Anti-inflammatory and 12 vs 24-hourly treatment intervals for mastitis

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Introduction

Mastitis is a common and important disease of dairy cattle which is predominantly of bacterial etiology. Under New Zealand management conditions Gram-positive cocci are the most common isolates from both clinical and subclinical mastitis cases (McDougall 1998; McDougall *et al.* 2007).

Currently there are several registered intramammary therapies that include corticosteroids, but these products were registered many years ago and there are limited published reports demonstrating the benefit of corticosteroids in these formulations or the data is on gram-negative infections (Sipka *et al.* 2013).

Hydrocortisone aceponate (HCA) is a synthetic glucocorticoid. It has dose-dependent anti-inflammatory and immunosuppressive properties (FDA 2011). No previous randomized controlled studies have determined the effect of the addition of HCA to an antimicrobial infusion for the treatment of cows with clinical or subclinical mastitis. In the current study, a non-inferiority design was used to assess the effect of the addition of HCA to intramammary treatment with penicillin alone to establish that addition of the corticosteroid did not reduce the probability of bacteriological cure associated with immune suppression. Additionally, as approximately 10% of dairy herds in New Zealand milk cows only once daily throughout lactation and further 35% milk once daily during part of the lactation (Lopez-Villalobos *et al.* 2023), the effect of once daily treatment and twice daily milking was compared with twice-daily treatment and milking.

Materials and methods

Ruakura Animal Ethics Committee study numbers 15385 and 15148.

For the clinical arm of the study, cows were enrolled if there was gross evidence of clinical mastitis, i.e., presence of heat or swelling of the udder or of changes in milk composition. These cows were examined by a technician and those with grossly evident signs of systemic disease, or a history of treatment with antimicrobials, corticosteroids or non-steroidal anti-inflammatory drugs (NSAID) in the 28 days preceding presentation were excluded. Once evidence of clinical mastitis was confirmed, milk samples were collected and treatment initiated immediately, as described below.

For the subclinical arm of the study, cows were eligible if they had a recorded cow-level SCC >200,000 cells/mL at the most recent herd (DHI) test and no history of treatment with antimicrobials, corticosteroids or NSAID in the preceding 28 days. Cows with one or more quarters from which bacteria were isolated were enrolled.

For clinical mastitis cases, cows meeting the inclusion criteria were assigned at the time of enrolment to be treated with 1g of penicillin and 20mg of HCA (Intracillin Milking Cow Platinum, Virbac New Zealand Ltd., Hamilton, New Zealand, ACVM registration number A012053; IVP) or 1g penicillin alone (Intracillin 1000 Milking Cow, Virbac New Zealand Ltd., Hamilton, New Zealand, ACVM registration number A007787; CP) on three occasions.

Cows meeting the inclusion criteria for the subclinical mastitis arm of the study were blocked by age (2 vs. >2 years) within farm, ranked by SCC at the most recent herd test, and then randomly assigned be treated by intramammary infusion on three occasions with IVP or CP at 12 or 24h intervals.

Cows were examined and sampled at multiple timepoints up to 28 days after the start of treatment. Bacteriological cure was defined as having occurred where the milk samples collected on Days 7, 14, and 21 after initiation of treatment did not result in isolation of the same bacterial species that had been present prior to initiation of treatment (Day 0).

Bacteriological cure rate was initially modeled using chi-squared analyses. Factors associated ($P<0.2$) were then offered to a logistic regression model with cow nested in herd as a random effect. Estimated marginal means and 95% confidence intervals (CI) were calculated to provide estimates of main effects, having adjusted for covariates in the model.

For the subclinical arm, bacteriological cure rate was analyzed on a non-inferiority basis. Using the final model for bacteriological cure, a difference in estimated marginal means for the cure rate of each treatment was calculated and 95% CI estimated for this difference. The IVP would be declared non-inferior to CP if the lower 95% CI for the difference in bacteriological cure (CP–IVP) was >-10%.

Quarter-level log10 SCC, clot scores and heat or swelling scores were analyzed on a superiority basis. The ordinal clots score (i.e. 0, 1, 2) were collapsed into presence or absence of clots and, with heat or swelling score, was analyzed using binary logistic regression models.

Results

For the clinical arm 60 cows (64 quarters) were enrolled in the CP group, and 60 cows (67 quarters) were enrolled in the IVP group from 11 herds. For the subclinical arm, 703 quarters form 420 cows were enrolled across 18 herds.

In the final model, the estimated marginal mean cure rate did not differ between quarters treated with CP (65.1%; 95% CI 49.2–78.2%) or IVP (66.7%; 95% CI 51.6–79.0%) ($P=0.87$), in quarters with clinical mastitis.

The bacteriological cure rate for subclinically infected quarters did not differ between quarters treated with CP (66.2%; 95% CI 58.8–72.9%) or IVP (64.9%; 95% CI 57.4–71.8%) ($P=0.77$).

The bacteriological cure rate of subclinically infected quarters after treatment with IVP was non-inferior to CP, as the marginal difference in cure rates between treatments was estimated to be -1.2% with 95% CI of -6.8% to 9.2%, so the lower limit of the difference was <10% absolute cure rate.

The bacteriological cure rate of subclinical mastitis cases did not differ between quarters treated at intervals of 12 h (64.6%; 95% CI =57.2–71.4%) or 24h (66.5%; 95% CI =59.1–73.3%) ($P=0.65$). Treatment at 24h intervals was non-inferior to treatment at 12 h intervals as the marginal difference was estimated to be 1.8% with 95% CI of -5.7% to 9.4%, so the lower limit of the difference was <10%.

For quarters diagnosed with clinical mastitis, treatment with IVP resulted in a lower overall estimated marginal mean (±SE) $\log_{10}$ SCC (3.02±0.07) compared with those treated with CP (3.15±0.07). Estimated marginal mean $\log_{10}$ SCC was lower 14, 21 and 28 d after initiation of treatment for quarters treated with IVP than those treated with CP ($P<0.05$).

For infected quarters of cows with subclinical mastitis, quarter-level $\log_{10}$ SCC did not differ following treatment with CP or IVP ($P=0.38$).

For clinical cases, the odds ratio for the proportion of quarters with clots present was 0.51 (95% CI = 0.22–1.15, $P=0.1$) (Figure 4) for IVP treated quarters vs. CP treated quarters. In the final model, clot score varied across time ($P<0.001$), but there was no time by treatment interaction ($P=0.48$).

The proportion of quarters diagnosed with clinical mastitis with heat or swelling was reduced following treatment with IVP compared with CP (P=0.01). There was a treatment by time interaction (P=0.01).

Discussion

Intramuscular or intramammary infusion of corticosteroids in cases of mastitis has been shown to result in a more rapid decline in clinical or subclinical markers of inflammation compared to not using corticosteroids (Sipka *et al.* 2013). However, the relatively small size of those studies precluded assessment as to whether use of corticosteroids might have reduced the bacteriological cure rate. Where the NSAID meloxicam was given concurrent with intramammary infusion of antimicrobials, an increased bacteriological cure rate of mild to moderate clinical mastitis was demonstrated (McDougall *et al.* 2016). In the current study we have demonstrated that there was no reduction in the bacteriological cure rate amongst clinical or subclinical cases of mastitis treated with HCA in conjunction with penicillin, compared with penicillin alone.

Once a day milking is practiced throughout lactation in 10%, and for part of the lactation in a further 35% of herds in New Zealand (Lopez-Villalobos *et al.* 2023). This study demonstrated that treatment frequency (i.e. 12 hourly vs. 24 hourly) did not affect bacteriological cure rate when milking twice a day providing flexibility for producers milking and treating once or twice a day. Frequency of administration did not affect bacteriological cure and did not interact with treatment group. Penicillin is a time dependent antibiotic so that the pharmacokinetic parameter to maximize is the time above minimum inhibitory concentration as a percentage of treatment period (Toutain *et al.* 2002). Antimicrobial concentrations in the udder are expected to reduce at each milking (Whittem *et al.* 2012) and then be elevated as another dose is administered. In this study, bacteriological cure rate was not depressed by 24h treatment intervals and twice daily milking, which likely resulted in the removal of antibiotic via the milking process between doses. This may be related to the comparatively large dose (1g) of penicillin administered, meaning that the concentration may not have fallen below the minimum inhibitory concentration for significant periods within the inter-dosing interval. The clinical significance of this finding is that Intracillin Milking Cow products (IVP or CP) may be infused at 24h intervals when twice daily milking occurs. For managerial reasons, herd owners may prefer that all treatments occur once daily to minimise risks of incorrect treatment intervals occurring or the risk that withholding periods are not observed.

The authors would not advocate for the routine treatment of subclinical infections during lactation, in this study subclinical infections were included as a research model so sufficient cases could be enrolled efficiently.

For clinical cases, inclusion of HCA was associated with a reduction in outcomes associated with inflammation. IVP treatment reduced quarter-level $\log_{10}$ SCC at 14, 21 and 28 days after initiation of treatment, but not at earlier times. Addition of HCA tended to reduce the proportion of quarters with clots within seven days after treatment and significantly reduced the proportion of quarters with heat or swelling in the first three days after treatment. Presence of clots or heat and/or swelling of the udder are used by herdowners to diagnose clinical mastitis and to assess the response to treatment. Therefore, a reduction in severity of clinical signs is likely to lead to reduced numbers of unnecessary repeat treatments hence reducing overall antimicrobial usage and reducing exposure of commensal bacteria to antimicrobials.

We conclude that the bacteriological cure rate of subclinical mastitis was non-inferior following treatment with penicillin and HCA compared to penicillin alone, and for 24 hourly compared with 12 hourly treatment intervals. There was some evidence of reduced severity of local inflammation in cases of clinical mastitis when HCA was combined with penicillin compared to treatment with penicillin alone.

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