# Effect of extended cefquinome treatment on clinical persistence or recurrence of environmental clinical mastitis

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**Abstract**

The efficacy of antibiotic treatment of clinical mastitis (**CM**) is classically evaluated using bacteriological cure. This provides a concise and objective way of assessing efficacy, but unfortunately does not reflect the field situation where persistence or recurrence of clinical signs lead to perceived treatment failure. If clinical signs persist or recur, intramammary (**IMM**) treatment is often extended or supplemented with parenteral treatment, in the expectation of a more efficient elimination of clinical signs or a lower probability of recurrence of clinical signs. The objective of this study was to evaluate the efficacy against clinical persistence or recurrence of three cefquinome treatment regimes, standard 1.5-day intramammary (**SIMM**), 5-day extended intramammary (**EIMM**) and combination of EIMM plus 5-day extended parenteral (**ECOMBO**) treatment. The study was conducted on three dairy farms with a high recurrence rate of environmental mastitis. Efficacy was evaluated using a multi-level model at the quarter and at the cow level, based on the persistence or recurrence of clinical signs at any time during a 105-day period following the end of the initial treatment, independent of pathogen. The most prevalent pathogens were *E. coli* (16.9%) and *S. uberis* (11.97%). EIMM and ECOMBO significantly decreased the persistence or recurrence of CM by 8 % and 6 % at the quarter level and by 9 % and 8 % at the cow level, respectively. ECOMBO may not reduce the persistence or recurrence of CM beyond EIMM. Whilst extended treatment regimens offered an improved outcome in this study, the producer and practitioner need to carefully consider such regimens from the perspective of prudent antibiotic use.

*Keywords***:** Bovine; Lactation; Mammary gland; Extended treatment; Antibiotic

## Introduction

Environmental pathogens, particularly *Streptococcus uberis* and *Escherichia coli*, can be a cause of persistent intramammary infection (Van Eennenaam et al., 1995, Döpfer et al., 1999, Bradley and Green, 2001). On some farms, with a low bulk milk somatic cell count (**BMSCC**) and high incidence of clinical mastitis (**CM**), a significant proportion of CM may occur in a limited number of animals as a result of a high level of recurrence (Houben et al., 1993, Lam et al., 1996, Zadoks et al., 2001). Recurrent CM cases have been described as being as severe as index cases, with comparable impact on milk yield and probability of death (Bar et al., 2007). Moreover, cows with recurrent CM are at a higher risk for culling (Bar et al., 2008).

Recurrent CM is usually defined by initial disappearance and subsequent re-occurrence of clinical signs after a preset number of days. Using this definition, recurrent CM can be due to a recrudescence of a persistent IMM infection due to failure to cure (Pinzón-Sánchez et al., 2011), or as a result of re-infection of the quarter after successful cure. However, differentiating between persistence of infection and re-infection is not possible in the field. Generally, in practice, the disappearance of clinical signs is considered as cure, whereas persistence or recurrence of clinical signs is considered as treatment failure. This treatment failure is what is evaluated in this study.

One of the consequences of successful elimination of the causative bacteria is a shortened timeframe during which infection can spread to other cows in the herd, via the milking machine, the milker or the environment. Potentially, improving bacteriological cure rates decreases the infection pressure on healthy cows and, thus, prevents new CM cases. At the same time bacteriological cure also

prevents the recrudescence of persistent infections (Van Eenennaam et al., 1995). These indirect effects of cure may play a role in decreasing the overall incidence of CM.

A number of approaches to improve bacteriological cure of CM have been evaluated, such as extending treatment duration (Sol et al., 2000, Oliver et al., 2004, Milne et al., 2005) and additional parenteral therapy (Shpighel et al., 1997, Erskine et al., 2002, Wenz et al., 2005). However, such studies have not evaluated the long-term outcome of treatment, nor do they necessarily accurately reflect the field situation where CM treatment outcomes are assessed by the elimination of clinical signs, such as abnormal milk, swelling or redness of the udder. In the field, if clinical signs persist or recur, intramammary (**IMM**) treatment is often extended or reinstated with parenteral treatment in the expectation of a more effective elimination of clinical signs, leading to the use of additional antibiotic on farm. However, there are few reports on the effects of extended treatment, with or without parenteral treatment, on CM persistence or recurrence.

Clinical mastitis can be treated with different types of antibiotics. Cefquinome is a broad-spectrum β-lactam antibiotic for the treatment of CM, via the IMM and parenteral routes and is licensed as a combination therapy for *E. coli* mastitis in the UK. Concurrent use of IMM and parenteral cefquinome in CM has been evaluated (Shpigel et al., 1997, Ehinger et al, 2006). In herds in which environmental mastitis predominates, the etiology is necessarily diverse thereby demanding a broad-spectrum antibiotic for first treatment of CM in the absence of previous identification of the causative pathogen.

The aim of this study was to evaluate the effect of different cefquinome treatment regimes in a field based context on the likelihood of clinical persistence or recurrence of CM in dairy herds with high recurrence rates of environmental mastitis.

## Material and Methods

### *Farms*

Three commercial dairy farms in Somerset, UK, were selected on the basis of access to electronic records, a history of a high rate of recurrence of CM and a predominance of environmental mastitis (Table 1). CM cases were sampled from August 2009 until November 2010. Monthly milk production, individual cow somatic cell count (SCC) and all CM cases had been recorded for at least 12 months prior to the start of the study. Milking protocols were comparable between farms, post milking teat disinfection, pre-dipping or pre-wiping and inspection for CM was practiced on all farms in all cows throughout lactation. Milking procedures and equipment did not change during the study period. All three farms used blanket antibiotic dry cow treatment.

### *Animals*

Lactating Holstein Friesian dairy cows with CM in one or more quarters were enrolled. Animal parity, yield, historic SCC, CM history, treatment history and relevant clinical data were recorded contemporaneously onto data capture forms or retrieved from on-farm software.

*Inclusion and exclusion criteria*

Cows were eligible for the study if they were in good general health and had four functional quarters free from clinically significant udder, teat and teat orifice lesions. Cows were followed for 105 days after treatment and when cows were dried off or removed from the herd earlier, right censoring was used. Data from animals that were dried off or removed from the herd due to death or culling were analysed until the day of dry off or removal.

*Treatment allocation*

Cows were randomly allocated to a treatment group, by the herdspersons based on line numbers. Line numbers were allocated randomly on farm at the moment animals joined the herd. Cows that developed CM were sampled aseptically before treatment, according to their pre-assigned treatment group. When clinical signs did not resolve ('treatment failure') during the 105 day period after the last treatment of an animal's first enrolled clinical case, or if clinical signs disappeared and recurred at any time point during that period, the cows were treated again with the same treatment regime on all subsequent occasions.

*Treatment*

All treatments were administered by farm personnel and three different regimes were evaluated; 1) 1.5-day IMM treatment with cefquinome 75 mg (Cobactan LC, MSD Animal Health), twice on the first day, at two consecutive milkings and once, at the morning milking on the following day (SIMM),

2) 5-day IMM treatment with cefquinome 75 mg, six times, twice on the first day, at two consecutive milkings, 4 times once a day, at the morning milking (EIMM),

3) 5-day combination treatment with cefquinome 75 mg IMM, six times, twice on the first day at two consecutive milkings and once, at the morning milking on the following 4 days, plus cefquinome sulphate suspension (1 mg/kg, Cobactan 2.5%, MSD Animal Health) by intramuscular injection five times at 24-hour intervals (ECOMBO).

*Post admission withdrawal*

Animals were withdrawn post admission due to missing data, injury or disability or abnormalities, or concomitant disease or disease other than CM requiring antibiotic or anti-inflammatory treatment.

*Detection of CM, persistence of clinical signs and milk sampling*

CM was defined as a quarter with any visible change of milk aspect and was identified by farm personnel, who had been trained and assessed in the detection, classification and sampling of CM. Individual cases were assessed for persistence or recurrence of clinical signs at every milking (twice daily on two units and thrice daily on one unit). The severity of CM was classified using a three-grade scale: Grade 1, mild (only clots in the milk); Grade 2, moderate (milk aspect changes in colour and / or consistency and / or presence of clots, heat, pain and/or swelling of the udder); and Grade 3, severe (milk aspect changes in colour and / or consistency and / or presence of clots, fever, depression, anorexia, very swollen udder).

Any concurrent treatments were also recorded. Prior to treatment farm personnel collected milk samples from affected quarters. Milk samples were frozen (-20 °C) and collected for submission to the laboratory on a weekly basis.

*Laboratory Methods*

Microbiological investigation and SCC were carried out using the standard milk sample examination techniques, according to the standard recommended by the International Dairy Federation (Bulletin No 132, 1981), International Standard 13366-1:1997 (E) and 13366-2:1997 (G). More specifically, 3 plates were used, 10 μL of secretion was inoculated onto sheep blood agar and Edward's agar; 100 μL of secretion was inoculated onto MacConkey agar to enhance the detection of *Enterobacteriaceae* before incubation at 37 ºC. All plates were read at 24, 48, and 72 h. Organisms were identified and quantified using standard laboratory techniques (NMC, 1999; Quinn et al., 1994). *Escherichia coli* was identified by colony morphology, oxidase, and indole tests; other *Enterobacteriaceae* were identified using a microtube identification system (RapiD 20 E, bioMérieux, Basingstoke, UK).

*Efficacy of treatment*

Treatment was considered effective if clinical signs had resolved after the last treatment and did not recur in the 105 day period after treatment, independent of the bacteria involved. To allow assessment of the potential benefits of systemic treatment on concurrently infected (but not clinically affected) quarters, efficacy was assessed at the quarter and cow level. At the quarter level, lack of

efficacy was based on clinical persistence or clinical recurrence of CM in the same quarter. At the cow level, lack of efficacy was based on clinical persistence or clinical recurrence of CM in the same cow, irrespective of the quarter involved.

*Data handling and Statistical analysis*

In this randomized, positive controlled, unmasked, three treatment group study, the null hypothesis was that there was no difference in time to clinical recurrence of CM between groups. This hypothesis was analyzed in a multi-level model. The clinically affected quarter was the experimental unit, with the subsequent analysis taking into account the effect of clustering of cases within quarters, and quarters within cows. Inevitably in studies such as this some cows were allocated to treatment group incorrectly. In order to ensure compliance in a large field based study such as this, farmers were allowed some discretion in individual cow treatment allocation. Analysis explored the impact of deviations from the predefined treatment protocols.

Cow and farm data were transferred to a database (Microsoft Access 2003) and all fields were checked for unusual or impossible entries. Data fields were coded as categorical or continuous, as appropriate, and data transformations carried out for continuous data to normalize distribution, when necessary.

The outcome variable of interest was the persistence or recurrence of clinical signs of mastitis after the end of treatment. Initial analysis consisted of descriptive statistics and graphical assessment. Conventional Kaplan-Meier survival curves were constructed to provide a visual display of clinical

persistence or recurrence ('treatment failure') of CM. To construct this curve, the 105 day post treatment study period was divided into 7-day blocks. Each case in each block was coded as recurrent or persistent (CM=1) or not recurrent or persistent (CM=0) at the quarter level. Cows were censored at the end of the 105-day follow-up period, at the end of lactation, or after death or culling. Discrete time survival models with random effects were specified so that correlations within the data (cases within quarters and quarters within cows) were accounted for as appropriate in a (frailty) model. The model took the form;

$$CM_{tijk} \sim \text{Bernoulli probability (mean} = \mu_{tijk})$$

$$\text{Logit}(\mu_{tijk}) = \alpha + \log t_{tijk} + \log t_{tijk}^2 + \log t_{tijk}^3 + \log t_{tijk}^4 + \beta_1 X_{ijk} + \beta_2 X_{jk} + \beta_3 X_k + u_{jk} + v_k$$

where t is the week of lactation after previous CM, i, j and k denote the $i^{th}$ CM case in the $j^{th}$ quarter of the $k^{th}$ cow, $\pi_{ijk}$ the fitted probability of clinical persistence or recurrence of CM after treatment for case i in quarter j of cow k, $\alpha$ the regression intercept, $X_{ijk}$ the vector of covariates at case level, $\beta_1$ the coefficients for covariates $X_{ik}$, $X_{jk}$ the vector of quarter level covariates, $\beta_2$ the coefficients for covariates $X_{jk}$, $X_k$ the vector of cow level covariates, $\beta_3$ the coefficients for covariates $X_k$, $u_j$ the random effect to reflect residual variation between quarters and $v_k$ the random effect to reflect residual variation between cows (both random effects assumed to be normally distributed with mean = 0 and variances $\Omega_u$ and $\Omega_v$ respectively).

The distributions of covariates were assessed and transformations or re-categorization carried out as deemed appropriate on biological grounds. Model building was carried out using MLwiN with penalized quasi-likelihood for parameter estimation (Rasbash et al., 2010). To avoid the potential biased estimates that can arise from quasi-likelihood methods (Browne and Draper, 2006) final models were

selected using Markov chain Monte Carlo (MCMC) for parameter estimation in WinBUGS (Spiegelhalter et al., 2004) using methods described in detail previously (Green et al., 2004). Covariates remained in the model when the 95% credibility intervals for the odds ratios did not include 1.00. Biologically plausible interactions between significant covariates were tested and included when the 95% credibility intervals for the odds ratio of the interaction term did not include 1.00.

Predictions of the survival time to clinical persistence or recurrence of CM after treatment were made using posterior predictive assessments (Gelman et al., 1996, Green et al., 2007). This incorporates the full model posterior predictive distribution, and was used to evaluate model fit and to illustrate the predicted impact of treatment on time to recurrence of CM. The effects of additional treatments were evaluated statistically by including terms for the extra treatments in multivariate models.

**Results**

CM occurred in 1008 cases on the three study farms, of which 994, mainly mild to moderate cases, were enrolled (Table 2). Fourteen cases (1008-994) were excluded due to missing data. Ninety-three cows were incorrectly allocated to treatment group, 124 received a NSAID concurrently and 106 received additional systemic antibiotics. These data were included in the statistical analysis and included as covariates in the initial analysis. There was a large variety of pathogens obtained from the samples and they are listed in Table 3. The most frequently isolated pathogens were *E. coli* (16.9%) and *Strep. uberis* (12%). These pathogens can be associated with typical environmental CM aetiology which was seen in both first and recurrent cases. Milk production, parity, underlying mastitis pathogens, CM history and treatment history did not differ significantly between groups.

*Quarter level*

Clinical persistence or recurrence at the quarter level is shown in Table 4. EIMM and ECOMBO treatment reduced the clinical persistence or recurrence of CM by 8% and 6%, respectively (EIMM, OR= 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72]). ECOMBO did not further decrease clinical persistence or recurrence when compared to EIMM alone. The time to quarter level clinical persistence or recurrence for the three treatment groups is illustrated in Fig. 1. A posterior prediction of treatment effects is shown in Fig. 2, which is comparable to the survival curve in Fig. 1, demonstrating good model fit, illustrates the predicted outcome for the three treatment groups based on the final model. Other significant covariates for quarter level clinical persistence or recurrence were farm, quarter location, parity and NSAID treatment. Infection severity was not a risk for persistence or recurrence (Table 6).

*Cow level*

Cow level clinical persistence or recurrence rates are presented in Table 5. The reduction in CM persistence or recurrence was 9% for EIMM and 8% after ECOMBO (EIMM, OR= 0.55, 95% CI [0.38-0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93]). The final multivariate model showed that, apart from treatment regime, the significant covariates for clinical persistence or recurrence of CM at the cow level were farm, quarter location, parity, and *Strep. uberis* infection compared to *E. coli* infection (Table 7).

## Discussion

Bacteriological cure is classically used to assess mastitis treatment efficacy because it is a concise and objective parameter. On farm, treatment efficacy is evaluated based on resolution of clinical signs and lack of recurrence. Persistence or recurrence of clinical signs often results in extended intramammary treatment or additional parenteral treatment, expecting a more efficient elimination of clinical signs and/or a lower probability of recurrence of clinical signs.

In this study, EIMM and ECOMBO were associated with a significant decrease in the probability of persistent or recurrent CM, both at the quarter level and at the cow level when compared to SIMM, in line with other recent findings (Pinzón-Sánchez et al., 2011). This suggests that extended and more 'aggressive' treatment regimens can be beneficial for individual cows when clinical persistence or recurrence rates are high. A reduction in persistent or recurrent CM can be due simply to higher bacteriological cure after extended treatment (Sol et al., 2000, Oliver et al., 2004, Pinzón-Sánchez et al. 2011), though it could also be as a result of a decrease in the risk of re-infection with another pathogen.

Our study differs from earlier studies in that *E. coli* was the most frequently isolated pathogen (Table 3). Döpfer et al. (1999) and Bradley and Green (2001) used molecular methods to demonstrate that clinical *E. coli* mastitis can recur, and that recurrent *E. coli* strains may be cow adapted. We found that *Strep. uberis* CM was nearly twice as likely to clinically persist or recur as *E. coli* CM. We also found a numerical reduction in clinical persistence or recurrence of *E. coli* CM after EIMM compared to SIMM (data not shown), although the difference was not statistically significant. This is in contrast to

297 studies that show that antibiotic treatment of *E. coli* CM should be avoided because it is not effective
298 (Pyörälä et al., 1998) or not expected to be effective in recurrent cases (Schukken et al., 2004).
299
300 An often overlooked, indirect effect of increasing bacteriological cure is a lower infection
301 pressure, simply because cured quarters are no longer able to spread infection to other quarters or other
302 cows (Swinkels et al., 2005a, 2005b; Barlow et al., 2009). This means extended treatment may not only
303 result in a higher bacteriological cure but may also have an indirect effect in a herd, such as an overall
304 lower re-infection rate and thus, less persistence or recurrence of CM. Because treatment strategies were
305 compared within herds, the 'infection pressure' for each treatment group was the same and could not
306 have influenced differences between treatment groups.
307
308 Clinical persistence or recurrence of CM was higher at the level of the cow (58%) than the
309 quarter (43%). This was expected, because at the cow level, CM can occur in any of the four quarters.
310 The difference between clinical persistence or recurrence at the cow and quarter level was not large and
311 indicates that the probability of CM recurrence in the other three quarters was relatively low and shows
312 persistence or recurrence mainly occurred in the originally affected quarter. This can be either caused by
313 the fact that chronically infected quarters may 'flare-up' after treatment (Houben et al., 1993, Lam et al.,
314 1996, Zadoks et al., 2001) and/or that previously infected quarters are more susceptible to new infection
315 (Zadoks et al., 2001).
316
317 Our model was not built to compare ECOMBO and EIMM treatment directly as both were
318 compared to SIMM. However, we believe ECOMBO treatment was not likely to have reduced
319 persistence or recurrence of CM, at the quarter or cow level, beyond EIMM treatment. In contrast, the

probability of clinical persistence or recurrence at the cow level was numerically higher in the ECOMBO group than in the EIMM group (Figure 1). However, it is possible that additional parenteral treatment in the ECOMBO group contributed to removal of subclinical infections (with minor pathogens) in the same cow (Sérieys et al., 2005), making those quarters more susceptible to new infections, thereby increasing the likelihood of subsequent CM and recurrence at the cow level. This is in line with the findings of Wenz et al. (2005), who concluded that parenteral treatment with a cephalosporin in addition to standard IMM cephalosporin treatment had no effect on recurrence of mild *E. coli* CM. Our results suggest that ECOMBO treatment with cefquinome may have no added value over EIMM treatment on farms with a high rate of recurrence where environmental pathogens predominate.

For quarters in cows with an additional NSAID treatment, clinical persistence or recurrence was significantly higher (Table 6) compared to quarters in cows where NSAID treatment was not used. This is unexpected, because clinical symptoms are assumed to resolve more quickly after NSAID treatment. It may be that farm personnel used additional NSAID treatment in cows which they suspected clinical symptoms to resolve more slowly or which they perceived to be more sensitive to mastitis and thus more likely to recur.

Prudent antibiotic use is a pre-requisite in modern agriculture and demands evidence-based justification for extended treatment. Exposure of bacteria to antibiotics increases the risk of selection for antibiotic resistance. EIMM treatment led to increased antibiotic use (from three to six tubes per case) and an increase in the duration of exposure to antimicrobials, which was not compensated by the 8-9% decrease in antibiotics used for recurrent cases. Thus, EIMM treatment led to an overall increase in

antibiotic exposure, albeit that that exposure was largely confined to the mammary microbiome. The ECOMBO approach clearly increased antibiotic exposure compared to EIMM, as well as resulting in exposure of the gut flora to antimicrobial activity, and did not seem to lower persistence or recurrence of CM beyond EIMM. This study challenges the perception that additional parenteral treatment will improve the outcome of all CM and re-enforces the need for such approaches to only be used for known pathogens. Whilst research suggests the use of parenteral antibiotics in severe mastitis cases may be helpful (Wenz et al., 2001, Erskine et al., 2002), further research is needed to better define the need and/or criteria for the use of systemic antibiotics in the treatment of mild and moderate cases of CM.

In conclusion, both EIMM and ECOMBO cefquinome treatment significantly reduced the persistence or recurrence of CM on farms with a high incidence of mild and moderate environmental mastitis. Because additional extended parenteral treatment beyond EIMM may not reduce clinical persistence or recurrence, the producer and practitioner need to carefully consider such regimes from the perspective of prudent antibiotic use.

## Conflict of interest statement

The study was sponsored by MSD Animal Health.

## Acknowledgement

The authors wish to thank Rinse Jan Boersma for his critical comments and Linda Horspool for writing assistance.

## References


Bar, D., Gröhn, Y.T., Bennett, G., Gonzalez, R.N., Hertl, J.A., Schulte, H.F., Tauer, L.W., Welcome, F.L., Schukken, Y.H., 2007. Effect of repeated episodes of generic clinical mastitis on milk yield in dairy cows. Journal of Dairy Science 90, 4643-4653.

Bar, D., Tauer, L.W., Bennett, G., Gonzalez, R.N., Hertl, J.A., Schukken, Y.H., Schulte, H.F., Welcome, F.L., Gröhn, Y.T., 2008. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. Journal of Dairy Science 91, 2205-2214.

Barlow, J.W., White, L.J., Zadoks, R.N., Schukken, Y.H., 2009. A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds. Preventive Veterinary Medicine 90, 31–42.

Bradley, A.J., Green, M.J., 2001. Adaptation of *Escherichia coli* to the bovine mammary gland. Journal of Clinical Microbiology 39, 1845-1849.

Browne, W.J., Draper D., 2006. A comparison of bayesian and likelihood-based methods for fitting multilevel models. Bayesian Analysis 1, 473-514.

Döpfer, D., Barkema, H.W., Lam, T.J.G.M., Schukken, Y.H., Gaastra, W., 1999. Recurrent clinical mastitis caused by *Escherichia coli* in dairy cows. Journal of Dairy Science 82, 80-85.

Ehinger, A.M., Schmidt, H., Kietzmann, M., 2006. Tissue distribution of cefquinome after intramammary and ‘‘systemic’’ administration in the isolated perfused bovine udder. The Veterinary Journal 172, 147-153.

Erskine, R.J., Bartlett, P.C., VanLente, J.L., Phipps, C.R., 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. Journal of Dairy Science 85, 2571-2575.

Gelman, A., Meng, X.L., Stern, H., 1996. Posterior predictive assessment of model fitness via realized discrepancies. Statistica Sinica 6, 733-807.

Green, M.J., Burton, P.R., Green, L.E., Schukken, Y.H., Bradley, A.J., Peeler, E.J., Medley, G.F., 2004. The use of Markov chain Monte Carlo for analysis of correlated binary data: patterns of somatic cells in milk and the risk of clinical mastitis in dairy cows. Preventive Veterinary Medicine 16, 157-174.

Green, M.J., Bradley, A.J., Medley, G.F., Browne, W.J., 2007. Cow, farm and management factors during the dry period that determine the rate of clinical mastitis after calving. Journal of Dairy Science 90, 3764-3776.

Houben, E.H.P., Dijkhuizen, A.A., van Arendonk, J.A.M., Huirne, R., 1993. Short- and long-term production losses and repeatability of clinical mastitis in dairy cattle. Journal of Dairy Science 76, 2561-2578.

Lam, T.J.G.M., Lipman, L.J., Schukken, Y.H., Gaastra, W., Brand, A., 1996. Epidemiological characteristics of bovine clinical mastitis caused by *Staphylococcus aureus* and *Escherichia coli* studied by DNA fingerprinting. American Journal of Veterinary Research 57, 39-42.

Milne, M.H., Biggs, A.M., Barrett, D.C., Young, F.J., Doherty, S., Innocent, G.T., Fitzpatrick, J.L., 2005. Treatment of persistent intramammary infections with *Streptococcus uberis* in dairy cows. Veterinary Record 157, 245-250.

NMC, 1999. Laboratory Handbook on Bovine mastitis. National Mastitis Council Inc, Madison, WI.

Oliver, S.P., Almeida, R.A., Gillespie, B.E., Headrick, S.J., Dowlen, H.H., Johnson, D.L., Lamar, K.C., Chester, S.T., Moseley, W.M., 2004. Extended ceftiofur therapy for treatment of experimentally-induced *Streptococcus uberis* mastitis in lactating dairy cattle. Journal of Dairy Science 87, 3322-3329.

Pinzón-Sánchez C., Ruegg, P.L., 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. Journal of Dairy Science 94, 3397-3410.

Pyörälä S.H., Pyörälä, E.O., 1998. Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows. Journal of the American Veterinary Medical Association 212, 407-412.

Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R., 1994. Clinical Veterinary Microbiology. Wolfe, London, England.

Rasbash, J., Browne, W.J., Healy, M., Cameron, B., Charlton, C., 2010. MLwiN Version 2.22. In multilevel models project (Centre for multilevel modeling, Bristol).

Schukken, Y.H., Dogan, B., Klaessig, S., Simpson, K., Almeida, R., Srinivasan, V., Gillespie, B., Oliver, S., 2004. Chronic and recurrent coliforms, implications for lactation therapy. In: Proceedings of the Annual Meeting of the National Mastitis Council, pp. 35-40.

Sérieys, F., Raguet, Y., Goby, L., Schmidt, H., Friton., G., 2005. Comparative efficacy of local and systemic antibiotic treatment in lactating cows with clinical mastitis. Journal of Dairy Science 88, 93-99.

Shpigel, Y., Levin, D., Winkler, M., Saran, A., Ziv, G., Böttner, A., 1997. Efficacy of cefquinome for treatment of cows with mastitis experimentally induced using *Escherichia coli*. Journal of Dairy Science 80, 318-323.

Sol, J., Sampimon, O.C., Barkema, H.W., Schukken, Y.H., 2000. Factors associated with cure after therapy of clinical mastitis caused by *Staphylococcus aureus.* Journal of Dairy Science 83, 278–284.

Spiegelhalter, D.J., Thomas, A., Best, N., 2004. WinBUGS Version 1.4.1. (Cambridge, UK, MRC Biostatistics Unit).

Swinkels, J.M., Hogeveen, H., Zadoks, R.N., 2005a. A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. Journal of Dairy Science 88, 4273-4287.

Swinkels, J.M., Rooijendijk, J.G.A., Zadoks, R.N., Hogeveen, H., 2005b. Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae.* Journal of Dairy Research, 72, 75-85.

Van Eenennaam, A.L., Gardner, A., Holmes, J., Perani, J.L., Anderson, R.J., Cullor, J.S., Guterbocks, W.U., 1995. Financial analysis of alternative treatments for clinical mastitis associated with environmental pathogens. Journal of Dairy Science 78, 2086-2095.

Wenz, J.R., Barrington, G.M., Garry, F.B., McSweeney, K.D., Dinsmore, R.P., Goodell, G., Callan, R.J., 2001. Bacteraemia associated with naturally occurring acute coliform mastitis in dairy cows. Journal of the American Veterinary Medical Association 219, 976-981.

Wenz, J.R., Garry, F.B., Lombard, J.E., Elia, R., Prentice, D., Dinsmore, R.P., 2005. *Short Communication:* Efficacy of parenteral ceftiofur for treatment of systemically mild clinical mastitis in dairy cattle. Journal of Dairy Science 88, 3496-3499.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Wellenberg, G.J., Gröhn, Y.T., Schukken, Y.H., 2001. Cow- and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. Journal of Dairy Science 84, 2649-2663.

**Table 1.** The characteristics of the three herds involved in the study

| Farm ID | C | H | S |
|---|---|---|---|
| Number of dairy cows | 560 | 239 | 308 |
| BMSCC (x 1,000) | 248 | 201 | 158 |
| ICRM | 85 | 116 | 76 |
| Predominant Housing | Cubicles | Cubicles /pasture | Cubicles |
| Predominant Breed | HF | HF | HF |
| Approx 305 Day Yield (L) | 9159 | 9003 | 11,309 |
| Milking Frequency | 2X | 2X | 3X |

BMSCC, bulk milk somatic cell count, 12 months rolling mean; IRCM, incidence rate of clinical mastitis (number of quarter cases per 100 cows per year).

**Table 2.** Severity of clinical mastitis, indicated in numbers of cases and percentages in parenthesis

| Clinical signs | SIMM | EIMM | ECOMBO | Total |
|---|---|---|---|---|
| Grade 1 | 175 (57) | 192 (60) | 222 (60) | 589 (59) |
| Grade 2 | 109 (36) | 100 (31) | 125 (34) | 334 (34) |
| Grade 3 | 21 (7) | 26 (8) | 24 (6) | 71 (7) |
| Total | 305 (100) | 318 (100) | 371 (100) | 994 (100) |

Grade 1, mild (only clots in the milk); Grade 2, moderate (milk aspect changes in colour and / or consistency and / or presence of clots, heat, pain and/or swelling of the udder); and Grade 3, severe (milk aspect changes in colour and / or consistency and / or presence of clots, fever, depression, anorexia, very swollen udder); SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days.

**Table 3**. Aetiology of clinical mastitis cases per treatment group

| | Treatment | | | | |
|---|---|---|---|---|---|
| Diagnosis (n) | SIMM | EIMM | ECOMBO | Total | Total (%) |
| *E. coli* | 54 | 55 | 59 | 168 | 16.90 |
| *S. uberis* | 31 | 41 | 47 | 119 | 11.97 |
| *S. dysgalactiae* | 14 | 13 | 22 | 49 | 4.93 |
| *S. aureus* | 11 | 17 | 14 | 42 | 4.23 |
| *Bacillus* spp. | 9 | 14 | 14 | 37 | 3.72 |
| Yeast spp. | 14 | 13 | 7 | 34 | 3.42 |
| *Enterococcus* spp. | 10 | 8 | 6 | 24 | 2.41 |
| *Klebsiella* spp. | 6 | 8 | 3 | 17 | 1.71 |
| *Prototheca* spp. | 11 | 3 | 1 | 15 | 1.51 |
| *A. pyogenes* | 2 | 1 | 10 | 13 | 1.31 |
| *Enterobacter* spp. | 4 | 2 | 3 | 9 | 0.91 |
| *Aerococcus* | 3 | 1 | 4 | 8 | 0.80 |
| *Pseudomonas* spp. | 2 | 3 | 3 | 8 | 0.80 |
| Other major pathogens | 8 | 12 | 8 | 28 | 2.81 |
| Any Enterobacterial involvement | 76 | 83 | 77 | 236 | 23.74 |
| Mixed aetiology (major pathogens) | 16 | 22 | 30 | 68 | 6.84 |
| *Corynebacterium* spp. | 17 | 19 | 30 | 66 | 6.64 |
| Coagulase negative *Staph* | 12 | 11 | 30 | 53 | 5.33 |
| Mixed aetiology (minor pathogens) | 9 | 11 | 13 | 33 | 3.32 |
| Contaminated | 11 | 11 | 8 | 30 | 3.02 |
| No growth | 61 | 53 | 59 | 173 | 17.40 |
| Grand Total | 305 | 318 | 371 | 994 | 100.00 |

SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days.

**Table 4.** Numbers and percentages (between brackets) of persistence or recurrence of clinical mastitis at the quarter level within 105 days after the end of treatment of the initial clinical mastitis, irrespective of the isolated bacterial species

| | Treatment | | | |
|---|---|---|---|---|
| Recurrence | SIMM | EIMM | ECOMBO | Total |
| No (%) | 158 (52) | 192 (60) | 216 (58) | 566 (57) |
| Yes (%) | 147 (48) | 126 (40)* | 155 (42)* | 428 (43) |
| Total | 305 | 318 | 371 | 994 |

SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days; *, The odds ratio (OR) of recurrence after EIMM and after ECOMBO treatment were statistically significantly different from the SIMM treatment group (EIMM, OR = 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72].

**Table 5**. Numbers and percentages (between brackets) of persistence or recurrence of clinical mastitis at the cow level within 105 days after the end of treatment of the initial clinical mastitis, irrespective of the isolated bacterial species

| | Treatment | | | |
|---|---|---|---|---|
| Recurrence | SIMM | EIMM | ECOMBO | Total |
| No (%) | 111 (36) | 144 (45) | 162 (44) | 417 (42) |
| Yes (%) | 194 (64) | 174 (55)* | 209 (56)* | 577 (58) |
| Total | 305 | 318 | 371 | 994 |

SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days; *, the Odds Ratio (OR) of recurrence of EIMM and ECOMBO treatment were statistically significantly different from the SIMM treatment group (EIMM, OR= 0.55, 95% CI [0.38-0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93].

**Table 6.** Final model outcome of the multivariate analysis of probability of clinical persistence or recurrence at the quarter level

| Model term | OR | 95% CI | |
|---|---|---|---|
| Intercept=-3.2 | | | |
| Ref= SIMM | | | |
| EIMM | 0.26 | 0.12 | 0.50 |
| ECOMBO | 0.38 | 0.19 | 0.72 |
| Ref = farm C* | | | |
| Farm H | 2.58 | 1.35 | 5.32 |
| Farm S | 0.79 | 0.36 | 1.73 |
| Ref= quarter LF | | | |
| Quarter LH | 0.66 | 0.35 | 1.24 |
| Quarter RF | 0.49 | 0.25 | 0.93 |
| Quarter RH | 1.08 | 0.56 | 2.09 |
| Ref=Parity 1 | | | |
| Parity 2 | 1.31 | 0.52 | 3.27 |
| Parity 3 | 3.23 | 1.25 | 8.25 |
| Parity 4 | 4.74 | 1.73 | 13.17 |
| Parity 5+ | 3.29 | 1.32 | 8.26 |
| Ref= Grade 1[f] | | | |
| Grade 2 | 1.04 | 0.63 | 1.73 |
| Grade 3 | 1.62 | 0.51 | 5.23 |
| Ref= Yes NSAID[g] | | | |
| No NSAID | 0.30 | 0.11 | 0.74 |

Ref, reference parameter; SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days; *, for farm characteristics, see Table 1; LF, left front; RF, right front; LH, left hind; LF, left front; Grade 1, Mild; Only clots in the milk, Grade 2, Moderate; Heat, pain and/or swelling of the udder, Grade 3, Severe; Fever, depression, anorexia, very swollen udder; yes NSAID, non-steroidal anti-inflammatory drug given in addition to antibiotics.

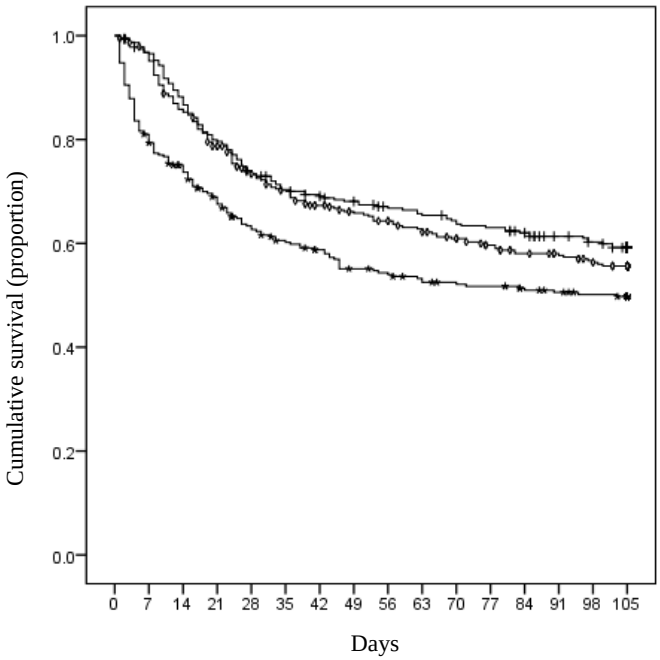
**Table 7.** Final outcome of the multivariate model of the probability of clinical persistence or recurrence at the cow level

| Model term | OR | 95% CI | |
|---|---|---|---|
| Intercept= -4.0 | | | |
| Ref= SIMM | | | |
| EIMM | 0.55 | 0.38 | 0.77 |
| ECOMBO | 0.66 | 0.47 | 0.93 |
| Ref = farm C* | | | |
| Farm H | 1.74 | 1.22 | 2.48 |
| Farm S | 0.90 | 0.59 | 1.34 |
| Ref=Parity 1 | | | |
| Parity 2 | 1.80 | 1.10 | 3.03 |
| Parity 3 | 3.10 | 1.85 | 5.55 |
| Parity 4 | 3.31 | 1.93 | 6.10 |
| Parity 5+ | 3.32 | 2.04 | 5.71 |
| Ref= *E. coli*† | | | |
| *S. uberis* | 1.96 | 1.21 | 3.20 |

Ref, reference parameter; SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days; *, for farm characteristics, see Table 1; †, recurrence of clinical mastitis causing pathogen - recurrence of other bacteria was not significantly different from the reference pathogen, *E. coli*.

**Figure 1.** Legend

**Figure 1** Kaplan Meier survival curve, illustrating cumulative survival (=no persistence or recurrence) at the quarter level, during 105 days after initial treatment for the 3 different treatment groups

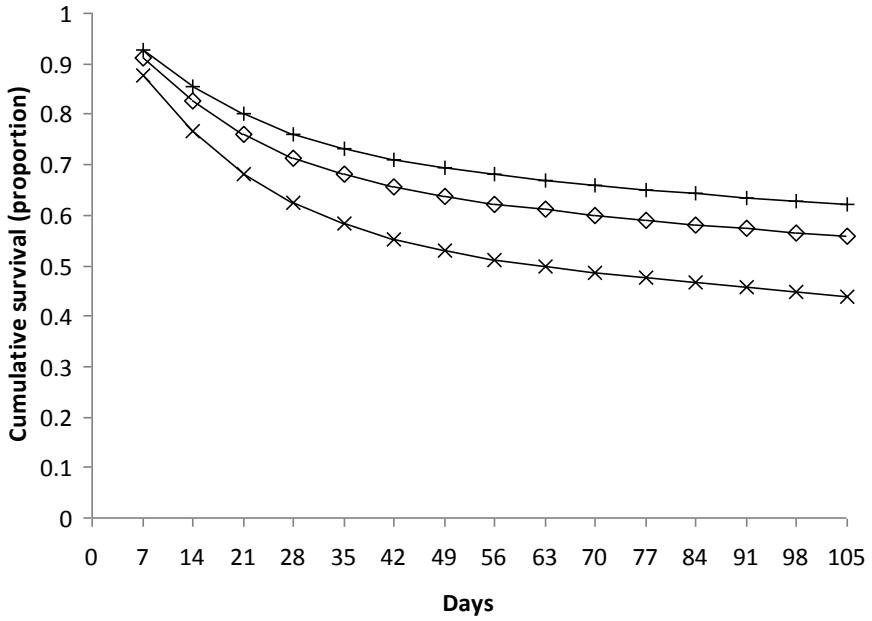



x, short intramammary cefquinome treatment (SIMM) for 1.5 days; +, extended intramammary (EIMM) cefquinome treatment for 5 days; ◊, extended combined intramammary and parenteral cefquinome treatment (ECOMBO) for 5 days.

Both EIMM and ECOMBO treatment reduced persistence or recurrence of clinical mastitis significantly compared to the SIMM (EIMM, OR = 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72].

**Figure 2.**

The Bayesian prediction of survival (=no persistence or recurrence) of clinical mastitis after initial treatment from the multilevel model. The prediction is made for every quarter assuming it could receive each treatment




**X,** short intramammary cefquinome treatment (SIMM) for 1.5 days; +, extended intramammary (EIMM) cefquinome treatment for 5 days; ◊, extended combined intramammary and parenteral cefquinome treatment (ECOMBO) for 5 days.