Efficacy of extended cefquinome treatment of clinical *Staphylococcus aureus* mastitis

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ABSTRACT

Clinical *Staphylococcus aureus* mastitis is difficult to cure. Extended antimicrobial treatment is often advocated as a practical approach to improve cure rates; however, scientific evidence of this hypothesis is lacking. A multi-centered, nonblinded, randomized, positive-controlled clinical trial was conducted in 5 European countries—France, Hungary, Italy, the Netherlands, and the United Kingdom—to study the efficacy of an extended intramammary cefquinome treatment (5 d) compared with a standard intramammary cefquinome treatment (1.5 d) of *Staph. aureus* clinical mastitis. Least squares means estimates of bacteriological cure during lactation were 34% [standard error (SE) = 9.9%] for the standard treatment group and 27% (SE = 8.4%) for the extended treatment group. In the final model, extended therapy was not significantly better. The only factor predicting bacteriological cure was pretreatment cow somatic cell count (SCC). Cows with >250,000 cells/mL in milk before treatment were less likely to cure. Least squares means of clinical cure during lactation was 60% (SE = 19%) for the standard treatment group and 82% (SE = 12%) for the extended treatment group. In the final model, clinical cure after extended treatment was significantly better. Pretreatment cow udder firmness predicted clinical cure. Firm udders were less likely to cure clinically. Irrespective of treatment regimen, new infection rates with pathogens other than *Staph. aureus* were higher (42%) after bacteriological cure than after nonbacteriological cure (22%) and cured cows had a significantly lower SCC. In conclusion, independent of the treatment protocol, cows with an SCC <250,000 cells/mL before treatment showed a higher probability of bacteriological cure. It appears that successful treatment of clinical *Staph.*

aureus mastitis with cefquinome is associated with an increased number of new infections with coagulase-negative staphylococci. Extended treatment improved clinical, but not bacteriological, cure rates compared with the standard treatment. These results indicate that extending treatment of clinical *Staph. aureus* mastitis with cefquinome should not be recommended.

Key words: dairy cow, lactation, mammary gland, antimicrobial

INTRODUCTION

Contagious mastitis has been successfully controlled in recent decades on many farms in many countries due to the large scale adoption of the 5-point plan: optimization of milking and milking machine, blanket dry cow treatment, postmilking teat disinfection, treatment of clinical mastitis, and culling of chronically infected cows (Neave et al., 1969). As a result, the incidence of contagious pathogens, such as *Streptococcus agalactiae* and *Staphylococcus aureus*, has declined, resulting in a dramatic decrease of the average bulk milk SCC (**BM-SCC**) in the majority of European countries. Despite this, *Staph. aureus* remains an important mastitis-causing pathogen on many farms, even when BMSCC is low (Barkema et al., 1998; Olde Riekerink et al., 2010).

An important reason the prevalence of *Staph. aureus* remains high in herds is the degree of difficulty to cure existing infections, leading to both repeat clinical episodes and spreading of bacteria to herd mates. Bacteriological cure (**BC**) of *Staph. aureus* mastitis during lactation is generally low, both for subclinical (Sol et al., 1997) and clinical cases (Sol et al., 2000; Bradley and Green, 2009), and is mainly dependent on the duration of infection, the bacterial strain, and the duration of treatment (Barkema et al., 2006). Also, some *Staph. aureus* strains have a stronger clinical manifestation than others (Zadoks et al., 2000; Haveri et al., 2005; Fournier et al., 2008), a lower persistence, and a higher probability of cure (Haveri et al., 2005).

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Subclinical *Staph. aureus*-infected cows can be selected for treatment based on their estimated probability of cure. In *Staph. aureus* clinical mastitis (CM), however, treatment is necessary to fight the clinical symptoms and to bring the cow back into production as soon as possible. Increased duration of treatment is often used in practice because it is an easy way to possibly improve the probability of cure. Research showing the beneficial effect of extended antimicrobial treatment of subclinical *Staph. aureus* mastitis during lactation are numerous and conclusive (Gillespie et al., 2002; Oliver et al., 2004; Deluyker et al., 2005). However, studies of extended antimicrobial treatment of clinical *Staph. aureus* mastitis are limited and less conclusive, showing a better effect (Jarp et al., 1989), only a numerical difference (Pyörälä and Pyörälä, 1998), or a significantly higher cure only for β -lactamase negative *Staph. aureus* cases (Sol et al., 2000). Thus, more research on the effects of extended treatment is needed for clinical *Staph. aureus* mastitis.

Cefquinome is a broad-spectrum cephalosporin which is commonly used for the intramammary (IMM) treatment of CM throughout Europe, where it has been approved for the treatment of CM caused by the major mastitis-causing pathogens, including *Staph. aureus*. The objective of this study is to compare different aspects of the efficacy of a standard versus an extended duration of IMM cefquinome treatment against clinical *Staph. aureus* mastitis.

MATERIALS AND METHODS

Study Design

This was a multi-centered, nonblinded, randomized, positive controlled clinical trial that was conducted in 5 countries: France, Hungary, Italy, the Netherlands, and the United Kingdom. The protocol was in accordance with the note for Guidance on statistical principles for veterinary clinical trials (EMA, 2002), the legal requirements in the countries where it was implemented, and the European Guideline for the conduct of efficacy studies for intramammary products for use in cattle (EMA, 2003).

Lactating cows with clinical *Staph. aureus* mastitis from different herds were randomly assigned to 2 treatment groups, receiving either a standard 1.5-d or an extended 5-d IMM cefquinome treatment. Milk samples were taken around 14 and 21 d after the last treatment to determine the primary efficacy criteria, BC, and clinical cure (CC).

Animals and Herds

No specific animal management or housing was required for the selection of herds. Herd-specific data,

such as herd size (number of dairy cows), herd management (housing, bedding, feeding), or udder health management (average milking interval, teat disinfecting practices, that is, pre- and postdipping and blanket, selective or no dry cow treatment), were recorded, as well as the 3 most recent BMSCC.

Criteria for Selection of Clinical Mastitis Cases

Lactating cows with CM in a single quarter were included in this study. Clinical mastitis was defined as a quarter with clinical signs (swelling, heat, pain) or any changes in the appearance of milk, with or without associated general clinical signs. For the included cows, historic cow data, such as breed, date of birth, number of lactation, date of last calving, estimated or measured milk yield at time of treatment, cow SCC, and history of previous mastitis cases, were recorded. Excluded were cows with CM occurring less than 30 d from the planned day of drying-off, cows with severe systemic signs needing other systemic treatment, cows with other intercurrent diseases at the time of CM, cows given systemic or IMM anti-inflammatory or antimicrobial treatments within a 30-d period before inclusion, cows with visible teat damage, and cows with daily milk yield less than 5 kg before the onset of clinical signs.

Postadmission Withdrawal

A cow was withdrawn after enrolment if the pretreatment milk sample was contaminated (>2 bacteria species) or if no *Staph. aureus* was isolated. If 2 bacteria were cultured pretreatment, *Staph. aureus* had to be the primary pathogen, having equal or more bacteria than the other pathogen based on semiquantitative analysis (low/moderate/high), to keep the cow in the study. Also withdrawn were cows experiencing an adverse event, injury, or illness, including mastitis in another quarter or treatment with another antimicrobial or anti-inflammatory. If any other significant deviation from the protocol occurred, the corresponding case was also withdrawn from the study.

Number of Animals

The tested hypothesis was that the extended treatment resulted in a higher cure rate (50%) than the standard treatment (25%). Based on a one-sided, Chi-squared test with type I error $\alpha = 0.05$ and type II error $\beta = 0.20$, the estimated sample size is 48 cows per treatment group. Assuming 70% bacteriological positive and noncontaminated milk cultures, a *Staph. aureus* prevalence among these of 10%, and 10% exclu-

sion for adverse events and major deviations, a total of approximately 1,500 cows should be included to reach the target sample size. However, during the study it became clear that the actual prevalence of *Staph. aureus* in noncontaminated bacteriological-positive cultures was much higher than estimated (not 10% but 17.7%); thus, the trial could be stopped before reaching the 1,500 included cows. Finally, 1,217 cows with CM were screened.

Treatment and Randomization

Eligible cows were treated IMM in the infected quarter with 75 mg of cefquinome sulfate (Cobactan LC, MSD Animal Health, Boxmeer, the Netherlands) according to one of the following treatment protocols: extended treatment group = 3 infusions of one tube at 12-h intervals, followed by 3 infusions of one syringe at 24-h intervals (5 d); or standard treatment group = 3 infusions of one tube at 12-h intervals between each infusion (1.5 d). To ensure the homogeneity of treatment groups, cows were allocated randomly to either the extended or the standard treatment group. A randomization list was established in advance for each country using SAS software (version 9.1; SAS Institute Inc., Cary, NC). Each investigator received a set of numbered therapeutic units containing either 6 or 3 tubes. When a new animal was enrolled, the investigator had to give the therapeutic unit with the lowest number as indicated on the randomization list. Any adverse event, whether or not considered treatment related, was recorded and thoroughly reviewed.

Milk Sampling

At inclusion, milk sampling for bacteriology was performed from the affected quarter of all clinical cases. First and second post-treatment quarter milk samples were only taken if *Staph. aureus* was cultured from the sample at inclusion. The first post-treatment milk sample was collected 14 to 17 d after the last IMM infusion, and the second was taken 7 to 10 d later. Therefore, in the extended treatment group, the first post-treatment milk sample was taken between d 18 and 21 and the second post-treatment sampling was taken between d 25 and 31. In the standard treatment group, the 2 post-treatment samplings were between d 15 and 18 and between d 22 and 28, respectively.

Quarter milk sampling was done by the farmer, a technician, or the local investigator from the foremilk before milking, after discarding 3 to 5 streams of milk, according to a strict hygiene protocol. All samples were kept cool (1–8°C) before transport, at ambient temperatures during transport, and kept cool again upon

arrival and during analysis in a centralized laboratory (IDEXX, Alfortville, France). In case the shipment was delayed (i.e., sample taken during the weekend), samples were frozen until transport. Two quarter milk samples for SCC were taken, 1 at inclusion and 1 just after the second post-treatment milk sample for bacteriology. At least 2 mL of milk were collected, identified properly, and sent to the laboratory.

Laboratory Procedures

Milk samples were plated with an inoculum size of 0.1 mL (Walker et al., 2010) and bacterial colonies were identified according to National Mastitis Council guidelines (NMC, 1999). Also, semiquantitative analysis (low/moderate/high) of the isolated bacterial species was performed.

A milk sample was considered contaminated if 3 or more bacterial species were isolated. In case of a contaminated sample at inclusion, the animal was withdrawn from the study. If one other bacterial species, besides *Staph. aureus*, was isolated as well, the additional bacterium was identified.

To determine cefquinome resistance in vitro, all the *Staph. aureus* strains isolated from pretreatment milk samples were shipped to MSD Animal Health Research and Development for the determination of their MIC of cefquinome. The MIC were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) document M31-A3 (CLSI, 2008). Additionally, the production of β -lactamase by the *Staph. aureus* strains was investigated by the nitrocefin disc-based test (Cefinase disc, Becton Dickinson, Franklin Lakes, NJ). All SCC determinations were performed using a DeLaval (Tumba, Sweden) cell counter. If the SCC of a sample could not be determined due to clotting in the milk, a value equal to the upper limit of quantification of the method ($4,000 \times 1,000$ cells/mL) was used for calculations.

Clinical Examination

At inclusion and after the first and second post-treatment milk sampling, a clinical examination was performed by the local investigator. Rectal temperature, general condition, udder swelling (assessed by observation), udder firmness and induration (assessed by palpation), udder pain (assessed by the cow's reaction), and milk quality (assessed by observation) were scored on a clinical examination form ranging from normal (score 0), slightly (score 1), moderately (score 2), and severely affected (score 3). At least 7 d were allowed between the first and second post-treatment clinical examination.

Definitions

Bacteriological cure was defined as the absence of *Staph. aureus* in both post-treatment milk samples. The presence of *Staph. aureus* in one or in both of the post-treatment milk samples was considered a treatment failure. In case 1 or 2 of the post-treatment samples were noninterpretable (>2 pathogens in a sample or noncompliance to the treatment protocol), the case was excluded from BC analysis.

A new infection was defined as the presence, in 1 or 2 of the post-treatment samples, of a pathogen other than *Staph. aureus* and a pathogen other than any pathogen isolated pretreatment. Clinical cure was defined as a cow in normal condition having no clinical sign of mastitis in milk quality or udder aspects (firmness, swelling, pain) at the first post-treatment evaluation. The presence of any clinical sign in any clinical parameter at the first post-treatment sample was considered a clinical treatment failure. Quarter SCC cure was defined as an SCC of $\geq 200,000$ cells/mL of the clinically affected quarter at inclusion that was reduced to $< 200,000$ cells/mL in the second post-treatment sample.

Statistical Analysis

All data were reviewed by the study supervisor to check for clinical consistency. They were entered electronically by an automatic system of data lecture (FORMS 5.2; ReadSoft, Helsingborg, Sweden). All the electronically entered data (100%) used for evaluation of BC were checked against raw data, and 10% of electronically entered data of the CC and SCC were checked against the raw data.

Bacteriological culturing was analyzed using a mixed model logistic regression analysis. For all regression models the linear predictor was given by

$$\begin{aligned} \text{Logit (cure)} = & \text{lactation} + \text{DIM} + \text{herd size} \\ & + \text{BMSCC} + \text{pretreatment SCC} + \text{Staph. aureus} \\ & \text{MIC} + \text{Staph. aureus CFU} + \text{Staph. aureus} \\ & \text{Bet lact} + \text{treatment} + \text{herd (random)} + e, \end{aligned}$$

where cure is either CC or BC; lactation is in 3 categories (1, 2, and 3+); DIM is in three 100-d categories; herd size is in categories of 100 animals; BMSCC is the BMSCC before treatment; pretreatment SCC is the cow SCC before treatment; *Staph. aureus* MIC is the MIC value at 0.25, 0.5, 1, or 2 $\mu\text{g/mL}$; *Staph. aureus* CFU is an indicator for cfu of *Staph. aureus* at the initial sampling; *Staph. aureus* Bet lact is an indicator variable indicating the presence or absence of β -lactamase;

and treatment indicated extended or standard duration of treatment. All of these factors were included as a fixed effect, and e was a binomial error term. β -Lactamase production of *Staph. aureus* is included in the model because it is a potential confounder due to its association with a lower probability of cure (Osterås et al., 1999; Sol et al., 2000). A random herd effect was included in the model with herd as a unique herd identifier. The CC model also included the results of the clinical investigation at the time of diagnosis. The clinical investigation included udder firmness, swelling, pain, milk characteristics, body temperature, and cow attitude.

A backward, stepwise regression analysis was performed. In the final model, all 2-way interactions were evaluated. For all outcome variables, treatment was compared with the control group with significance defined as $P > 0.05$.

RESULTS

Enrolled Cows

The cows in the study were from a diverse population. They were from 5 different European Union countries and originated from 68 different herds with a wide range of geometric BMSCC (237,000–520,000 cells/mL) and clinical *Staph. aureus* incidence (Table 1). A total of 1,217 cows with CM were sampled pretreatment for bacteriological examination. In 646 milk samples other bacteria than *Staph. aureus* were cultured, 286 samples showed no growth, and 70 samples were contaminated, leaving 215 cows with confirmed clinical *Staph. aureus* mastitis. This corresponds to an average prevalence of *Staph. aureus* of 17.7% among CM samples (215/1,217). After inclusion, another 9 cows were withdrawn from the study due to noncompliance to the study protocol. A total of 206 clinical *Staph. aureus* mastitis cases were included, 161 containing *Staph. aureus* only and 45 mixed cultures containing an additional pathogen. Additional pathogens to *Staph. aureus* were *Streptococcus uberis* (16), *Streptococcus dysgalactiae* (9), *Streptococcus dysgalactiae* (6), CNS (4), *Pseudomonas* spp. (4), *Escherichia coli* (2), *Enterobacteriaceae* spp. (2), *Klebsiella pneumonia* (1), and *Arcanobacterium pyogenes* (1). Finally, these 206 included cases, 114 in the extended treatment group and 92 in the standard treatment group, completed the study and were included in the statistical analysis (Table 1).

Homogeneity of Treatment Groups

The data of the 2 treatment groups were compared and tested statistically with a Student's t -test or a

Table 1. Description of country-specific traits and total traits

Item	Country ¹					Total
	FR	HU	IT	NL	UK	
No. of herds	35	8	14	4	7	68
Herd size ²	45	385	135	67	135	
BMSCC ³ ($\times 1,000$ cells/mL)	309	520	313	237	256	
Total of clinical cases (No.)	349	184	433	94	157	1,217
<i>Staph. aureus</i> CM ⁴ (No.)	53	58	64	28	12	215
<i>Staph. aureus</i> CM analyzed (No.)	50	58	63	24	11	206
Extended treatment (No.)	30	33	32	14	5	114
Standard treatment (No.)	20	25	31	10	6	92
BC ⁵ extended, No. (%)	8 (31)	2 (8)	13 (52)	7 (58)	0 (0)	30 (33)
BC standard, No. (%)	2 (11)	4 (18)	14 (61)	5 (62)	0 (0)	25 (33.3)
New infections, No. (%)						47/166 (28)
After BC, No. (%)						23/55 (42)
After non-BC, No. (%)						24/111 (22)
CC ⁶ extended, No. (%)	20 (67)	7 (24)	31 (100)	14 (100)	3 (75)	75/108 (69.4)
CC standard, No. (%)	9 (45)	4 (18)	29 (96.7)	7 (88)	3 (75)	52/84 (61.9)
QSCC ⁷ cure extended (%)						27/88 (30.7)
QSCC cure standard (%)						17/72 (23.6)
β -Lactamase positive, No. (%)	31 (59)	23 (41)	30 (48)	2 (7)	4 (33)	92 (43)

¹FR = France, HU = Hungary, IT = Italy, NL = the Netherlands, UK = United Kingdom.

²Median herd size.

³Bulk milk somatic cell count, geometric mean.

⁴Clinical mastitis.

⁵Bacteriological cure.

⁶Clinical cure.

⁷Quarter somatic cell count cure, clinically affected quarters at inclusion that had a QSCC <200,000 cells/mL after the second posttreatment sample.

Kruskal-Wallis one-way ANOVA for homogeneity (Table 2). No significant differences ($P > 0.05$) were found between the age, parity, DIM, milk production, BW, CM history, cow SCC, rectal temperature, or β -lactamase production between the extended and the standard treatment group.

Antimicrobial Susceptibility In Vitro

The MIC of cefquinome for the isolated *Staph. aureus* strains were distributed over a narrow range, from 0.25 to 2 μ g/mL, with 98% of the strains (209/213) having

an MIC <1 μ g/mL. None of the strains was resistant to cefquinome. For most of the countries the MIC₅₀ was 0.5 μ g/mL, except Italy, where it was one dilution step higher (1 μ g/mL). Despite this slight difference, strains collected in Italy had a good susceptibility against cefquinome, with 96% of the strains (60/64) having an MIC <1 μ g/mL.

BC

Twenty-three clinical cases in the extended treatment group and 17 cases in the standard treatment group

Table 2. Homogeneity of data between the two treatment groups¹

Item	Standard	Extended	<i>P</i> -value
Age (y)	5.2 (2.0)	4.9 (2.1)	0.35
Parity	2 (2–4)	2 (2–4)	0.43
DIM (d)	149.8 (87.3)	147.2 (93.6)	0.86
Milk production (kg)	26.7 (8.4)	26.4 (8.4)	0.82
BW (kg)	649.4 (71.0)	646.5 (73.4)	0.78
CM ² history (No.)	1 (0–2)	1 (0.1)	0.83
Log cow SCC	6.1 (1.4)	6.0 (1.4)	0.65
Rectal temperature (°C)	38.6 (0.4)	38.6 (0.4)	0.96

¹Most parameters were tested by Student's *t*-test and are shown as the mean and the SD in parentheses. For parity and clinical mastitis history, a Kruskal-Wallis one-way ANOVA was performed to better describe the data, these are shown as medians with their 25% and 75% intervals, respectively, in parentheses.

²Clinical mastitis.

Table 3. Final mixed model logistic regression model of bacteriological cure

Effect	Estimate	SE	df	t-value	P-value	Odds ratio	95% CI
Intercept	2.2196	1.3325	15	1.67	0.12		
Extended treatment							
Yes vs. no	−0.4247	0.4457	108	0.95	0.34	0.66	0.27–1.67
DIM ¹							
0–100 d	−0.8261	0.5882	108	1.40	0.16	0.44	0.14–1.41
101–200 d	−0.7820	0.5761	108	1.36	0.18	0.46	0.15–1.43
>200 d reference							
Parity ¹							
1	−0.3854	0.6060	108	−0.64	0.53	0.68	0.21–2.26
2	−0.6280	0.5378	108	−1.17	0.25	0.53	0.18–1.56
3+ reference							
Pretreatment							
Log SCC	−0.8657	0.4189	108	−2.07	0.041	0.42	0.18–0.97

¹Days in milk and parity were kept in the model because they acted as confounders.

were excluded from the analysis of BC due to noninterpretable bacteriological results, leaving 91 (114–23) cases in the extended treatment group and 75 (92–17) in the standard treatment group. The overall BC showed no difference between treatment groups: 33.0% (30/91) after extended treatment and 33.3% (25/75), after standard treatment (Table 1). Least squares means estimates for BC of *Staph. aureus* infections during lactation were 34% (SE 9.9%) for the standard treatment group and 27% (SE 8.4%) for the extended treatment group. Logistic regression results of BC are shown in Table 3. In the final model, extended therapy was not significantly different from the control treatment [odds ratio (OR) = 0.66, 95% CI (0.27–1.67)].

At the country level, BC also showed no difference per treatment group, except for France, where the cure after extended treatment was significantly better (31%) than after the standard treatment (11%; Table 1). Bacteriological cure at the country level showed a wide variety, with low cure rates in Hungary and the United Kingdom, ranging from 0 to 18%, and high cure rates in Italy and the Netherlands, ranging from 52 to 62%.

Both DIM and parity were nonsignificant risk factors for BC (Table 3). The only significant predictor variable was the pretreatment cow SCC; a higher pretreatment cow SCC indicated a lower probability of cure (OR = 0.42, 95% CI [0.18–0.97]). None of the herd-level variables indicated in the Material and Methods section were statistically significant. Parity was maintained in the final model as it acted as a confounder. Removing lactation number from the model did not affect the significance of the treatment variable. The random effect for herd was estimated at 1.1 (SE 0.78) and was not statistically significant. The overall percentage of β -lactamase-producing *Staph. aureus* strains was 43%, but varied widely between countries, from a noticeably low percentage of 7% in the Netherlands to 59% in France (Table 1).

Irrespective of treatment protocol, the BC for β -lactamase-positive strains was lower (28.8%) than for β -lactamase-negative strains (36.6%), although the difference was not significant (Chi-squared, $P = 0.29$). Extended treatment did not have an effect compared with standard treatment within β -lactamase-negative (35.8 and 37.5%, respectively) nor within β -lactamase-positive (28.9 and 28.6%, respectively) *Staph. aureus* strains.

CC

Six cases were excluded from the analysis of the CC in the extended treatment group; 4 cases related to mastitis observed in another quarter, 1 case related to missing data, and 1 case that was noncompliant with the treatment protocol. Eight cases were excluded in the analysis of the CC in the standard treatment group; 3 cases related to mastitis observed in another quarter, 1 case related to missing data, and 4 cases were noncompliant with the treatment protocol. One-hundred and eight cases (114–6) were left for analysis in the extended treatment group and 84 (92–8) in the standard treatment group.

The CC in the standard versus the extended treatment groups was 61.9 (52/84) and 69.4% (75/108), respectively (Table 1). Logistic regression results of CC of *Staph. aureus* mastitis during lactation were 60% (SE 19%) for the standard treatment group and 82% (SE 12%) for the extended treatment group. The final model for CC is shown in Table 4. In the final model, CC after extended treatment was significantly better compared with the standard treatment (OR = 3.03, 95% CI [1–9.17]). The only significant predictor variable for CC was the pretreatment cow udder firmness (OR = 0.40, 95% CI [0.20–0.79]). A firm udder at palpation at the time of diagnosis of CM predicted a lower probability of CC. None of the herd-level variables were statisti-

Table 4. Final mixed model logistic regression model of clinical cure

Effect	Estimate	SE	df	t-value	P-value	Odds ratio	95% CI
Intercept	2.9376	1.0879	16	2.70	0.016		
Extended treatment							
Yes vs. no	1.1082	0.5166	14	-2.15	0.050	3.03	1-9.17
Parity ¹							
1	-0.4102	0.7043	23	-0.58	0.57	0.66	0.16-2.85
2	-1.3802	0.6376	23	-2.16	0.041	0.25	0.07-0.94
3+ reference							
DIM ¹							
0-100 d	0.000302	0.6385	25	0.00	0.9996	1	0.27-3.7
101-200 d	0.5725	0.6829	25	0.84	0.41	1.78	0.43-7.24
>200 d reference							
Udder firmness							
Yes vs. no	-0.9256	0.3494	136	-2.65	0.0090	0.40	0.20-0.79

¹Parity and DIM were kept in the model because they acted as confounders.

cally significant. The DIM and parity categories were maintained in the final model because they acted as a confounder. Removing the lactation number from the model did not affect the significance of the treatment variable. The random effect for herd was estimated at 7.1 (SE 4.4) and was borderline statistically significant. Further evaluation of herds indicated that herds in one country (Hungary) had a marked lower CC rate than the other countries (Table 1).

SCC

The overall median SCC in the clinically affected quarters at 21 to 27 d after the last treatment was lower for the 5-d treatment (1,233,000 cells/mL) than for the 1.5-d treatment (2,076,000 cells/mL), but the difference was not significant due to the large variance. The median SCC of the bacteriologically cured quarters (33.5%), for 5- and 1.5-d treatment was low (112,000 and 130,000 cells/mL, respectively), when compared with nonbacteriologically cured quarters (66.5%; 2,520,000 and 2,954,000 cells/mL, respectively). No difference in quarter SCC was found between treatment groups. The percentage of quarters with an SCC <200,000 cells/mL (quarter SCC cure) after treatment was low: 30.7% after 5-d treatment and 23.6% after 1.5-d treatment (Table 1), which was 3 to 10% lower than BC.

New Infections

The percentage of new infections was comparable and therefore nonsignificant between the treatment groups: 28.6% (26/91) after the extended treatment and 28% (21/75) after the standard treatment (Table 1). Interestingly, irrespective of treatment protocol, the percentage of new infections was much higher for bacteriologically cured quarters (42% [23/55]) than for the noncured quarters (22% [24/111]; Table 1). The major-

ity (around 40%) of the new infections were caused by CNS irrespective of treatment protocol.

DISCUSSION

This study showed that standard IMM treatment of clinical *Staph. aureus* mastitis with cefquinome resulted in a BC rate of 33.3%. This BC rate is comparable to the standard treatment cure rates of clinical *Staph. aureus* mastitis found in the study of Bradley and Green (2009; 31.9%), Pyörälä and Pyörälä (1998; 29%), Jarp et al. (1989; 38.8%), and Sol et al. (2000; 38%, β -lactam-positive strains) but lower than the 48% (β -lactam-negative strains) cure rates found in the study of Sol et al. (2000) after standard treatment. However, a comparison of cure rates between trials has to be done with care because of different definitions for cure or different conditions in different areas. As in our study, the study of Bradley and Green (2009) was performed in multiple European countries (United Kingdom, Germany, and France), whereas the study of Sol et al. (2000) was performed in a more restricted area (the eastern part of the Netherlands), suggesting that local results cannot always be extrapolated to larger geographic areas. Also, differences were observed in our study in cure rates between countries. The problem in extrapolating outcomes of *Staph. aureus* treatment studies from one geographical area to another can be caused by many factors, such as differences in *Staph. aureus* strains. This was confirmed by the large differences in the prevalence of β -lactamase-producing *Staph. aureus* strains between countries (Table 1).

Our finding that extended treatment of clinical *Staph. aureus* did not result in an improved overall BC cure compared with standard treatment is in contrast with reports from studies on clinical (Jarp et al., 1989; Pyörälä and Pyörälä, 1998; Sol et al., 2000) and sub-clinical *Staph. aureus* mastitis (Gillespie et al., 2002;

Oliver et al., 2004; Deluyker et al., 2005) that describe an increased cure after extended treatment. Based on these previous studies, it seems that extended treatment is the best choice when treating *Staph. aureus* mastitis (Roy et al., 2009). Including high BMSCC *Staph. aureus* problem farms, because such farms are more interested to participate in the study, increased the probability of isolating *Staph. aureus*. On this type of farms, infection pressure of *Staph. aureus* is relatively high, possibly resulting in more reinfection of initially cured cows, which, in case the reinfection was with *Staph. aureus*, were recorded as noncured.

We found a much higher new infection rate after BC (42%) when compared with non-BC (22%; Table 1), confirming an increased quarter sensitivity for a new IMI after cure, as previously shown by Zadoks et al. (2001). We hypothesized that, to prevent new infections after cure, extended treatment should be restricted to farms with low infection pressure, likely corresponding to good udder health management, as has been previously suggested in a theoretical model by Barlow et al. (2009). Further research is needed to substantiate this hypothesis in the field. Interestingly, the majority ($\pm 40\%$) of the new infections after treatment were caused by CNS, which is similar to the findings of Bradley and Green, (2009) and Sampimon et al. (2010), who reported that CNS becomes more important on farms with low BMSCC and a low prevalence of *Staph. aureus*. It seems likely that the absence of *Staph. aureus* or, as in our study, the clearance of *Staph. aureus* by treatment creates a window of opportunity for opportunistic CNS to invade the udder. It has been reported that 30% of the CNS species *Staphylococcus epidermidis* carry the *mecA* gene (Sampimon et al., 2011), much higher than has been reported in *Staph. aureus*. The possible relation between *mecA*-positive CNS and *Staph. aureus* needs to be further clarified.

Additionally, on *Staph. aureus* problem farms where BMSCC is high, *Staph. aureus* infections are not only more prevalent, but also more chronic, resulting in lower cure rates (Barkema et al., 2006). This may explain the low BC rates in Hungary, where the average BMSCC was high (520,000 cells/mL; Table 1). These observations at the bulk tank level are likely to be related to the findings at the individual cow level, where we did find a significantly lower (OR = 0.42, 95% CI [0.18–0.97]) BC in cows with a high pretreatment SCC ($\geq 250,000$ cells/mL) compared with cows with a low pretreatment SCC ($< 250,000$ cells/mL) independently of treatment protocol (Table 3).

A lack of antimicrobial susceptibility to cefquinome could potentially have contributed to low BC in both treatment groups. Although isolated *Staph. aureus* strains had comparable low MIC levels in all countries,

showing good susceptibility in vitro, this does not necessarily correspond to good susceptibility in vivo. Another explanation for the apparent therapy resistance of *Staph. aureus* in vivo is biofilm formation, hindering the antibiotic to reach the site of infection (Melchior et al., 2006). Also, methicillin-resistant *Staph. aureus* (MRSA) caused by the expression of the *mecA* gene is of particular interest due to its susceptibility for β -lactam antibiotics in vitro, but resistance to almost all β -lactam antibiotics in vivo. Cattle are a potential reservoir for MRSA for the human population and, if transferred, can cause infections in humans that are difficult to cure (Holmes and Zadoks, 2011). Reports on MRSA in dairy cattle in different European countries, however, show that the prevalence is relatively low (Hendriksen et al., 2008). A more recent report shows the prevalence of MRSA to be below 2% at the quarter level (Vanderhaeghen et al., 2010). Because we did not test for the *mecA* gene in our *Staph. aureus* isolates, we are not able to determine its prevalence in our study. Potentially, presence of *mecA* resistance could have contributed to a lower bacteriological cure. However, we do not think that *mecA* resistance had a big influence on the comparison in efficacy between treatment groups because of the expected low prevalence of methicillin resistance and the likely equal distribution among treatment groups.

Our study shows an improvement in CC of *Staph. aureus* mastitis after extended treatment compared with the standard treatment. It was surprising to find a difference between CC and BC, as one would expect these parameters to be correlated. We hypothesized that extended treatment reduced the number of *Staph. aureus* cfu to a level at which clinical signs disappeared while leaving enough bacteria for a positive culture. Because we only registered the number of cfu in the milk samples before but not after treatment, this hypothesis could not be evaluated. Another explanation for the discrepancy between CC and BC is reinfection with *Staph. aureus*. Clinical cure was measured 14 d post-treatment, whereas BC was measured both on d 14 and 21 post-treatment. Equal BC and CC at d 14 post-treatment may, after subsequent reinfection with *Staph. aureus* in the 7 consecutive days, result in a relatively lower BC than CC. Of the clinical parameters measured at inclusion, only udder firmness remained in the final model, indicating that a firm udder at palpation at the time of diagnosis of CM predicted a lower probability of CC. The practical implication of this finding for treatment decisions of CM is not clear, although it could be helpful in predicting the resolution of clinical signs after treatment.

Extending exposure of bacteria to antimicrobials potentially increases the selection of resistant bacteria.

Because extended treatment only showed an effect on CC, which is based on subjective judgment and not on BC, extending treatment of *Staph. aureus* CM cannot be justified.

To minimize bias, this positively controlled field trial should have been double-blinded. This was not possible because the treatment groups had a different number of tubes, which could not be hidden from the investigators and the farmers. As laboratory personnel were unaware of treatment group allocations, the absence of a double-blind design is unlikely to have seriously affected the outcome of the BC. For the CC, however, this was different because the same investigator, who randomly selected the treatment protocol and applied the first tube, could potentially have remembered the treatment protocol while scoring the CC 2 and 3 wk later. Although we cannot exclude bias of the investigators judgment for CC, we assumed that in the majority of cases they did not remember the treatment protocol while judging CC later on.

CONCLUSIONS

In conclusion, independent of the treatment protocol, cows with SCC <250,000 cells/mL before treatment showed a higher probability of bacteriological cure. It appears that successful treatment of clinical *Staph. aureus* mastitis is associated with an increased number of new infections with CNS. Extended treatment of clinical *Staph. aureus* with cefquinome resulted in a higher CC but not BC than did standard treatment. These results indicate that extending treatment of clinical *Staph. aureus* mastitis should not be recommended.

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