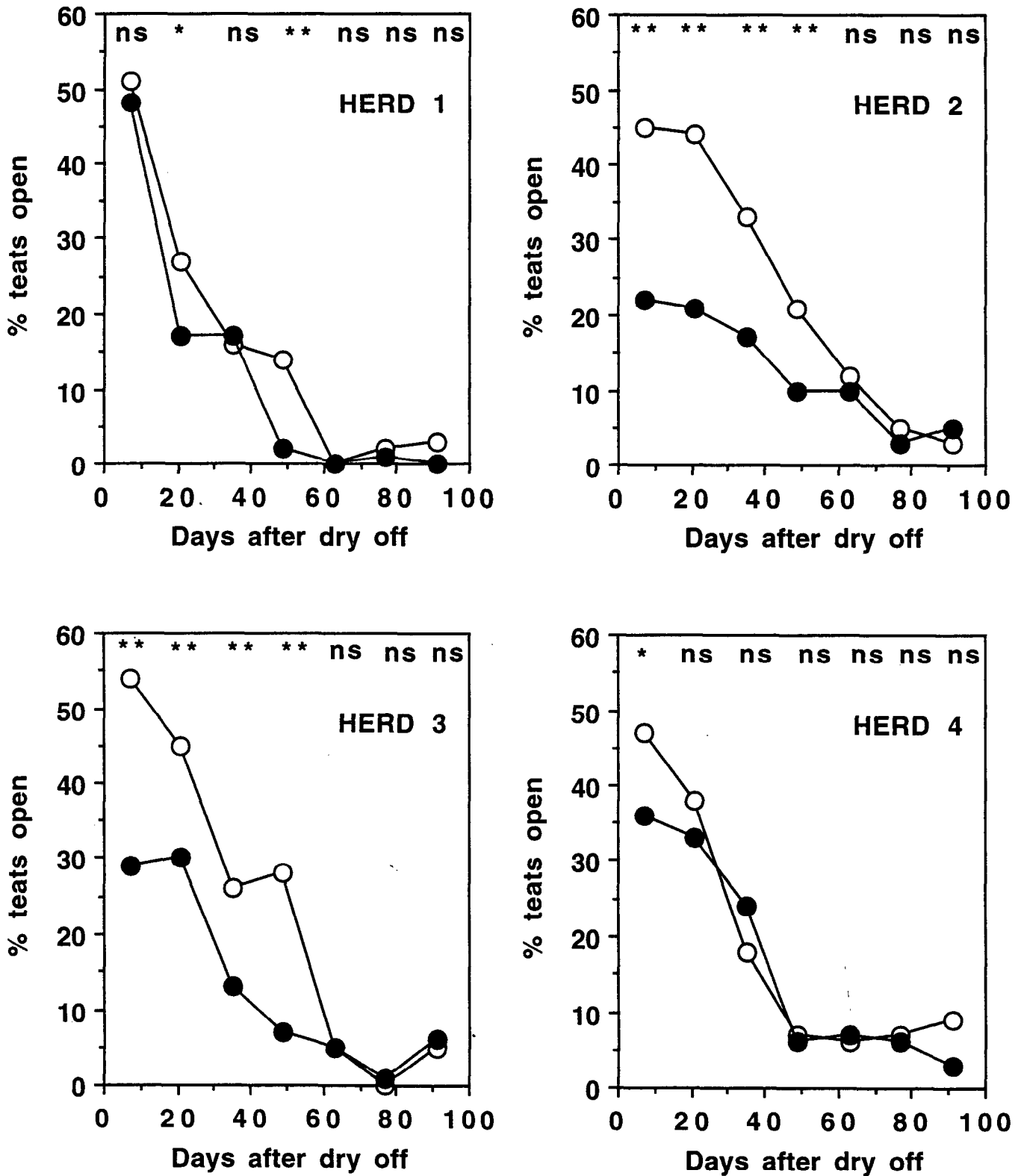


Figure 2. Trends in the percentage teats open throughout the dry period for each of the four farms. -●- = NI - AT and I - TC groups; -○- = NI - NT and I - TQ groups. ns = p > 0.05; \* = p < 0.05; \*\* = p < 0.01.

**Dry period teat sealing**

Trends in the percentage of open teats over the dry period are plotted individually for the four farms for both DCT-treated and untreated control quarters (Figure 2). These data have been pooled for both infected and uninfected cow groups.

The incidence of open teats was highest for the

untreated controls, using data pooled across all four farms, up to 49 days after drying off (p<0.05). In the case of Herds 2 and 3, the incidence of open teats in untreated quarters was typically double (p<0.01) that of the DCT-treated quarters, until about 50 days after drying off. For Herds 1 and 4, the decline in the incidence of open teats was very similar for treated and

untreated groups, although on a few occasions significantly fewer open teats ( $p < 0.05$ ) were recorded in the treated groups. Cows in all four herds showed similar initial percentages of open teats at 7 days after drying off (range 45-55%) for untreated quarters and about 95% of teats were observed to be closed at 60 days.

For all groups, there was typically a linear decline in the percentage of open teats over an initial period of 50-60 days. Over the period from 50-90 days after drying off, both DCT-treated and untreated groups had a residual incidence of from 3% to 5% of open teats and were not significantly different.

On all observation dates, the incidence of open teats was found to be higher for rear quarters than for front quarters, although the differences analysed by a logit regression model were significant ( $p < 0.01$ ) on only two occasions.

The repeatability of the observations on whether teats were open was analysed by comparing sequential pairs of observation sessions, pooled across treatments. In the first two sessions after drying off, 10% of teats classified initially as closed were classified subsequently as open at the following session. This proportion declined progressively to about 3% for the last two sessions.

#### Dry period infection pattern

Overall, 83% of all clinical IMIs during the dry period occurred within 21 days of drying off. Most of the difference in the incidence of IMIs between DCT-treated and untreated quarters developed over this period. Of the clinical IMIs identified, 97% were in quarters which at that time had an open teat canal.

#### Data from the following lactation

##### Somatic cell counts

Mean SCCs in the following lactation for the four experimental groups are given for all four herds in Table III. Trends for the four farms were similar, in that DCT treatment of all four quarters at drying off (NI-

AT and I-TC groups) was in most cases associated with lower SCC levels in the following lactation, although the differences were not always significant.

For cows in the uninfected group, the reduction in mean SCC associated with DCT treatment was about 30% using data pooled across tests and herds. Cows in the infected groups had higher SCCs, but also showed a reduction (49%) in count as a consequence of treating all quarters (I-TC).

The relatively low SCCs for the two infected cow groups is a consequence of the high overall cure rate achieved by the DCT at drying off.

##### Clinical mastitis after calving

The incidence of clinical mastitis recorded by the farmers in the following lactation was lower for DCT-treated quarters. The incidence of clinical IMIs for cows uninfected at drying off was 21% for the untreated (NI-NT) group and 12% for those in the treated (NI-AT) group ( $p < 0.05$ ). The comparable figures for cows infected at drying off were 38% and 22% for untreated (I-TQ) and treated (I-TC) cows respectively ( $0.05 < p < 0.1$ ).

These data relate to the 8-month period to the end of March 1993 for Herds 1, 3 and 4 but only to the end of Sept 1992 for Herd 2.

##### Production levels

There were no significant differences ( $p > 0.05$ ) between milk, milk fat or protein production levels for the NI-NT v. NI-AT or for the I-TQ v. I-TC groups.

## Discussion

The administration of the DCT treatment to uninfected quarters at drying off reduced the number of new clinical IMIs due to *Streptococcus uberis* during the dry period, regardless of whether a cow was classified as infected or uninfected at drying off. This treatment was also associated with a lower incidence of clinical mastitis after calving. These findings strongly suggest benefits in using DCT as a whole herd prophylactic strategy. Most of the new clinical IMIs in the untreated controls occurred within the first 21 days of the dry period. Studies<sup>(10)</sup> of changes in the histology of the teat canal after drying off suggest an initial dilation of the canal lumen for up to 7 days, with a keratin plug then forming over the following 14-21 days. Taken together, these observations suggest an initial period of susceptibility during which antibiotic resident in the mammary gland may inhibit the entry or multiplication of pathogens.

Quarters identified as having clinical IMIs during the dry period were treated with antibiotic and about 80% were cured. Had these quarters not been treated, some may have spontaneously self-cured, while others would have persisted and appeared as subclinical or clinical IMIs after calving. It is not known how persistent *Streptococcus uberis* IMI may be during the dry period under New Zealand conditions. Consequently, it is possible that through treating all clinical dry-period IMIs, the observed incidence of new post-calving IMIs was reduced and the apparent incidence of dry-period clinical IMIs increased.

**Table III. Weighted somatic cell counts for individual herds and treatment groups on three occasions during the lactation following treatment. Levels of significance are interpolated between the pairs of values which were compared<sup>a</sup>**

Herd	Test	Month	Somatic cell count ('000 cells/ml)				
			NI-NT	NI-AT	I-TQ	I-TC	
1	1	Sep	108	*	59	122 ns	48
	2	Nov	62	ns	42	92 ns	59
	3	Feb	48	ns	41	98 ns	50
2	1	Aug	116	ns	92	244 **	82
	2	Sep	78	ns	58	176 **	52
	3	Oct	83	ns	102	238 **	63
3	1	Sep	82	ns	51	122 ns	88
	2	Nov	98	*	64	185 ns	156
	3	Feb	102	ns	74	132 ns	198
4	1	Sep	114	**	35	130 **	57
	2	Nov	82	*	49	191 **	59
	3	Feb	85	ns	68	249 **	96

<sup>a</sup> ns =  $p > 0.05$ ; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$

The incidence of new IMIs due to *Streptococcus uberis* after calving also appeared to be significantly reduced by the DCT treatment. However, it is probable that, while some of these *Streptococcus uberis* infections in the NI-NT and I-TQ groups may well have originated close to calving, others would have persisted subclinically through from the dry period undetected by the periodic udder palpations. We could not quantify these effects.

Clinical IMIs in the ensuing lactation were significantly lower for the DCT blanket-treated cow groups. While this is in agreement with the survey data of Laycock *et al.*<sup>(2)</sup>, it conflicts with other reports<sup>(6)(7)(8)</sup>. It is possible that sanitising the end of the teats before infusing antibiotics is important and may account for the variable results reported.

The lower SCCs for the blanket treatment groups (NI-AT and I-TC), compared with the selective quarter groups (NI-NT and I-TQ), may indicate a lower carry-over of subclinical infection from the dry period into lactation as a consequence of effective prophylaxis.

Comparing the incidence of IMIs for the NI-NT and I-TQ quarter groups showed that cows classified as infected at drying off were no more susceptible to dry-period *Streptococcus uberis* IMI than were uninfected cows. The validity of this comparison depends on the assumption that the probability of a new dry-period IMI is independent of a cow's infection status at drying off. Binomial probabilities for the expected incidence of independent multiple quarter infections did not differ significantly from observed dry-period values. A significant departure ( $p < 0.05$ ) from the independence model occurred in the post-calving period and this suggests that predisposing within-cow factors were more important during lactation.

An important new observation from our study was the earlier closure of the teat canal after drying off for quarters which received DCT treatment. This effect was pronounced on two of the four farms. The clear implication from these teat closure observations was that physical sealing of the teat canal after drying off was facilitated in some way by the DCT treatment.

From udder palpations during the dry period, Day<sup>(9)</sup> reported that quarters with closed teat canals were rarely found to be clinically infected. The present study confirms these findings, and suggests that once a physical keratin seal has formed in the teat canal after drying off, an uninfected quarter has a very low risk of infection over the remainder of the dry period. It should be noted, however, that quarters were not routinely bacteriologically sampled during the dry period. Instead, udder palpations were relied on to detect abnormal quarters, and only these were subsequently bacteriologically sampled. Some closed teats may have developed infections which were not detected by udder palpations, but which were subsequently identified bacteriologically as post-calving infections.

The dry period examinations indicated that it took 30-40 days for a 50% decline in the incidence of open teats. Other studies of the involuting teat canal indicated that the canal became occluded with a solid keratin mass over 30 days or more, although in some cows a functional plug had formed by 16 days<sup>(10)</sup>.

The mechanism(s) responsible for the relationship which we observed between DCT treatment and teat canal closure are not obvious. One possibility is that occlusion of the teat canal lumen with keratin may be impeded by any resident bacterial colonisation. Such colonisation has been observed within the keratin layer during involution of the canal<sup>(10)</sup>. Bacterial enzymes and associated lipolytic or other degradation effects within the canal may slow the build-up of a solid mass of desquamated cells. The killing of such bacteria by antibiotic left in the teat canal or teat sinus at the time of DCT may thus increase the rate of sealing. Whether any residual antibiotic is retained in the lumen of the canal may also be dependent on the infusion technique<sup>(11)</sup>.

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