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A comparison of broad-spectrum and narrow-spectrum dry cow therapy used alone and in combination with a teat sealant

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ABSTRACT

The dry period is a critical time in the lactation cycle, offering the optimum time for cure of existing intramammary infection (IMI), while also encompassing the periods of highest susceptibility to new intramammary infection. Until recent years, intramammary infection in the dry period has been controlled with the use of antibiotic dry cow therapy. The aim of this study was to investigate 3 different dry cow therapy regimens, in low-somatic cell count (SCC; bulk milk SCC < 250,000 cells/mL) herds in southwest England. A total of 489 cows was recruited to the study and randomly allocated to receive either the broad-spectrum antibiotic cefquinome, a combination treatment comprising the narrow-spectrum antibiotic cloxacillin and an internal teat sealant, or the narrow-spectrum antibiotic cloxacillin alone. All quarters were sampled for bacteriology at drying off and again in the week immediately postcalving; 2 quarters were also sampled 2 wk before the estimated calving date to allow an assessment of infection dynamics during the dry period. Quarters were subsequently monitored for clinical mastitis for the first 100 d of lactation. Conventional multilevel (random effects) models were constructed to assess the efficacy of products in preventing IMI. Survival analysis was used to examine factors that influenced the risk of clinical mastitis using conventional Cox proportional hazards models. No differences were identified between the treatment groups in terms of cure of IMI caused by the major pathogens. Quarters in both the combination and cefquinome-treated groups were more likely to be free of a major pathogen or enterobacterial pathogen postcalving. With respect to clinical mastitis, the cefquinome-treated group was less likely to develop clinical mastitis than was the cloxacillin treated group.

Key words: dry cow therapy, cefquinome, intramammary infection, broad spectrum

INTRODUCTION

The dry period has long been acknowledged as a crucial period in mastitis control; classically this has focused on control of contagious mastitis pathogens and culminated in the development and adoption of antibiotic dry cow therapy as a cornerstone of the 5-point plan in the 1960s (Neave et al., 1966, 1969; Kingwill et al., 1970). Recent research has again highlighted the importance of the dry period in mastitis epidemiology (Smith et al., 1985; Green et al., 2002a), though following the control of the classic contagious mastitis pathogens, the environmental pathogens such as *Escherichia coli* and *Streptococcus uberis* have become significantly more important (Bradley and Green, 2004; Bradley et al., 2007). This shift in emphasis is as a result in the change in the demographic of cows at drying off, away from those with an IMI toward those without. This change in demographic, coupled with recent research, has clearly demarcated the 2 separate roles of dry cow therapy, namely the cure of existing and prevention of new IMI (Green et al., 2002b; Bradley et al., 2003; Bradley and Green, 2004).

As outlined above, antibiotic dry cow therapy has historically been focused on the control of gram-positive pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*. To this end, many early products were narrow spectrum and if gram-negative activity was present, it was initially introduced to ameliorate against poor infusion technique and accidental inoculation of such pathogens at drying off. Following the resurgence of interest in the role of the dry period, one such formulation (Ubro Red, Boehringer Ingelheim, Bracknell, UK) was assessed for its ability to control gram-negative infections. This product was formulated containing the aminoglycoside framycetin to enhance activity against penicillin-resistant *Staph. aureus*. Coincidentally, framycetin is highly persistent and also active against gram-negative organisms and its efficacy in gram-negative control in the dry period has been demonstrated (Bradley and Green, 2001). A logical progression from this initial research was to aim to re-

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duce antibiotic use and instead, attempt to prevent new infections by supplementing the cow's own defenses; to this end, the potential role of internal teat sealants was investigated and these have also been shown to reduce new IMI in cows uninfected at drying off (Berry and Hillerton, 2002; Huxley et al., 2002).

This initial research raised a clear dilemma for the practitioner. The optimal therapy for cure of persistent infections was unlikely to be the optimal therapy for prevention of new intramammary infection on a given unit. This dilemma led to the use of antibiotics and teat sealants in combination and this approach has been demonstrated to be efficacious (Godden et al., 2003; Newton et al., 2008). This approach has clear merit; however it also has several drawbacks, namely cost (as 2 products are being used, rather than 1), an increased risk of damage to the streak canal and inoculation of pathogens (2 infusions rather than 1), and the resistance of some producers to take this approach, especially those supplying cheese manufacturers that may have experienced problems with the phenomenon described and reported as "black spot defect" in Cheddar cheese (Lay et al., 2007).

A recent development among dry cow therapies in the EU has been the introduction of the broad-spectrum fourth-generation cephalosporin, cefquinome (Cephaguard Dry Cow, Virbac Ltd., Bury St. Edmunds, UK). This product potentially offers persistent, broad-spectrum activity against both gram-positive and gram-negative pathogens throughout the dry period, affording the opportunity to combine optimal cure rates with adequate control of new IMI through to the transition period. The aim of the research outlined in this paper was to investigate the efficacy of this novel product in the control of IMI in the dry period, in low bulk milk SCC herds, while concurrently investigating the importance of infections acquired in the early dry and transition periods and their effect on the incidence of clinical mastitis in the first 100 d of the subsequent lactation.

MATERIALS AND METHODS

Herd Selection

Eight commercial dairy farms in the southwest of England were enrolled in the study. Herds were eligible for inclusion in the study if they had appropriate cow identification and recording of health data, were enrolled in an individual cow milk recording scheme to allow collation of historic SCC data, and had a bulk milk SCC <300,000 cells/mL. Herds were then selected on the basis of existing records and an anticipated acceptable level of compliance. Each site was visited by a veterinarian, to provide suitable training to ensure protocol compliance.

Cow Selection

Prior to enrollment, cows were inspected by a member of the study team and were eligible if they were in good health and had 4 functional quarters free of significant teat lesions (e.g., cuts and deformities). Cows that had received systemic or intramammary antibiotics or anti-inflammatories in the 30 d before the last milking were not enrolled in the study.

Study Protocol

Enrollment. Farms were visited weekly, and cows were enrolled in the study on the day of drying off. At enrollment, key cow details including parity, estimated milk yield, individual cow SCC history, treatment history and estimated calving date, were collated from farm records. Prior to the final milking in the lactation and before treatment administration, each animal was identified and physically examined by a member of the study team for suitability on the basis of the exclusion criteria. Duplicate milk samples were then collected for bacteriological examination and a single sample for SCC evaluation was made from each quarter of each eligible animal using a method described previously (Bradley and Green, 2000).

Treatment Allocation and Administration. On the day of dry off, cows were randomly allocated to receive one of the 3 treatments. The 3 treatment groups comprised either broad-spectrum antibiotic treatment with the fourth-generation cephalosporin cefquinome (treatment group **CDC**; 150 mg of cefquinome; Cephaguard Dry Cow, Virbac Ltd.), combination treatment comprising narrow-spectrum cloxacillin and an internal teat sealant (treatment group **COMBO**; 600 mg of cloxacillin; Orbenin Extra Dry Cow, Pfizer Animal Health, Sandwich, UK) and (65% of bismuth subnitrate in a mineral oil base; OrbeSeal, Pfizer Animal Health), or narrow-spectrum antibiotic treatment comprising cloxacillin alone (treatment group **OEDC**; 600 mg of cloxacillin; Orbenin Extra Dry Cow, Pfizer Animal Health). Within herds, cows were randomly allocated to a treatment group using a randomization table and a block design to ensure a "tight" temporal allocation of treatments on individual units— this was necessary to ensure a comparable environmental challenge in each treatment group. Dry cow therapy was administered by a member of the study personnel following aseptic precautions. Following treatment administration, quar-

ters were dipped with a postmilking disinfectant and confined to a loafing yard for at least 30 min before moving to accommodation.

Dry Period Management and Sampling. Following treatment, and until calving, study animals were subjected to the usual dry cow husbandry practices for the farm and regularly observed by the owner/herdsperson or other suitably qualified person. Any disease or concurrent treatments were recorded. All cases of clinical mastitis were recorded and sampled.

At approximately 2 wk before the estimated date of calving, duplicate samples for bacteriology were collected from 2 ipsilateral quarters of each of the cows according to line number (i.e., odd numbered cows sampled on the left and even numbered cows sampled on the right)—this approach was necessary to address any potential proclivity, due to housing design, of cows to lie on 1 side and any temporal variation in challenge, given that environmental pathogens are the most likely cause of infection in the dry period in the modern dairy herd. Samples were collected using a strict aseptic technique after which quarters were dipped with a postmilking disinfectant and confined to a loafing yard for at least 30 min before returning to their accommodation.

At Calving. Cows were managed following normal husbandry practices on each of the participating farms. Animals were inspected by the herdsperson/owner immediately postcalving, with particular attention paid to udder health. Any physical abnormalities detected or conditions treated were recorded.

Postcalving. Duplicate quarter milk samples were collected for bacteriological examination at the first weekly visit after each cow had calved. At the same time, a milk sample was taken from each quarter for enumeration of the quarter SCC. Samples collected more than 10 d after calving were excluded from the analysis of efficacy as measured by the cure or acquisition of IMI during the dry period.

Clinical Mastitis Monitoring. Farm personnel, trained in the detection and aseptic sampling of clinical mastitis, monitored cows for the presence of clinical mastitis throughout the study period (from dry off until 100 d postcalving) and collected a pretreatment quarter milk sample, using aseptic technique, when cases occurred. These samples were frozen on the farm and stored until the next routine visit.

Laboratory Procedures

All milk samples collected were maintained at or below 8°C during transport to the laboratory for analysis. Microbiological investigation and somatic cell counts were carried out using the standard milk sample examination techniques, which exceeded the standard recommended by the International Dairy Federation (Bulletin No 132, 1981), International Standard 13366–1:1997 (E) and 13366–2:1997 (G). A more detailed description of these techniques is outlined below.

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, 48, and 72 h. Organisms were identified and quantified using standard laboratory techniques (Quinn et al., 1994; NMC, 1999). *Escherichia coli* were identified by colony morphology, oxidase and indole tests. Other *Enterobacteriaceae* were identified using a microtube identification system (RapiD 20 E, bioMérieux, Basingstoke, UK).

Somatic Cell Counting. Somatic cell count was determined using the Fossomatic method (Delta CombiScope—Model FTIR 400, Drachten, the Netherlands), according to the FIL .IDF 148 A: 95 norm.

Assessment of Efficacy

Isolation of an organism was taken to be indicative of IMI. A sample was considered contaminated if more than 3 pathogens were cultured from a sample. If this occurred, then the duplicate sample was submitted for bacteriological analysis (Bradley and Green, 2000). Several outcomes were assessed as outlined below.

Cure of Existing IMI. The overall and species-specific percentage cured was estimated and compared between groups. A cure was defined as the absence of a pathogen in the postcalving sample that had been present at drying off.

Acquisition of New IMI. The overall and species-specific percentages of new infections were estimated and compared between groups. A new infection was defined as the presence of a pathogen in the postcalving sample that had not been present at drying off. Therefore, a quarter infected with 1 pathogen at drying off was eligible to acquire a new infection with a different pathogen.

Successful Dry Period Outcome. Successful dry period outcomes were estimated and compared between groups using methods described previously (Newton et al., 2008). A successful outcome was defined in 2 ways; first, as the absence of a major pathogen from the postcalving sample and second, as the absence of any mastitis pathogen (major or minor) from the postcalving sample.

Incidence of Clinical Mastitis in the First 100 Days of the Subsequent Lactation. The overall

and species-specific incidences of clinical mastitis were assessed in the first 100 d of lactation and compared between treatment groups.

Analysis of Events Occurring Within the Dry Period

In addition to the analyses described above, a more in-depth exploration of the data was undertaken to describe the apparent dynamics of intramammary infection during the dry period; this analysis was undertaken for descriptive purposes only. More specifically, in the subset of quarters sampled during the dry period (between 7 and 21 d precalving) the change in infection status was assessed between drying off and the transition sample and also between the transition sample and calving, allowing a description of the timing of apparent cures and new IMI within the dry period according to the criteria outlined for the entire dry period as outlined above.

Data Collation and Statistical Analyses

Data were collated and initially analyzed using Excel and Access 2003 (Microsoft Corp., Redmond, WA) and Minitab 15.1 (Minitab Inc., State College, PA). Descriptive and graphical analyses were carried out to explore the data. Mann-Whitney and Wilcoxon tests were used to compare the treatment groups where appropriate. Univariable analysis of treatment efficacy was performed using the Chi-Square test to investigate differences in proportions between groups; a layered Bonferroni correction was used to allow for multiple comparisons where appropriate (Darlington, 1990).

When assessing the efficacy of products in control of intramammary infection, conventional multilevel (random effects) models (Goldstein, 1995) were specified so that correlations within the data (quarters within cows) were accounted for appropriately. Model specifications were

$$\begin{aligned} Y_{ij} &\sim \text{Bernoulli}\left(\text{probability} = \pi_{ij}\right) \\ \text{Logit}\left(\pi_{ij}\right) &= \alpha + \beta_1 X_{ij} + \beta_2 X_j + herd_k + u_j \\ u_j &\sim N\left(0, \sigma_u^2\right), \end{aligned}$$

where the subscripts i and j denote the ith quarter and the jth cow, respectively, α the regression intercept, X_{ij} the vector of covariates at quarter level, β_1 the coefficients for covariates X_{ij}, X_j the vector of cow level covariates, β_2 the coefficients for covariates X_j, $herd_k$ is a fixed effect for the kth herd, and u_j is a random effect to reflect residual variation between cows that is normally distributed with mean = 0 and variance = σ_u^2.

Covariate assessment and selection was carried out using MLwiN with penalized quasi-likelihood for parameter estimation (Rasbash et al., 2005). Covariates remained in the model when the 95% confidence intervals for the odds ratios did not include 1.00. Investigation of model fit was made from plots of cumulated fitted probabilities and residuals, as described previously (Green et al., 2004).

Survival analysis was used to examine factors that influenced the risk of clinical mastitis at the cow level in the first 100 d of lactation. A conventional Cox proportional hazards model was specified (Collett, 1994). The standard model can be summarized as

$$\lambda_i = \lambda_0 \times \exp\left(\beta' X'\right),$$

where λ_i = hazard function (instantaneous risk of clinical mastitis in cow i at time t, where t is the time from calving to 100 d postcalving), λ_0 = baseline hazard, and $\beta' X'$ = linear predictor containing a series of explanatory covariates X', with regression coefficients β'.

Parameter estimates were made using Egret (Vs 2.0.3, Cytel Software Corp., Cambridge, MA). The significance probability (α) was set at 0.05 and parameter significance assessed using the likelihood ratio test. In order to check that the assumption of proportionality of hazards was correct (Collett, 1994), a visual assessment was performed of the log-transformed cumulative hazard for the explanatory variables.

RESULTS

A total of 489, predominantly Holstein-Friesian, cows were enrolled between February and June from the 8 farms in the study; 161, 164, and 164 to the CDC, COMBO, and OEDC groups, respectively. Details of the number of cows recruited from each farm and salient farm management details are outlined in Table 1. Data from 449 and 460 cows were incorporated into the analyses pertaining to dry period IMI and clinical mastitis, respectively. Twenty-nine animals were not available for inclusion in the analyses. Reasons for this were: animals were either not pregnant or calved beyond the end of the study (n = 18) and animals not sampled within 10 d postcalving (n = 11). Animals that were concurrently treated after the postcalving sample had been collected were included in the clinical mastitis survival analysis, but were censored at the time of treatment.

Univariable Analysis

The key indices and parameters relating to animals included in the final analysis of efficacy are summarized in Table 2. No significant differences were detected between treatment groups in any of the key indices such as parity, yield before drying off, dry period length, or SCC at the end of the previous lactation.

Prevalence of Infection. The prevalence of the key mastitis pathogens at drying off and postcalving in each of the treatment categories are outlined in Table 3. No significant differences between the treatment groups in the prevalence of infection at dry off were identified. However, significant differences were found in the prevalence of pathogens present postcalving. Quarters in the CDC and COMBO groups were less likely to be infected with *Strep. uberis* than those in the OEDC group (11/584, 13/616 vs. 28/596, respectively; $P < 0.05$), an enterobacterial organism (20/584, 17/616 vs. 37/596, respectively; $P < 0.05$), or with a major pathogen overall (64/584, 52/616 vs. 96/596, respectively; $P < 0.05$). Quarters in the COMBO group, although not significantly different from those in the CDC group, were less likely to be infected with *E. coli* than were quarters in the OEDC group (20/584, 14/616 vs. 34/596, respectively; $P < 0.05$). When the likelihood of being free of any pathogen postcalving was considered, then quarters in the COMBO group were more likely (427/616 vs. 357/596; $P < 0.05$) to be pathogen-free posttreatment than quarters in the OEDC group; results from CDC-treated quarters were not significantly different from either of the other treatment groups.

Apparent Percentage of Dry Period Cures. The pathogen-specific apparent number of cures for the key mastitis pathogens are outlined in Table 4. No significant differences in the apparent percentage of cures were identified for any of the major pathogens; however, the apparent percentage of cures of CNS were significantly higher in the COMBO group (169/202 COMBO vs. 138/189 CDC, 139/194 OEDC; $P < 0.05$) compared with those in either of the other groups.

Apparent Dry Period Percentage of New IMI. The pathogen-specific apparent number of new IMI for the key mastitis pathogens are outlined in Table 5. No significant differences in the apparent percentage of new infections were identified for any of the gram-positive major pathogens. However, the apparent percentages of new infections for *E. coli* (20/562 CDC, 14/602 COMBO vs. 31/575 OEDC; $P < 0.05$) and all enterobacteriaceae combined (20/559 CDC, 17/598 COMBO vs. 35/575 OEDC; $P < 0.05$) were lower in the COMBO group compared with those of the OEDC group, whereas they were not significantly different from those of the CDC group.

Dry Period Outcomes. Dry period outcomes for each of the treatment groups are summarized in Table 6. Whereas no significant difference existed in the proportion of quarters acquiring an infection during the dry period, a difference existed in the proportion of quarters experiencing an apparent dry period cure and in the proportion of quarters pathogen-free posttreatment. Quarters in the COMBO group were more likely to experience a dry period cure (305/613 vs. 255/588; $P < 0.05$) and be pathogen-free postcalving (426/613 vs. 356/588; $P < 0.05$) than were quarters in the OEDC-treated quarters. Results for the quarters in the CDC group were not significantly different from those in either of the other treatment groups.

Clinical Mastitis. A total of 158 quarter cases of clinical mastitis occurred in the 460 cows eligible for inclusion in the analysis: 46, 48, and 64 in the quarters in the CDC, COMBO, and OEDC groups, respectively. Clinical cases with enterobacterial involvement were the most common, accounting for 58% of cases from which a major pathogen was identified; contagious mastitis pathogens were rarely identified. For the purposes of assessing efficacy, only the first case to occur in a quarter during the study period was considered to mitigate the risk of confounding effects of treating a case in 1 quarter on outcomes in another in the same cow. Table 7 summarizes the findings from the first case of clinical mastitis to occur in each cow, at which point they would have been censored from the study. Univariable analysis of the first case of clinical mastitis to occur in each cow revealed that cows in the COMBO group were significantly less likely to develop clinical enterobacterial mastitis than were cows in the OEDC group (4/154 vs. 15/155, respectively; $P < 0.05$). Results from cows in the CDC group were not significantly different from those in either of the other treatment groups.

Description of Events Occurring During the Dry Period

Transition samples were collected from 586 quarters across all 3 treatment groups; samples were not collected from approximately 300 eligible quarters due to the cows being unavailable for sampling (at distant pasture) or due to cows calving earlier than expected. A summary of the findings are outlined in Table 8. Unfortunately, insufficient data were available to allow a meaningful comparison between treatment groups and care needs to be taken in interpreting the findings. However, investigation of the available data suggests that the majority of apparent cures occurred in the early dry period and that, in this study, few infections persisted from drying off until the subsequent lactation. The apparent new infection rate before transition was

Table 1. Key characteristics of the 8 study farms

Item	Farm identification							
	B	C	F	G	H	R	S	T
Approximate herd size (cows in milk)	310	480	200	280	220	420	150	610
Proportion of recruited animals at the end of lactation 1	0.11	0.47	0.19	0.36	0.39	0.35	0.43	0.43
Number of animals recruited	18	96	53	61	41	63	54	103
Geometric mean bulk milk SCC (×1,000 cells/mL) in the 5 mo before the study	213	194	172	162	218	195	163	163
Dry cow winter housing[1]	Y	C, Y	C, Y	C, Y	Y	C, Y	C	Y
Predominant dry cow summer housing[1]	P	P	C, Y	P	P	P	P	Y
Predominant dry cow bedding	Straw	Sand	Sand	Straw	Straw	Straw	Straw	Straw

[1]C = freestalls; Y = covered straw yards; P = pasture.

Table 2. Summary of data from cows and quarters included in the analysis of product efficacy as measured by cure and acquisition of IMI during the dry period[1]

Item	CDC (n = 146, q = 584)				COMBO (n = 154, q = 616)				OEDC (n = 149, q = 596)			
	Mean	Med.	Min.	Max.	Mean	Med.	Min.	Max.	Mean	Med.	Min.	Max.
Parity	2.59	2	1	10	2.45	2	1	8	2.51	2	1	11
Yield at dry off (L)	15.2	15	1	40	15.3	15	2	43	15.7	15	2	40
Dry period length (d)	62.3	59	18	171	64.3	59	27	225	62.7	57	26	169
ICSCC[2] 1 mo before dry off (×1,000 cells/mL)	311	168	10	2,558	237	147	14	1,656	261	157	13	1,663
ICSCC 2 mo before dry off (×1,000 cells/mL)	205	105	10	3,742	183	110	7	2,875	216	106	8	2,100
ICSCC 3 mo before dry off (×1,000 cells/mL)	155	99	13	1,489	142	104	10	929	190	105	5	1,946

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone; q = number of quarters; Med. = median; Min. = minimum; Max. = maximum.

[2]Individual cow SCC.

Table 3. The prevalence of mastitis pathogens (n; % in parentheses) at drying off and postcalving, restricted to quarters and cows eligible for assessment of product efficacy[1]

	CDC (q = 584)		COMBO (q = 616)		OEDC (q = 596)	
Item	Dry off	Postcalving	Dry off	Postcalving	Dry off	Postcalving
Streptococcus uberis	16 (2.74)	11 (1.88)[a]	10 (1.68)	13 (2.18)[a]	16 (2.60)	28 (4.55)[b]
Streptococcus dysgalactiae	2 (0.34)	1 (0.17)	3 (0.50)	0 (0)	7 (1.14)	3 (0.49)
Coagulase-positive staphylococci	5 (0.86)	2 (0.34)	4 (0.67)	2 (0.34)	3 (0.49)	4 (0.65)
Escherichia coli	15 (2.57)	20 (3.42)[ab]	11 (1.85)	14 (2.35)[a]	13 (2.11)	34 (5.52)[b]
Other enterobacteriaceae	3 (0.51)	0 (0)	4 (0.67)	3 (0.50)	0 (0)	3 (0.49)
Aerococcus spp.	22 (3.77)	10 (1.71)	27 (4.53)	6 (1.01)	27 (4.38)	15 (2.44)
Arcanobacterium pyogenes	0 (0)	1 (0.17)	0 (0)	1 (0.17)	0 (0)	5 (0.81)
Other major pathogens	10 (1.71)	20 (3.42)	10 (1.68)	16 (2.68)	18 (2.92)	13 (2.11)
All enterobacteriaceae	18 (3.08)	20 (3.42)[a]	15 (2.52)	17 (2.85)[a]	13 (2.11)	37 (6.01)[b]
All major pathogens[2]	70 (11.99)	64 (10.96)[a]	66 (10.71)	52 (8.44)[a]	82 (13.76)	96 (16.11)[b]
CNS	189 (32.36)	133 (22.77)	202 (33.89)	113 (18.96)	194 (31.49)	141 (22.89)
Corynebacterium spp.	308 (52.74)	72 (12.33)	340 (57.05)	56 (9.40)	340 (55.19)	77 (12.50)
All minor pathogens[2]	329 (56.34)	150 (25.68)	376 (61.04)	135 (21.92)	348 (58.39)	143 (23.99)
No growth	178 (30.48)	370 (63.36)[ab]	172 (27.92)	427 (69.32)[a]	158 (26.51)	357 (59.90)[b]
Contaminated	7 (1.20)	0 (0)	2 (0.32)	1 (0.16)	8 (1.34)	0 (0)

[a,b]Values in columns between treatment groups and within sampling time points with different superscripts differ ($P < 0.05$).

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone; q = number of quarters.

[2]Totals may not equal sum of the individual pathogens due to mixed infections.

not dissimilar to that seen in the transition period, though importantly, few of these apparent infections persisted until the postcalving sample, with most appearing to resolve spontaneously. On the basis of the data collected in this study, no gram-negative infections appeared to originate from the early dry period, whereas between 10 and 50% of gram-positive infections originated from before the transition period.

Multivariable Analysis

Dry Period Outcomes. Two different outcomes were modeled: the likelihood of a quarter being free of a major mastitis pathogen postcalving (Table 9) and of being infected with an enterobacterial pathogen postcalving (Table10). The potential confounding influence of the presence of infection at drying off was tested in all models, as was the influence of yield at drying off, dry period length, parity, farm, SCC before drying off, and the influence of collection of a transition sample. Compared with quarters in the OEDC group, quarters in the COMBO group (odds ratio, **OR**, 2.01; 95% CI, 1.32 to 3.07) and CDC group (OR 1.61; 95% CI 1.07 to 2.43) had increased the odds of being free of a major mastitis pathogen postcalving; no difference between the COMBO and CDC groups existed. Parity 1 cows entering their second parity had increased odds

Table 4. The apparent dry period number and percentage of cures of the key mastitis pathogens[1]

	CDC		COMBO		OEDC	
Item	n[2]	%	n	%	n	%
Streptococcus uberis	16	100	10	80	16	75
Streptococcus dysgalactiae	2	100	3	100	7	100
Coagulase-positive staphylococci	5	100	4	100	3	66.67
Escherichia coli	15	100	11	100	13	92.31
Other enterobacteriaceae	3	100	4	100	0	NA[3]
Aerococcus spp.	22	100	27	100	27	100
Arcanobacterium pyogenes	0	NA	0	NA	0	NA
Other major pathogens	10	100	10	100	18	100
CNS	189	73.02[a]	202	83.7[b]	194	71.65[a]
Corynebacterium spp.	308	87.99	340	91.8	340	85.59

[a,b]Values in columns with different superscripts differ ($P < 0.05$).

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone.

[2]n = number of quarters infected at drying off.

[3]NA = not applicable.

Table 5. The apparent dry period number and percentage of new IMI for the key mastitis pathogens[1]

	CDC			COMBO			OEDC		
Item	n[2]	New IMI[3]	%	n	New IMI	%	n	New IMI	%
Streptococcus uberis	561	12	2.14	603	11	1.82	572	24	4.20
Streptococcus dysgalactiae	575	1	0.17	610	0	0.00	581	3	0.52
Coagulase-positive staphylococci	572	2	0.35	609	2	0.33	585	2	0.34
Escherichia coli	562	20	3.56[ab]	602	14	2.33[a]	575	31	5.39[b]
Other enterobacteriaceae	574	0	0.00	609	3	0.49	588	4	0.68
Aerococcus spp.	555	10	1.80	586	6	1.02	561	15	2.67
Arcanobacterium pyogenes	580	1	0.17	613	1	0.16	588	5	0.85
Other major pathogens	567	20	3.53	603	16	2.65	570	13	2.28
All enterobacteriaceae	559	20	3.58[ab]	598	17	2.84[a]	575	35	6.09[b]
CNS	388	81	20.88	411	79	19.22	394	84	21.32
Corynebacterium spp.	269	34	12.64	273	28	10.26	248	27	10.89

[a,b]Values in columns with different superscripts differ ($P < 0.05$).

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone.

[2]n = number of quarters uninfected with the pathogen at drying off.

[3]New IMI = number of new IMI acquired during the dry period.

of being free of a major mastitis pathogen compared with the odds for parity 3 and above cows. Other factors were found not to be influential.

When compared with quarters in the OEDC group, quarters in the COMBO (OR 0.39; 95% CI 0.24 to 0.61) and CDC (OR 0.53; 95% CI 0.34 to 0.82) groups had reduced odds of being infected with an enterobacterial mastitis pathogen postcalving; no difference was found between the COMBO and CDC groups. Parity 1 and 2 cows entering their second and third parities had reduced odds of being infected with an enterobacterial mastitis pathogen than did parity 3 and above cows. Irrespective of treatment group, significant variation existed between farms. Collection of a transition sample within 1 wk of calving significantly increased the risk of a cow being infected with an enterobacterial pathogen postcalving, though again this was irrespective of treatment group; interestingly, further analysis suggested that combination treated quarters were least likely to be affected by transition sampling. Other factors, such as SCC before drying off, yield at drying off, dry period length, and previous clinical mastitis history were not found to be influential.

Clinical Mastitis. Multivariable analysis was conducted at the cow level, using only the 2 quarters that had not been sampled during the transition period, thereby controlling for this potentially confounding factor. A conventional Cox proportional hazards model was specified, a summary of which is outlined in Table 11. A survival plot is illustrated in Figure 1. Cows in the CDC group were the least likely to develop clinical mastitis. Results from cows in both the CDC (hazard ratio 0.49; 95% CI 0.25 to 0.93) and COMBO (HR 0.57; 95% CI 0.31 to 1.03) groups were similar and at lower risk of developing clinical mastitis than those from cows in the OEDC group. Parity, farm, and the presence of a major pathogen present at drying off had no significant effect on the likelihood of developing clinical mastitis in the early part of the next lactation. Other factors, such as SCC before drying off, yield at drying off, dry period length, and previous clinical mastitis history were also found not to be influential.

Table 6. A summary of the quarter level dry period outcomes for each of the treatment groups[1]

Item	CDC	COMBO	OEDC
Quarters treated (n)	577	613	588
Quarters infected at drying off (n)	399	441	430
Quarters experiencing an apparent dry period cure (n)	248[ab]	305[a]	255[b]
Quarters uninfected at drying off (n)	178	172	158
Quarters becoming infected during the dry period (n)	56	51	57
Quarters uninfected postcalving (n)	370[ab]	426[a]	356[b]

[a,b]Values in columns with different superscripts differ ($P < 0.05$).

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone. Values may differ from Tables 3, 4, and 5 due to exclusion of data from quarters where a sample was contaminated either at drying off or postcalving.

Table 7. Summary of first quarter cases of clinical mastitis occurring in each cow, in cows recruited to the study and eligible for inclusion in the analysis of efficacy as measured by the occurrence of clinical mastitis[1]

Diagnosis	CDC	COMBO	OEDC
Number of quarters (cows) at risk	604 (151)	616 (154)	620 (155)
Escherichia coli	6	4	12
Streptococcus uberis	1	3	2
Streptococcus dysgalactiae	1	3	
Staphylococcus aureus	1		1
Klebsiella spp.			1
Yeast spp.	1		
Bacillus spp.		1	1
Aerococcus spp.	1		2
Arcanobacterium pyogenes	1	1	2
E. coli and *Strep. uberis*	1		1
Klebsiella spp. and *Aerococcus* spp.	1		
Strep. uberis and *Staph. aureus*	1		
E. coli and *Strep. dysgalactiae*			1
Total cases with mixed etiology	3	0	2
Total cases with enterobacterial involvement	8[ab]	4[a]	15[b]
Total major pathogens	15	12	23
CNS	2		
Corynebacterium spp.		1	
Total minor pathogens	2	1	0
No growth	1	6	
No sample	7	12	14
Total cases	25	31	37

[a,b]Values in columns with different superscripts differ ($P < 0.05$).

[1]CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone.

DISCUSSION

The data and results outlined in this study demonstrate the efficacy of the fourth-generation cephalosporin, cefquinome when incorporated in a dry cow formulation (Cephaguard Dry Cow, Virbac Ltd) in the treatment and prevention of IMI in nonlactating dairy cows. In addition, cefquinome has been demonstrated to significantly reduce the likelihood of a cow developing clinical mastitis in the first 100 d of the subsequent lactation compared with that from the use of a narrow-spectrum cloxacillin-containing formulation. Much of the apparent enhanced benefit compared with cloxacillin appeared to be as a result of control of infection with enterobacterial species and a subsequent decrease in enterobacterial clinical mastitis in the early part of the subsequent lactation. This study has investigated the efficacy of the very broad-spectrum fourth-generation cephalosporin, cefquinome. It would be of great value to the practitioner to know if earlier generation cephalosporins could offer similar benefits over narrow-spectrum dry cow products. One would expect increasing efficacy against gram-negative pathogens as one moved through the cephalosporin generations and the spectrum of activity increased; however, given the importance of persistence of activity to the back end of the dry period, this would inevitably be affected by duration of activity, which is also likely to be formulation dependant—this is an area in need of further research to enable the practitioner to make more evidence-based decisions on product selection.

The use of intramammary dry cow therapy with persistent gram-negative activity has previously been demonstrated to control enterobacterial clinical mastitis in the subsequent lactation (Bradley and Green, 2001). This is the first study to have compared the use of an internal teat sealant, either alone or in combination with antibiotic, with a broad-spectrum dry cow therapy with persistent activity. Interestingly, the cefquinome formulation appeared to have similar efficacy to that of narrow-spectrum dry cow therapy used in combination with an internal teat sealant. This finding was surprising, as it necessitates the persistence of gram-negative activity until the transition period, a fact reinforced by the finding that those gram-negative infections present at calving appear to have exclusively originated from the latter part of the dry period.

The dynamics of the cure and new IMI processes in this study are complex and not easy to elucidate. Whereas at the species level, no significant difference was found in percentage of cures, with the exception of the CNS, a significant difference was found in the efficacy of the products when measured by the likelihood of an infected quarter being free of a major pathogen. A strong rationale exists for taking this approach to assessment of dry cow therapy, because the clinical out-

come of most significance to the farmer is the proportion of quarters pathogen-free at calving. Arguably, this approach to assessment of efficacy of dry cow therapy is most relevant in herds with relatively lower bulk milk SCC, in which both the prevalence of infection at drying off is lower and the infectious pressure with contagious pathogens is reduced.

Whereas combination treatment and treatment with cefquinome exhibited similar efficacy against major mastitis pathogens, arguably, minor pathogens were better controlled using the combination treatment. This is of interest, as one might speculate that persistence of minor pathogens and the effect this may have on gland susceptibility, either as a result of moderate elevation in SCC or competitive exclusion, may, in part, explain the lower incidence of clinical mastitis in the cefquinome-treated group compared with that of the combination-treated group. Unfortunately, sufficient power in this study was not available to investigate this effect, but it could be a fruitful area of further research.

The relationship between intramammary infection in the nonlactating period and clinical mastitis in the early part of the subsequent lactation has been an area of recent research (Bradley and Green, 2000; Green et al., 2002a). This study, in common with several others, has reinforced the importance of the dry period in clinical mastitis epidemiology and should aid the practitioner in making evidence-based decisions about dry cow therapy selection.

This study, along with several other recent studies undertaken by the authors (Newton et al., 2008; Bradley et al., 2010), has demonstrated how effective antibiotic dry cow therapy is in removing persistent infections present at drying off in the modern low SCC dairy herd. These findings should reiterate to the practitioner the importance of prevention of new intramammary infection during the dry period, because less than 1.9% (4/212) of major pathogen infections present postcalving had apparently persisted from drying off. This observation should call into question the cost benefit of the use of additional parenteral antibiotic therapies as a supplement to dry cow therapy in such herds, and should instead focus the practitioner and herdsperson alike on minimizing challenge rather than focusing on cure.

CONCLUSIONS

This study has demonstrated the utility of a persistent broad-spectrum antibiotic dry cow therapy, containing cefquinome, in the treatment and prevention of intramammary infection during the nonlactating period of dairy cattle. It has also reiterated the importance of prevention of new intramammary infection in the dry

Table 8. Summary of the prevalence of infection, early and late dry period apparent cures, new IMI prevalence, and relative contribution of the early and late dry period to infections present at calving in quarters sampled during the transition period (number of quarters sampled = 586)

Item	Infection prevalence at drying off		Early dry period apparent cure		Early dry period apparent new infection		Infection prevalence at transition		Late dry period apparent cure		Late dry period apparent new infection		Infection prevalence postcalving		Proportion of infections apparently originating from the early dry period	Proportion of infections apparently originating from the late dry period
	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Streptococcus uberis	11	1.88	10	90.91	9	1.57	10	1.71	8	80.00	16	2.78	18	3.07	0.11	0.89
Streptococcus dysgalactiae	2	0.34	2	100	1	0.17	1	0.17	1	100	0	0	0	0		
Coagulase-positive staphylococci	2	0.34	2	100	4	0.68	4	0.68	3	75.00	1	0.17	2	0.34	0.50	0.50
Escherichia coli	16	2.73	16	100	19	3.33	19	3.24	19	100	19	3.35	19	3.24	0.00	1.00
Other enterobacteriaceae	1	0.17	1	100	4	0.68	4	0.68	4	100	2	0.34	2	0.34	0.00	1.00
Aerococcus spp.	22	3.75	21	95.45	7	1.24	8	1.37	8	100	10	1.73	10	1.71	0.00	1.00
Other major pathogens	16	2.73	14	87.50	40	7.02	42	7.17	40	95.24	12	2.21	14	2.39	0.14	0.86
CNS	194	33.11	115	59.28	149	38.01	228	38.91	169	74.12	73	20.39	132	22.53	0.45	0.55
Corynebacterium spp.	347	59.22	307	88.47	23	9.62	63	10.75	54	85.71	55	10.52	64	10.92	0.14	0.86

Table 9. Summary of the multilevel model, free of a major pathogen postcalving

Item	Coefficient	SE	Odds ratio	95% CI	
				Lower	Upper
Treatment (ref[1] OEDC)[2]					
COMBO	0.70	0.21	2.01	1.32	3.07
CDC	0.48	0.21	1.61	1.07	2.43
Pathogen identified					
Cow major @ DO[3]	−0.21	0.18	0.82	0.56	1.18
Parity (ref parity 3 and above)					
Parity 1	0.46	0.21	1.58	1.04	2.41
Parity 2	0.42	0.23	1.52	0.96	2.39
Farm (ref Farm_B)					
Farm_C	−0.38	0.55	0.68	0.23	2.03
Farm_F	−0.60	0.55	0.55	0.18	1.66
Farm_G	−0.50	0.55	0.61	0.20	1.82
Farm_H	−0.35	0.60	0.71	0.21	2.32
Farm_R	0.11	0.58	1.12	0.35	3.60
Farm_S	−0.62	0.56	0.54	0.17	1.65
Farm_T	−0.82	0.54	0.44	0.15	1.31
Sample collection time (ref TR TIME CAT_1)[4]					
TR TIME CAT_2	0.13	0.33	1.14	0.59	2.19
TR TIME CAT_3	0.31	0.34	1.36	0.69	2.68
TR TIME CAT_4	−0.07	0.36	0.93	0.45	1.92
TR TIME CAT_999	0.24	0.44	1.27	0.52	3.06

[1]Referent sample.

[2]OEDC = group treated with cloxacillin alone; COMBO = group treated with cloxacillin in combination with internal teat sealant; CDC = group treated with cefquinome.

[3]Cow major @ DO: a major pathogen identified in any quarter at drying off.

[4]TR TIME CAT_1: sample collected 1–7 d precalving; TR TIME CAT_2: sample collected 8–14 d precalving; TR TIME CAT_3: sample collected 15–21 d precalving; TR TIME CAT_4: sample collected >21 d precalving; TR TIME CAT_999: no transition period milk sample collected.

Table 10. Summary of the multilevel model, infected with an enterobacterial pathogen postcalving

Item	Coefficient	SE	Odds ratio	95% CI	
				Lower	Upper
Treatment (ref[1] OEDC)[2]					
COMBO	−0.95	0.23	0.39	0.61	0.24
CDC	−0.64	0.22	0.53	0.82	0.34
Parity (ref parity 3 and above)					
Parity_1	−0.23	0.22	0.79	1.23	0.51
Parity_2	−0.02	0.24	0.98	1.58	0.61
Farm (ref Farms_B and T)					
Farm_C	−0.40	0.59	0.67	2.18	0.21
Farm_F	0.33	0.59	1.39	4.53	0.43
Farm_G	−1.77	0.59	0.17	0.55	0.05
Farm_H	−0.64	0.65	0.53	1.93	0.16
Farm_R	−0.44	0.63	0.64	2.27	0.18
Farm_S	0.53	0.60	1.70	5.64	0.51
Sample collection time (ref TR TIME CAT_1)[3]					
TR TIME CAT_2	−0.47	0.34	0.63	1.23	0.32
TR TIME CAT_3	−0.94	0.35	0.39	0.79	0.19
TR TIME CAT_4	−0.52	0.38	0.59	1.27	0.28
TR TIME CAT_999	−1.74	0.49	0.18	0.47	0.07

[1]Referent sample.

[2]OEDC = group treated with cloxacillin alone; COMBO = group treated with cloxacillin in combination with internal teat sealant; CDC = group treated with cefquinome.

[3]TR TIME CAT_1: sample collected 1–7 d precalving; TR TIME CAT_2: sample collected 8–14 d precalving; TR TIME CAT_3: sample collected 15–21 d precalving; TR TIME CAT_4: sample collected > 21 d precalving; TR TIME CAT_999: No transition period milk sample collected.

Table 11. Summary of the clinical mastitis survival model

Item	Coefficient	SE	Hazard ratio	95% CI	
				Lower	Upper
Treatment (ref[1] OEDC)[2]					
CDC	−0.72	0.33	0.49	0.25	0.93
COMBO	−0.56	0.30	0.57	0.31	1.03
Pathogen identified					
Cow major @ DO[3]	0.18	0.27	1.20	0.70	2.05
Parity (ref parity 3 and above)					
Parity_1	−0.35	0.30	0.71	0.39	1.28
Parity_2	−0.19	0.34	0.83	0.43	1.60
Farm (ref Farm_B)					
Farm_C	1.07	1.04	2.92	0.38	22.54
Farm_F	1.39	1.05	4.02	0.51	31.45
Farm_G	1.13	1.06	3.08	0.39	24.52
Farm_H	1.03	1.08	2.81	0.34	23.51
Farm_R	0.48	1.10	1.62	0.19	14.00
Farm_S	0.17	1.16	1.19	0.12	11.54
Farm_T	0.91	1.04	2.49	0.32	19.16

[1]Referent sample.

[2]OEDC = group treated with cloxacillin alone; CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant.

[3]Cow major @ DO: a major pathogen identified in any quarter at drying off.

period and once again highlighted the pivotal role IMI during the dry period has on the incidence and etiology of clinical mastitis in the subsequent lactation.

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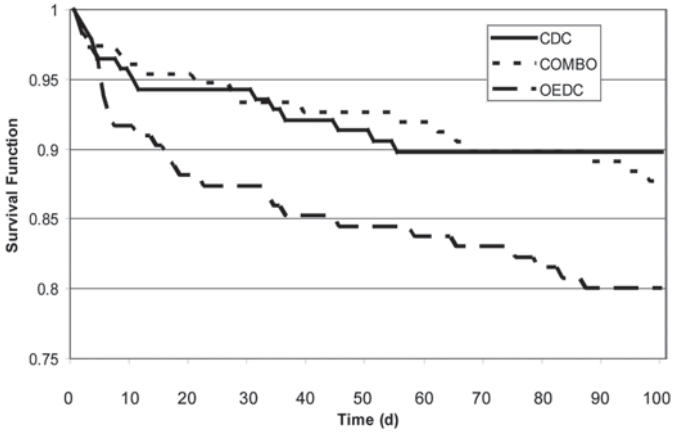

Figure 1. Clinical mastitis survival plot. CDC = group treated with cefquinome; COMBO = group treated with cloxacillin in combination with internal teat sealant; OEDC = group treated with cloxacillin alone.

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