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

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26 **Abstract**

27

28 The efficacy of antibiotic treatment of clinical mastitis (**CM**) is classically evaluated using  
29 bacteriological cure. This provides a concise and objective way of assessing efficacy, but unfortunately  
30 does not reflect the field situation where persistence or recurrence of clinical signs lead to perceived  
31 treatment failure. If clinical signs persist or recur, intramammary (**IMM**) treatment is often extended or  
32 supplemented with parenteral treatment, in the expectation of a more efficient elimination of clinical  
33 signs or a lower probability of recurrence of clinical signs. The objective of this study was to evaluate  
34 the efficacy against clinical persistence or recurrence of three cefquinome treatment regimes, standard  
35 1.5-day intramammary (**SIMM**), 5-day extended intramammary (**EIMM**) and combination of EIMM  
36 plus 5-day extended parenteral (**ECOMBO**) treatment. The study was conducted on three dairy farms  
37 with a high recurrence rate of environmental mastitis. Efficacy was evaluated using a multi-level model  
38 at the quarter and at the cow level, based on the persistence or recurrence of clinical signs at any time  
39 during a 105-day period following the end of the initial treatment, independent of pathogen. The most  
40 prevalent pathogens were *E. coli* (16.9%) and *S. uberis* (11.97%). EIMM and ECOMBO significantly  
41 decreased the persistence or recurrence of CM by 8 % and 6 % at the quarter level and by 9 % and 8 %  
42 at the cow level, respectively. ECOMBO may not reduce the persistence or recurrence of CM beyond  
43 EIMM. Whilst extended treatment regimens offered an improved outcome in this study, the producer  
44 and practitioner need to carefully consider such regimens from the perspective of prudent antibiotic use.

45

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

47

## 48 **Introduction**

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

57

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

65

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

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

72

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

83

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

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

94

## 95 **Material and Methods**

96

### 97 *Farms*

98

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

107

### 108 *Animals*

109

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

113

114 *Inclusion and exclusion criteria*

115

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

121

122 *Treatment allocation*

123

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

131

132 *Treatment*

133

134 All treatments were administered by farm personnel and three different regimes were evaluated;  
135 1) 1.5-day IMM treatment with cefquinome 75 mg (Cobactan LC, MSD Animal Health), twice on the  
136 first day, at two consecutive milkings and once, at the morning milking on the following day (SIMM),

137 2) 5-day IMM treatment with cefquinome 75 mg, six times, twice on the first day, at two consecutive  
138 milkings, 4 times once a day, at the morning milking (EIMM),  
139 3) 5-day combination treatment with cefquinome 75 mg IMM, six times, twice on the first day at two  
140 consecutive milkings and once, at the morning milking on the following 4 days, plus cefquinome  
141 sulphate suspension (1 mg/kg, Cobactan 2.5%, MSD Animal Health) by intramuscular injection five  
142 times at 24-hour intervals (ECOMBO).

143

#### 144 *Post admission withdrawal*

145

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

149

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

151

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



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

163

#### 164 *Laboratory Methods*

165

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

176

#### 177 *Efficacy of treatment*

178

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

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

186

#### 187 *Data handling and Statistical analysis*

188

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

197

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

202

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

206 persistence or recurrence ('treatment failure') of CM. To construct this curve, the 105 day post treatment  
 207 study period was divided into 7-day blocks. Each case in each block was coded as recurrent or persistent  
 208 (CM=1) or not recurrent or persistent (CM=0) at the quarter level. Cows were censored at the end of the  
 209 105-day follow-up period, at the end of lactation, or after death or culling. Discrete time survival models  
 210 with random effects were specified so that correlations within the data (cases within quarters and quarters  
 211 within cows) were accounted for as appropriate in a (frailty) model. The model took the form;

212

$$213 \quad CM_{ijk} \sim \text{Bernoulli probability (mean} = \mu_{ijk})$$

214

$$215 \quad \text{Logit}(\mu_{ijk}) = \alpha + \log t_{ijk} + \log t_{ijk}^2 + \log t_{ijk}^3 + \log t_{ijk}^4 + \beta_1 X_{ijk} + \beta_2 X_{jk} + \beta_3 X_k + u_{jk} + v_k$$

216

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

224

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

229 selected using Markov chain Monte Carlo (MCMC) for parameter estimation in WinBUGS  
230 (Spiegelhalter et al., 2004) using methods described in detail previously (Green et al., 2004). Covariates  
231 remained in the model when the 95% credibility intervals for the odds ratios did not include 1.00.  
232 Biologically plausible interactions between significant covariates were tested and included when the  
233 95% credibility intervals for the odds ratio of the interaction term did not include 1.00.

234

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

240

## 241 **Results**

242

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

252

253 *Quarter level*

254

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

265

266 *Cow level*

267

268 Cow level clinical persistence or recurrence rates are presented in Table 5. The reduction in CM  
269 persistence or recurrence was 9% for EIMM and 8% after ECOMBO (EIMM, OR= 0.55, 95% CI [0.38-  
270 0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93]). The final multivariate model showed that, apart  
271 from treatment regime, the significant covariates for clinical persistence or recurrence of CM at the cow  
272 level were farm, quarter location, parity, and *Strep. uberis* infection compared to *E. coli* infection (Table  
273 7).

274

275 **Discussion**

276

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

282

283 In this study, EIMM and ECOMBO were associated with a significant decrease in the probability  
284 of persistent or recurrent CM, both at the quarter level and at the cow level when compared to SIMM, in  
285 line with other recent findings (Pinzón-Sánchez et al., 2011). This suggests that extended and more  
286 ‘aggressive’ treatment regimens can be beneficial for individual cows when clinical persistence or  
287 recurrence rates are high. A reduction in persistent or recurrent CM can be due simply to higher  
288 bacteriological cure after extended treatment (Sol et al., 2000, Oliver et al., 2004, Pinzón-Sánchez et al.  
289 2011), though it could also be as a result of a decrease in the risk of re-infection with another pathogen.

290

291 Our study differs from earlier studies in that *E. coli* was the most frequently isolated pathogen  
292 (Table 3). Döpfer et al. (1999) and Bradley and Green (2001) used molecular methods to demonstrate  
293 that clinical *E. coli* mastitis can recur, and that recurrent *E. coli* strains may be cow adapted. We found  
294 that *Strep. uberis* CM was nearly twice as likely to clinically persist or recur as *E. coli* CM. We also  
295 found a numerical reduction in clinical persistence or recurrence of *E. coli* CM after EIMM compared to  
296 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

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

330

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

337

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



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

351

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

357

#### 358 **Conflict of interest statement**

359

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361

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363

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366

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

481

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

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

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526 **Table 2.** Severity of clinical mastitis, indicated in numbers of cases and percentages in parenthesis  
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Clinical signs	SIMM	EIMM	ECOMBO	Total
529 Grade 1	175 (57)	192 (60)	222 (60)	589 (59)
530 Grade 2	109 (36)	100 (31)	125 (34)	334 (34)
531 Grade 3	21 (7)	26 (8)	24 (6)	71 (7)
532 Total	305 (100)	318 (100)	371 (100)	994 (100)

533

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

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

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546 Diagnosis (n)	545 Treatment			546 Total	546 Total (%)
	546 SIMM	546 EIMM	546 ECOMBO		
547 <i>E. coli</i>	54	55	59	168	16.90
548 <i>S. uberis</i>	31	41	47	119	11.97
549 <i>S. dysgalactiae</i>	14	13	22	49	4.93
550 <i>S. aureus</i>	11	17	14	42	4.23
551 <i>Bacillus</i> spp.	9	14	14	37	3.72
552 Yeast spp.	14	13	7	34	3.42
553 <i>Enterococcus</i> spp.	10	8	6	24	2.41
554 <i>Klebsiella</i> spp.	6	8	3	17	1.71
555 <i>Prototheca</i> spp.	11	3	1	15	1.51
556 <i>A. pyogenes</i>	2	1	10	13	1.31
557 <i>Enterobacter</i> spp.	4	2	3	9	0.91
558 <i>Aerococcus</i>	3	1	4	8	0.80
559 <i>Pseudomonas</i> spp.	2	3	3	8	0.80
560 Other major pathogens	8	12	8	28	2.81
561 Any Enterobacterial involvement	76	83	77	236	23.74
562 Mixed aetiology (major pathogens)	16	22	30	68	6.84
563 <i>Corynebacterium</i> spp.	17	19	30	66	6.64
564 Coagulase negative <i>Staph</i>	12	11	30	53	5.33
565 Mixed aetiology (minor pathogens)	9	11	13	33	3.32
566 Contaminated	11	11	8	30	3.02
567 No growth	61	53	59	173	17.40
568 Grand Total	305	318	371	994	100.00

569

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

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

578

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

584

585 SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome  
586 treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome  
587 treatment for 5 days; \*, The odds ratio (OR) of recurrence after EIMM and after ECOMBO treatment  
588 were statistically significantly different from the SIMM treatment group (EIMM, OR = 0.38, 95% CI  
589 [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72].

590



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

594

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

599

600  
 601 SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended intramammary cefquinome  
 602 treatment for 5 days; ECOMBO, extended combined intramammary and parenteral cefquinome  
 603 treatment for 5 days; \*, the Odds Ratio (OR) of recurrence of EIMM and ECOMBO treatment were  
 604 statistically significantly different from the SIMM treatment group (EIMM, OR= 0.55, 95% CI [0.38-  
 605 0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93].

606 **Table 6.** Final model outcome of the multivariate analysis of probability of clinical persistence or  
 607 recurrence at the quarter level  
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609	Model term	OR	95% CI	
610	Intercept=-3.2			
611	Ref= SIMM			
612	EIMM	0.26	0.12	0.50
613	ECOMBO	0.38	0.19	0.72
614	Ref = farm C*			
615	Farm H	2.58	1.35	5.32
616	Farm S	0.79	0.36	1.73
617	Ref= quarter LF			
618	Quarter LH	0.66	0.35	1.24
619	Quarter RF	0.49	0.25	0.93
620	Quarter RH	1.08	0.56	2.09
621	Ref=Parity 1			
622	Parity 2	1.31	0.52	3.27
623	Parity 3	3.23	1.25	8.25
624	Parity 4	4.74	1.73	13.17
625	Parity 5+	3.29	1.32	8.26
626	Ref= Grade 1 <sup>f</sup>			
627	Grade 2	1.04	0.63	1.73
628	Grade 3	1.62	0.51	5.23
629	Ref= Yes NSAID <sup>g</sup>			
630	No NSAID	0.30	0.11	0.74

631  
 632 Ref, reference parameter; SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended  
 633 intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and  
 634 parenteral cefquinome treatment for 5 days; \*, for farm characteristics, see Table 1; LF, left front; RF,  
 635 right front; LH, left hind; LF, left front; Grade 1, Mild; Only clots in the milk, Grade 2, Moderate; Heat,  
 636 pain and/or swelling of the udder, Grade 3, Severe; Fever, depression, anorexia, very swollen udder; yes  
 637 NSAID, non-steroidal anti-inflammatory drug given in addition to antibiotics.  
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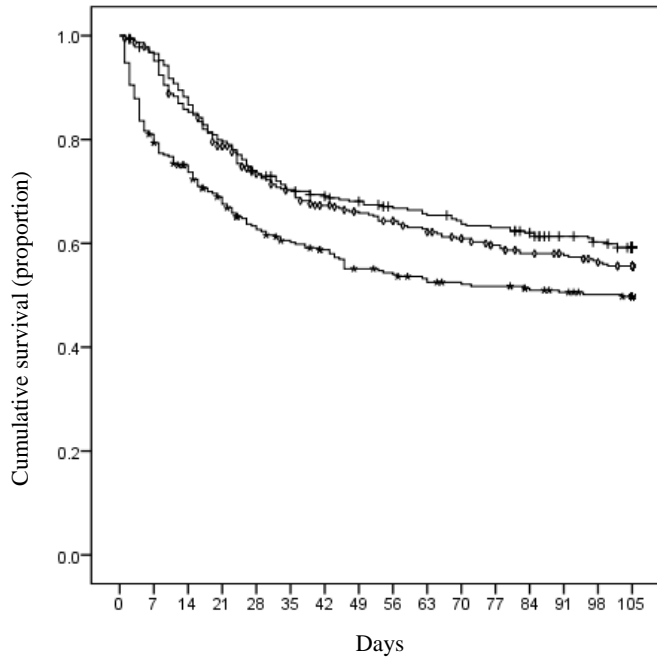
639 **Table 7.** Final outcome of the multivariate model of the probability of clinical persistence or recurrence  
 640 at the cow level

642 Model term	OR	95% CI	
643 Intercept= -4.0			
644 Ref= SIMM			
645 EIMM	0.55	0.38	0.77
646 ECOMBO	0.66	0.47	0.93
647 Ref = farm C*			
648 Farm H	1.74	1.22	2.48
649 Farm S	0.90	0.59	1.34
650 Ref=Parity 1			
651 Parity 2	1.80	1.10	3.03
652 Parity 3	3.10	1.85	5.55
653 Parity 4	3.31	1.93	6.10
654 Parity 5+	3.32	2.04	5.71
655 Ref= <i>E. coli</i> <sup>†</sup>			
656 <i>S. uberis</i>	1.96	1.21	3.20

657  
 658 Ref, reference parameter; SIMM, intramammary cefquinome treatment for 1.5 days; EIMM, extended  
 659 intramammary cefquinome treatment for 5 days; ECOMBO, extended combined intramammary and  
 660 parenteral cefquinome treatment for 5 days; \*, for farm characteristics, see Table 1; †, recurrence of  
 661 clinical mastitis causing pathogen - recurrence of other bacteria was not significantly different from the  
 662 reference pathogen, *E. coli*.

663 **Figure 1.** Legend  
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666 **Figure 1** Kaplan Meier survival curve, illustrating cumulative survival (=no persistence or recurrence) at  
 667 the quarter level, during 105 days after initial treatment for the 3 different treatment groups  
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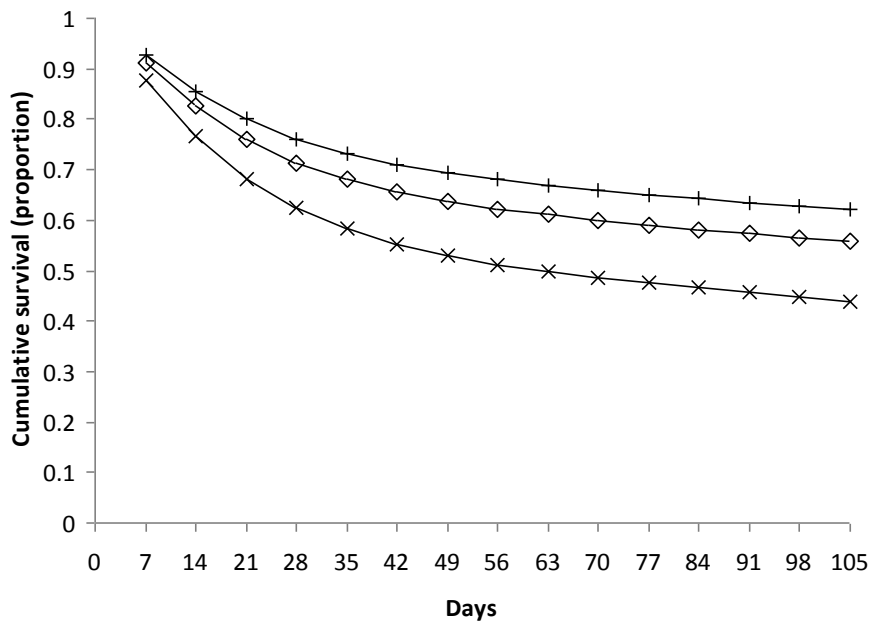
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 673 X, short intramammary cefquinome treatment (SIMM) for 1.5 days; +, extended intramammary  
 674 (EIMM) cefquinome treatment for 5 days; ◇, extended combined intramammary and parenteral  
 675 cefquinome treatment (ECOMBO) for 5 days.  
 676  
 677 Both EIMM and ECOMBO treatment reduced persistence or recurrence of clinical mastitis significantly  
 678 compared to the SIMM (EIMM, OR = 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI  
 679 [0.19-0.72].

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**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



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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.