Intramammary antimicrobial treatment of subclinical mastitis and cow performance later in lactation

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INTERPRETIVE SUMMARY

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Long term therapeutic effects of antimicrobial treatment of recently acquired subclinical mastitis in Dutch dairy cows were studied based on follow-up data from 2 linked randomized field trials. Antimicrobial treatment of recently acquired subclinical mastitis during lactation resulted in lower composite SCC during the remainder of the lactation as compared with untreated controls. No differences in clinical mastitis and milk yield during the remainder of the lactation were observed. Antimicrobial treatment of cows with recently acquired subclinical mastitis should not be the first option of choice when trying to improve udder health in dairy herds.

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19 **later in lactation**

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ABSTRACT

The aim of this study was to evaluate long term therapeutic effects of antimicrobial treatment of recently acquired subclinical mastitis (RASCM) during lactation. Quarter level clinical mastitis (CM) follow up, composite somatic cell counts (SCC), and cow level milk yield later in cows' lactation were evaluated using follow up data from 2 previously published linked randomized field trials. The first trial randomly assigned antimicrobial treatment with any intramammary product or negative control to culture-positive quarters of cows having a first elevated composite SCC after 2 consecutive low composite SCC measurements. Untreated cows that had a second elevated composite SCC at the next measurement and were staphylococci-positive (i.e., *Staphyloccocus aureus* or non-aureus staphylococci) were randomly assigned to treatment or control. Quarter level CM cases were reported by the participating herdsmen and milk yield and composite SCC data were obtained from the regular test day recording. Frailty survival models were used to evaluate the long term therapeutic effects of antimicrobial treatment of RASCM on quarter level CM follow up. Mixed linear regression models were applied to quantify the effect on milk yield and composite SCC. Data of 638 quarters from 486 cows in 38 herds were available for statistical analyses, of which 229 quarters of 175 cows received antimicrobial treatment for RASCM. Antimicrobial treatment culminated in reduced composite SCC levels later in lactation but did not result in different milk yield levels or CM follow up compared to control cows. Antimicrobial treatment of cows with RASCM should therefore only be considered in exceptional situations given the current focus on antimicrobial usage reduction in animal husbandry.

Key Words: antimicrobials, clinical mastitis, milk yield, somatic cell count, dairy cow

INTRODUCTION

Bovine subclinical mastitis (**SCM**) results in economic losses to the dairy farmer (Hogeveen et al., 2011). Cows with SCM produce less milk, have a higher composite SCC (**CSCC**), a higher probability of developing clinical mastitis (**CM**), and are culled earlier than their healthy herd mates (Reksen et al., 2006, 2007; van den Borne et al., 2011). Additionally, cows with IMI may be a source of infection to other cows because some pathogens are contagious and thus can spread between cows (Lam et al., 1996; Zadoks et al., 2001; Barlow et al., 2013).

Antimicrobial treatment of SCM is one of the options to improve udder health in dairy herds. It is most commonly applied at drying-off but SCM may also be treated during lactation (Barkema et al., 2006; Barlow, 2011). Bacteriological cure of SCM after lactational antimicrobial treatment is affected by the causative pathogen (Deluyker et al., 2005; Barlow, 2011), treatment factors (Barkema et al., 2006), cow factors (Sol et al., 1997; Sandgren et al., 2008; Salat et al., 2008), pathogen strain characteristics (van den Borne et al., 2010b), and chronicity of infection (van den Borne et al., 2010c).

Antimicrobial treatment of SCM during lactation aims to reduce duration of infection and prevent transmission of IMI to susceptible cows (Barkema et al., 2006; Barlow, 2011; Barlow et al., 2013). Bacteriological cure of subclinical IMI by antimicrobial treatment may also reduce SCC, culling and CM through clinical flare-ups, and may improve milk yield of the treated cow during the remainder of lactation (Barkema et al., 2006; Barlow, 2011). These beneficial indirect effects are thought to be cost-effective

for the antimicrobial treatment of SCM caused by contagious mastitis pathogens during lactation (Keefe, 1997; Barlow et al., 2009; van den Borne et al., 2010a). In the short term (i.e., during the follow up period until bacteriological cure had been evaluated), CM occurrence, culling, and milk yield did not differ between treated and non-treated cows (Deluyker et al., 2005; Sandgren et al., 2008; van den Borne et al., 2010c). In one study on SCM caused by streptococci, less CM cases were observed in treated animals (St.Rose et al., 2003). Studies evaluating these potential beneficial indirect effects in the long term (i.e., after bacteriological cure evaluation), however, are scarce. The few studies published on long term effects, focused either on *Staphylococcus aureus* IMI (Barlow et al., 2013) or on chronic SCM (St.Rose et al., 2003; Sandgren et al., 2008). Beneficial indirect effects of antimicrobial treatment later in lactation might differ between recently acquired SCM (**RASCM**) and chronic SCM cases.

The aim of this study was to investigate the effect of antimicrobial treatment of RASCM on CM follow up, CSCC, and milk yield later in lactation.

MATERIALS AND METHODS

Data on short term therapeutic effects of lactational antimicrobial treatment of bovine RASCM were obtained from 2 linked randomized field trials published previously (van den Borne et al., 2010c). Follow-up data on CM, culling, CSCC and milk yield were collected in addition to the 2 field trials as described below.

Description of Study Design and Short-Term Data

The 2 linked randomized field trials were conducted in 39 Dutch dairy herds, mainly consisting of Holstein-Friesian dairy cows, from December 2006 to May 2008. Herds were participating in the 4-weekly milk recording and had an average incidence of first elevated CSCC of more than 10% per test day. In trial 1, quarter milk samples were aseptically collected within 10 days after milk recording (d -7) from cows with an elevated CSCC (≥ 150,000 cells/mL for primiparae and ≥ 250,000 cells/mL for multiparae) after 2 test days with CSCC measurements below the parity-specific thresholds. Bacteriological culturing, quarter SCC (**QSCC**) determination, and β-lactamase testing of *Staph. aureus* isolates were initiated within 24 h after sample collection. Quarters culture-positive for *Staph. aureus*, *Streptococcus uberis, Streptococcus dysgalactiae,* other non-agalactiae streptococci, or non-aureus staphylococci (**NAS**), and having a quarter SCC ≥ 100,000 cells/mL were randomly allocated antimicrobial treatment or no treatment at the cow level. Pre-intervention milk samples from quarters with a QSCC ≥ 100,000 cells/mL were again taken at d 0 and antimicrobial treatment was initiated directly afterwards. Untreated control cows that had a second elevated CSCC measurement in the next milk recording were eligible for enrollment in trial 2. Untreated control cows that did not have a second elevated CSCC measurement remained in trial 1. In trial 2, quarter milk sampling and laboratory tests were repeated and cows with staphylococci (i.e., *Staph. aureus* or NAS) positive quarters were randomly allocated to treatment or untreated control. Streptococci-positive cows were not enrolled in the second trial. Cows were randomized in a ratio of 1:1 for treatment and control but a ratio of 1:4 was used for staphylococci-positive cows in trial 1 to have sufficient staphylococci-positive cows to enroll in trial 2 (van den Borne et al., 2010c). Treatment was administered by the farmers at d 0 in both trials with any registered

antimicrobial product commercially available for intramammary treatment. Milk samples from all treated and control quarters were taken at d 21 and d 28 after treatment allocation in both trials. Bacteriological cure of a quarter was defined as absence of a pathogen, that originally was present at d 0, in both milk samples post-treatment. Further details on study design and data collected within this 28 d follow-up period were described previously (van den Borne et al., 2010c). Laboratory results of milk samples were not communicated to the farmers during the trials, except when a cow had to be treated for the study. Farmers received all laboratory results after the last samples had been collected, accompanied with a written advice to improve the mastitis management in their herds.

Data Collection on Clinical Mastitis, Culling, CSCC and Milk Yield

In addition to the data collected in the previously described studies, data was collected after the 28 d follow up period to evaluate the performance of enrolled cows later in lactation. Farmers reported cow identification, date, and quarter location of all CM cases in their herds during the follow-up period. Clinical mastitis was defined as a quarter with visible abnormalities of the udder or milk or both. Farmers were instructed to aseptically collect a milk sample from each quarter with CM and to store it at -20°C upon collection. Standard bacteriological culturing procedures (Harmon et al., 1990) were performed to identify the causative pathogen. Farmers reported dates of culling and drying off. Data collection in each herd continued until 6 months after the last enrolled cow completed the follow-up period (d 28) and thus varied across herds (van den Borne et al., 2010c). Composite SCC and milk yield data of all enrolled cows were obtained from the regular test day recordings (CRV, Arnhem, the Netherlands).

153

154 ***Statistical Analysis***

155 Due to the study design, cows could participate in both trials. Data from trial 1
156 control cows that were also included in trial 2 were therefore excluded from the current
157 statistical analyses to avoid biased estimates in cows' performance later in lactation.

158 Data on CM occurrence, culling and drying-off was obtained from all participating
159 herds. However, 3 herds did not complete data reporting until the end of the study period
160 because of a lack of compliance by the farmer for this part of the study. Another 3 herds
161 installed an automatic milking system during data collection. Observations from those 6
162 herds were therefore censored when data reporting ended or when an automatic milking
163 system was installed. Finally, cows that were ≥ 400 DIM at intervention (d 0) were
164 excluded from analyses.

165 All statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, USA)
166 using PROC PHREG and PROC MIXED.

167 ***Clinical Mastitis.*** Quarter level Cox proportional hazards models were used to
168 evaluate the effect of antimicrobial treatment on the hazard of a first CM follow up case in
169 quarters with RASCM. Recurrent CM events or CM events in other quarters of the same
170 cow were not evaluated. A quarter became at risk for CM in the current study at the day
171 the last milk sample was collected (d 28). Its failure time was determined until the first CM
172 follow up case or censoring. Censoring occurred at the day of culling, drying off, when
173 cows were 180 d at risk in the same lactation or when the study ended. All available
174 covariates (Table 1) were tested in bivariable Cox proportional hazards models. Treatment
175 was forced into all models as the predictor of primary interest. All covariates associated

with CM follow up ($P < 0.25$), based on the Type 3 Test, were subsequently selected for multivariable survival analyses. Correlations between selected variables were determined using the Spearman correlation coefficient. The biological more meaningful variable was maintained to avoid collinearity if risk factor pairs showed an absolute correlation > 0.5. Multivariable survival analyses consisted of a forward selection procedure, starting with the covariate with the lowest P - value in the bivariable analysis, until newly added covariates did not significantly ($P < 0.10$) contribute to the model anymore. The forward selection procedure and the P - value < 0.10 were chosen because of low statistical power resulting from a low number of first CM follow up cases. All multivariable models were inspected for confounding which was defined to occur when estimates changed > 25% when a covariate was added to the model. All 2-way interactions between the covariates in the final model, including antimicrobial treatment, were tested. The effect of clustering of quarters within cows within herds was evaluated by adding shared herd and cow frailty effects one at a time to the final statistical model. The frailty effect with the largest variance was selected among competing models.

The proportional hazards assumption was evaluated by the Grambsch-Therneau test (Grambsch and Therneau, 1994) using the SCHOEN macro for SAS, by plotting the scaled Schoenfeld residuals against the survival time, and by the creation of time-dependent covariates of the variables contributing to the final statistical model. Model fit was evaluated by plotting the Cox-Snell residuals against the cumulative hazard. Proportional hazards assumption and model fit were evaluated using the final model but without the frailty effect included. Evaluations of the proportional hazards assumption and model fit gave no reasons for concern.

Milk yield and CSCC. The observational period for the statistical analyses of milk yield and CSCC started at the last test day recording before trial enrollment (d -7) and ended at culling, drying off, 180 d after treatment evaluation in the same lactation or when data collection at the herd level was terminated. Mixed linear regression models were used to evaluate the effect of treatment on milk yield and the natural logarithm of CSCC (**LnCSCC**) during lactation. A random intercept at the herd level was added to all models to adjust for clustering of cows within herds. A repeated effect was additionally added to adjust for correlation of multiple milk yield and LnCSCC measurements within cows. Based on the Akaike Information Criterion, the first-order autoregressive and moving average [arma(1,1)] correlation structure gave the best fit among 6 competing correlation structures (i.e., independent, compound symmetry, first order autoregressive, Toeplitz, first-order autoregressive moving average, and unstructured) for both outcome variables. All available covariates (Table 1) were tested one at the time using mixed linear regression models with treatment forced into the models. Test day parameters (Table 1) were included as time-varying predictors that could change at each test day. Days in milk and a correction for peak production ($e^{-0.05 \cdot DIM}$) were additionally added to the models for milk yield to depict the lactation curve (Wilmink, 1987). A backward selection procedure with variables having an unconditional association ($P < 0.25$) with milk yield or LnCSCC, based on the Type 3 test, was applied to identify covariates significantly ($P < 0.05$) contributing to the final mixed linear regression models. The interaction term of 'treatment' and 'test day measurement following trial enrollment' was evaluated in both statistical models for milk yield and LnCSCC to evaluate the treatment effect over time. The interaction term between 'DIM' and 'parity' was also evaluated for the statistical model for milk yield to correct for

potential lactation curve differences between primiparae and multiparae. Other interaction terms were deemed biologically irrelevant.

Homoscedasticity was graphically assessed by plotting standardized residuals against predicted values and by applying the Score Test (Breusch and Pagan, 1979). Normality of residuals was additionally assessed by graphical evaluation and determination of skewness and kurtosis.

RESULTS

The dataset contained 727 quarters with RASCM from 549 cows in 39 herds after removing trial 1 observations of cows that were enrolled in both trials. Of these, 89 quarter level observations were excluded because cows were > 400 DIM at SCM intervention (n = 31), had CM during one of the trials (n = 23) or were lost because the farmer stopped reporting information (n = 35). Thereafter, the quarter level dataset consisted of 638 quarters with RASCM from 486 cows in 38 herds, of which 229 quarters of 175 cows received an antimicrobial treatment. Because of missing values, observations had to be excluded from the statistical analyses for CM follow up (n = 4), milk yield (n = 18), and LnCSCC (n = 24), leaving 634 quarter level observations and 3,028 and 3,022 test day recordings available for the final statistical analyses of these indicators, respectively.

Clinical Mastitis

Median DIM at treatment allocation (d 0) was 90 d (range: 20 - 388) for quarters with CM follow up and 168 d (range: 15 - 399) for quarters without CM follow up. Twenty-seven quarters (4.2%) showed a CM follow up within 180 d after treatment evaluation.

245 Median time to CM follow up was 46 d (range: 3 – 165 d) while median time to censoring
246 was 162 d (range: 0 – 180) in quarters without CM follow up. Only seven milk samples for
247 bacteriological culturing were taken by farmers from these 27 CM follow up cases. The
248 same pathogen was determined as in the pre-intervention sample (d -7) in 3 of those
249 samples. The other 4 milk samples were culture-negative (n = 1), or the pathogen differed
250 from the pathogen cultured at d -7 (n = 3).
251 Antimicrobial treatment of RASCM was associated with time to quarter CM follow
252 up in an interaction term with "quarter IMI status pre-intervention" in the final frailty
253 model, as presented in Table 2. This interaction term is further illustrated by Kaplan-Meier
254 survival curves (Figure 1). Control quarters (without antimicrobial treatment) with multiple
255 milk samples culture-positive for the same pathogen before randomization had 4.7 times
256 higher hazards for CM follow up than quarters that were only culture-positive for the
257 pathogen at d -7. Quarters with multiple milk samples culture-positive for the same
258 pathogen before randomization, that received treatment did not significantly differ in their
259 CM follow up rates from quarters that were culture-positive only once (HR = 0.85) or that
260 did not receive antimicrobial treatment (HR = 0.53). Quarters in which a major pathogen
261 was identified at d 0 had a 3.1 times higher hazard for CM follow up than quarters with a
262 RASCM caused by NAS. Hazards for CM follow up increased 1.5 and 1.1 times for each
263 unit increase in LnQSCC-7 and daily milk yield, respectively.
264
265 ***Milk Yield***
266 The results from the final mixed linear regression model for daily milk yield are
267 presented in Table 3 and are illustrated in Figure 2. Antimicrobial treatment of RASCM

during lactation was not associated with milk yield following treatment allocation (P = 0.34; Table 3). There was also no evidence for a therapeutic effect over time as displayed by the non-significant interaction term between 'treatment' and 'test day measurement following trial enrollment' (P = 0.77; Figure 2). The final statistical model was adjusted for the difference in lactation curves between primiparae and multiparae because the interaction term between 'DIM' and 'parity' was significant.

The final linear mixed model for milk yield did not result in entirely normal distributed residuals. Removing 4 outliers, however, caused the residuals to become normally distributed and it only marginally affected point estimates (results not shown). Graphical observation of standardized residuals and the significant Score test indicated furthermore that some heteroscedasticity was present. Standardized residuals showed a lower variance at predicted milk yield values below 15 kg/d with a tendency towards positive values, indicating an underestimation of the model at lower milk yield levels.

LnCSCC

The results of the final mixed linear regression model for LnCSCC are presented in Table 4 and are illustrated in Figure 3. LnCSCC was lower after trial enrollment in both treated and control cows but LnCSCC reduction was more pronounced in cows receiving antimicrobial treatment. This was evident by the significant interaction term between 'Treatment' and 'Test day following trial enrollment' ($P < 0.0001$; Figure 3). The difference in LnCSCC between treated and control cows remained significant until the fourth test day record post trial enrollment. No difference could be observed thereafter. Multiparae and cows with a major pathogen identified in 1 of the enrolled quarters had a

291 significantly higher LnCSCC throughout the entire observational period. Cows originating
292 from trial 2 (that thus had two consecutive high CSCC measurement at trial enrollment
293 instead of one) finally, had a higher LnCSCC throughout the entire observational period
294 compared to cows from trial 1.
295
296 **DISCUSSION**
297 In the first month after treatment allocation, antimicrobial treatment of SCM
298 resulted in reduced quarter and composite SCC levels of the cows enrolled in the two linked
299 randomized field trials (van den Borne et al., 2010c). As was determined in the current
300 study, these reduced CSCC levels persisted until the fourth test day recording (i.e., 4
301 months) after antimicrobial treatment. Similar effects have been observed before (St.Rose
302 et al., 2003; Salat et al., 2008; Sandgren et al., 2008). It should be noted, however, that only
303 culture-positive quarters were treated in the trials used for the current study (van den Borne
304 et al., 2010c) whereas all quarters of enrolled cows were treated with antimicrobials, either
305 via the intramammary or systemically route, in the previously conducted studies (St.Rose
306 et al., 2003; Salat et al., 2008; Sandgren et al., 2008). Because IMI in untreated quarters
307 may have gone unnoticed at trial enrolment, SCC reduction at cow level may have been
308 underestimated. As expected, other covariates remained significant in the final model for
309 CSCC. The covariate 'Trial' can be interpreted as an indicator for the duration of infection
310 because CSCC measurement had to be high once or twice for trial 1 and 2, respectively.
311 Finally, differences between major and minor mastitis pathogens (Schukken et al., 2003)
312 and parities (de Haas et al., 2002) have been observed before too.

313 The relationship between SCM and CM follow up is well described in the literature
314 (e.g., Green et al., 2004; Reksen et al., 2006; van den Borne et al., 2011). We hypothesized
315 previously that 25% of all CM cases can be avoided if cows could be prevented from
316 developing a high CSCC or if a perfect intervention could be applied to high CSCC cows
317 (van den Borne et al., 2011). It was therefore anticipated that antimicrobial treatment of
318 RASCM would result in fewer CM follow ups, analogous to the studies of Barlow et al.
319 (2013) and Sandgren et al. (2008). However, no such effect was observed in the current
320 study. This likely is being caused by the late lactation stage most cows were in, resulting
321 in a low overall incidence risk of CM follow up (4%; Olde Riekerink et al., 2008; van den
322 Borne et al., 2010d). This, subsequently, resulted in low power to detect a statistically
323 significant difference. Moreover, study designs differed, hampering a true comparison
324 between the 3 investigations. In their study, Barlow and co-workers (2013) only treated
325 *Staph. aureus* IMI whereas other pathogens were also treated in the current study.
326 Genotypes of *Staph. aureus* isolates differed too (van den Borne et al., 2010b; Barlow et
327 al., 2013). Sandgren et al. (2008) observed a reduced cow level CM incidence risk in cows
328 that received an intramuscular antimicrobial treatment compared with untreated control
329 cows and cows that received an intramammary antimicrobial treatment. In the current
330 study, CM was followed up at the quarter level. An explorative survival analysis evaluating
331 the effect of antimicrobial treatment on time to CM follow up at the cow level did not
332 identify a significant relationship either ($P = 0.34$; results not shown). This probably results
333 from the low statistical power at cow level also (i.e., only 46 cases were observed with
334 incidence risks being 10.7% and 7.4% in control and treated cows, respectively). This cow
335 level performance indicator was therefore not scrutinized further.

Farmers were requested to take milk samples of all CM cases in their herd but only 7 out of 27 CM follow up cases were sampled due to low compliance of farmers on this study protocol item. Conclusions about the relationship of the identified pathogens in samples from clinical and subclinical mastitis IMI could therefore not be drawn. Control quarters with multiple culture-positive milk samples before treatment allocation had higher hazards for CM follow up than control quarters with a single culture-positive milk sample. This observation might be a result of the bacteriological status of the quarter and the test characteristics of bacteriological culturing, low shedding of bacteria, false-positive milk samples because of contamination, or spontaneous cure may explain this bacteriological status as discussed earlier (van den Borne et al., 2010c). The observed higher hazards for CM follow up with increased LnQSCC-7 and milk yield levels and with IMI caused by major pathogens in comparison with lower yields and NAS IMI matched earlier studies (Green et al., 2004; Taponen and Pyörälä, 2009; van den Borne et al., 2011).

Milk yield in cows with chronic SCM was not affected by antimicrobial treatment in the studies of St.Rose et al. (2003) and Sandgren et al. (2008). The current study, focusing on RASCM, also provided no further evidence. Milk yield losses because of IMI therefore seem to be irreversible. Thus, when trying to preserve milk yield, focus should be on preventing new IMI in susceptible cows rather than on treating them.

Cow level bioeconomic simulation models have been developed to investigate the cost-effectiveness of antimicrobial treatment of subclinical IMI caused by streptococci and *Staph. aureus* during lactation (Swinkels et al., 2005a,b; Steeneveld et al., 2007). These models showed that the cost-effectiveness depends on both cow and herd characteristics and that antimicrobial treatment only seems profitable at the cow level if transmission of

IMI to susceptible cows is high. Given the lack of difference in milk yield and CM follow up in the current study, antimicrobial treatment of RASCM is most likely not profitable. Lactational antimicrobial treatment of NAS IMI is not assumed to be cost-effective either (Bexiga et al., 2011). On the herd level, however, antimicrobial treatment of RASCM might be cost-effective for herds that receive a penalty for high bulk milk SCC because of the reduction in SCC. Moreover, antimicrobial treatment of SCM seems profitable for contagious pathogens because of the prevention of new IMI in susceptible cows (Keefe, 1997; Barlow et al., 2009; van den Borne et al., 2010a). The results from this study can be used to update the models developed previously.

Treating RASCM with antimicrobials during lactation results in an increased antimicrobial usage which does not coincide with the current focus on reducing antimicrobial usage in animal husbandry. Based on the results of our current and earlier work, we see no reason to propagate the routine use of antimicrobials to treat RASCM cases during lactation. Optimizing mastitis prevention should be the first approach in all situations. Only in exceptional cases, antimicrobial treatment of SCM caused by contagious pathogens during lactation should be considered.

CONCLUSIONS

Using follow-up data from 2 previously conducted linked randomized field trials, long term effects of antimicrobial treatment of RASCM were determined. Antimicrobial treatment of RASCM resulted in lower CSCC later in cows' lactation compared with untreated control cows. No evidence was found for a beneficial long-term effect of antimicrobial treatment of RASCM on CM follow up or milk yield. Lactational

antimicrobial treatment of cows with RASCM should therefore not be the first option of choice when trying to improve udder health in dairy herds.

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Figure captions

Figure 1. Kaplan-Meier survival plot of time to clinical mastitis follow up in 4 groups of quarters:

(———) control quarters with a single milk sample culture-positive pre-intervention

(--------) control quarters with multiple milk samples culture-positive pre-intervention

(— — —) treated quarters with a single milk sample culture-positive pre-intervention

(— · — ·) treated quarters with multiple milk samples culture-positive pre-intervention

Figure 2. Predicted milk yield at test day records after antimicrobial treatment of recently acquired subclinical mastitis in control cows (black) and treated cows (grey)

Figure 3. Predicted natural logarithm of composite SCC (lnCSCC) at test day records after antimicrobial treatment of recently acquired subclinical mastitis in control cows (black) and treated cows (grey)

511 Table 1. Covariates included (√) in the statistical models for quarter level clinical mastitis

512 (CM) follow up, cow level milk yield and composite SCC (CSCC)

Covariates	Category	CM	Milk yield	CSCC[1]
Test day record level				
Test day record relative to trial enrollment (d 0)	0 1 2 3 4 5 6 ≥ 7		√	√
Lactation stage	0 - 100 d 101 - 200 d ≥ 201 d		√[2]	√
Farmer received laboratory results of enrolled cows	Yes No		√	√
Cow level				
Treatment	Yes No	√	√	√
Lactation stage at d 0	0 - 100 d 101 - 200 d ≥ 201 d	√		
Parity	1 ≥ 2	√	√	√
No. of quarters infected	1 ≥ 2	√	√	√
Season at d -7	Housing (October - March) Pasture (April - September)	√	√	√
Trial	1 2	√	√	√
Milk yield at treatment allocation	Continuous	√		
Pathogen identified at d -7	Major mastitis pathogen[3] in ≥ 1 quarter Only non-aureus staphylococci		√	√
Cow IMI status pre-intervention	≥ 1 samples positive[4] 0 samples positive		√	√
History of CM in any quarter pre-intervention	Yes No		√	√
Quarter level				
LnQSCC-7[5]	Continuous	√		

Pathogen identified	Major	√
	Non-aureus staphylococci	
Quarter location	Front	√
	Rear	
Quarter IMI status pre-intervention	≥ 1 samples positive[6]	√
	0 samples positive	

[1]Natural logarithm of test day CSCC

[2]For this outcome variable, lactation stage was analyzed as a continuous variable and included a correction for peak production (Wilmink, 1987)

[3]*Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and other non-agalactiae streptococci

[4]The same pathogen (as identified at d -7) was also cultured from at least one other milk sample taken from the same cow pre-intervention

[5]Natural logarithm of quarter SCC at d -7

[6]Compared to the pathogen identified at d -7, the same pathogen was also cultured at other occasions in this quarter pre-intervention

524 Table 2. Factors associated with time to the first case of quarter level clinical mastitis (CM;
525 n = 27) follow up within 180 d after randomization of lactational antimicrobial treatment
526 to quarters with recently acquired subclinical mastitis (n = 634)

					90% CI	
Variable	Category	Frequency	% CM	HR	Lower	Upper
Treatment	No	405	4.4	Ref.	-	-
0 samples pos.[1]	Yes	85	3.5	2.98	0.64	13.89
≥ 1 samples pos.	Yes	144	4.2	0.53	0.24	1.19
Pathogen	Major	343	6.7	3.07	1.18	7.98
	Non-aureus staphylococci	291	1.4	Ref.	-	-
LnQSCC-7[2]	Continuous	mean = 6.2, SD = 1.1		1.49	1.11	2.00
Milk yield (kg/d)[3]	Continuous	mean = 27.0, SD = 8.0		1.06	1.01	1.11
Quarter IMI status pre-intervention[2]	0 samples pos.	243	2.1	Ref.	-	-
Control quarter	≥ 1 samples pos.	247	6.5	4.73	1.36	16.39
Treated quarter	≥ 1 samples pos.	144	4.2	0.85	0.25	2.82

527 Variance of the herd frailty effect was 0.49 (SE = 0.44)
528 Ref. = Reference
529 [1]Number of additional quarter milk samples culture-positive for the same pathogen pre-
530 intervention compared with the pathogen identified at d -7
531 [2]Natural logarithm of quarter SCC at moment of intervention (d -7)
532 [3]Milk yield at treatment allocation
533

534 Table 3. Factors associated with milk yield after randomization of antimicrobial treatment
535 to 482 cows with recently acquired subclinical mastitis according to the final mixed linear
536 regression model

Variable	Category	Frequency (%)	Estimate (kg/day)	SE	*P* - value
Intercept			32.06	0.85	< 0.01
Treatment	Yes	36.3	-0.43	0.44	0.34
	No	63.7	Ref.		
Test day record following trial enrollment	0	15.8	Ref.		
	1	15.8	-0.27	0.17	0.11
	2	15.2	-0.75	0.23	< 0.01
	3	13.5	-1.18	0.30	< 0.01
	4	12.1	-1.23	0.36	< 0.01
	5	10.7	-1.48	0.42	< 0.01
	6	9.3	-1.95	0.49	< 0.01
	≥ 7	7.6	-1.83	0.55	< 0.01
Parity	≥ 2	66.6	8.62	0.77	< 0.01
	1	33.4	Ref.		
DIM	Continuous		-0.04	0.003	< 0.01
Peak production function	Continuous		-15.07	0.92	< 0.01
DIM x parity	≥ 2		-0.03	0.003	< 0.01
	1				

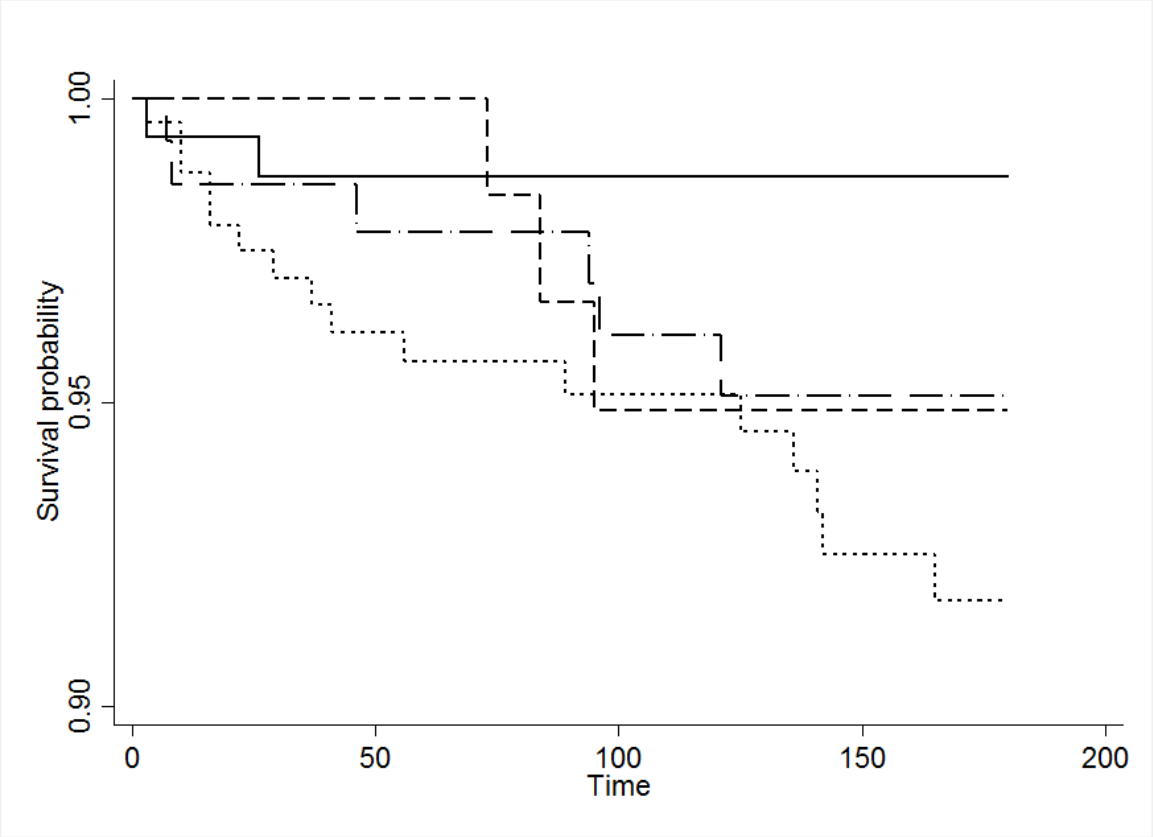
537 Variance of the repeated cow effect was 0.88 for ρ and 0.83 for γ. Variance of the random
538 herd effect was 10.84. Residual variance was 28.85
539 Ref. = Reference
540

541 Table 4. Factors associated with the natural logarithm of monthly composite SCC after
542 randomization of antimicrobial treatment to 483 cows with recently acquired subclinical
543 mastitis in the final mixed linear regression model

Variable	Category	Freq. (%)	Estimate (kg/day)	SE	*P* - value
Intercept			5.32	0.08	< 0.01
Treatment	Yes	36.2	0.15	0.08	0.08
	No	63.8	Ref.		
Test day record following trial enrollment	0	15.8	Ref.		
	1	15.8	-0.46	0.05	< 0.01
	2	15.1	-0.41	0.06	< 0.01
	3	13.5	-0.37	0.06	< 0.01
	4	12.1	-0.37	0.07	< 0.01
	5	10.7	-0.40	0.07	< 0.01
	6	9.4	-0.40	0.08	< 0.01
	≥ 7	7.6	-0.36	0.08	< 0.01
Treatment x test day record following trial enrolment	0		Ref.		
	1		-0.52	0.08	< 0.01
	2		-0.41	0.09	< 0.01
	3		-0.36	0.10	< 0.01
	4		-0.36	0.11	< 0.01
	5		-0.24	0.12	0.05
	6		-0.24	0.13	0.07
	≥ 7		-0.25	0.14	0.08
Parity	≥ 2	66.5	0.66	0.06	< 0.01
	1	33.5	Ref.		
Trial	2	26.3	0.30	0.06	< 0.01
	1	73.7	Ref.		
Pathogen	Major	62.3	0.27	0.06	< 0.01
	Non-aureus staphylococci	37.7	Ref.		

544 Variance of the repeated cow effect was 0.75 for ρ and 0.54 for γ. Variance of the random
545 herd effect was 0.03. Residual variance was 0.78
546 Ref. = Reference
547

548 van den Borne et al. Figure 1.



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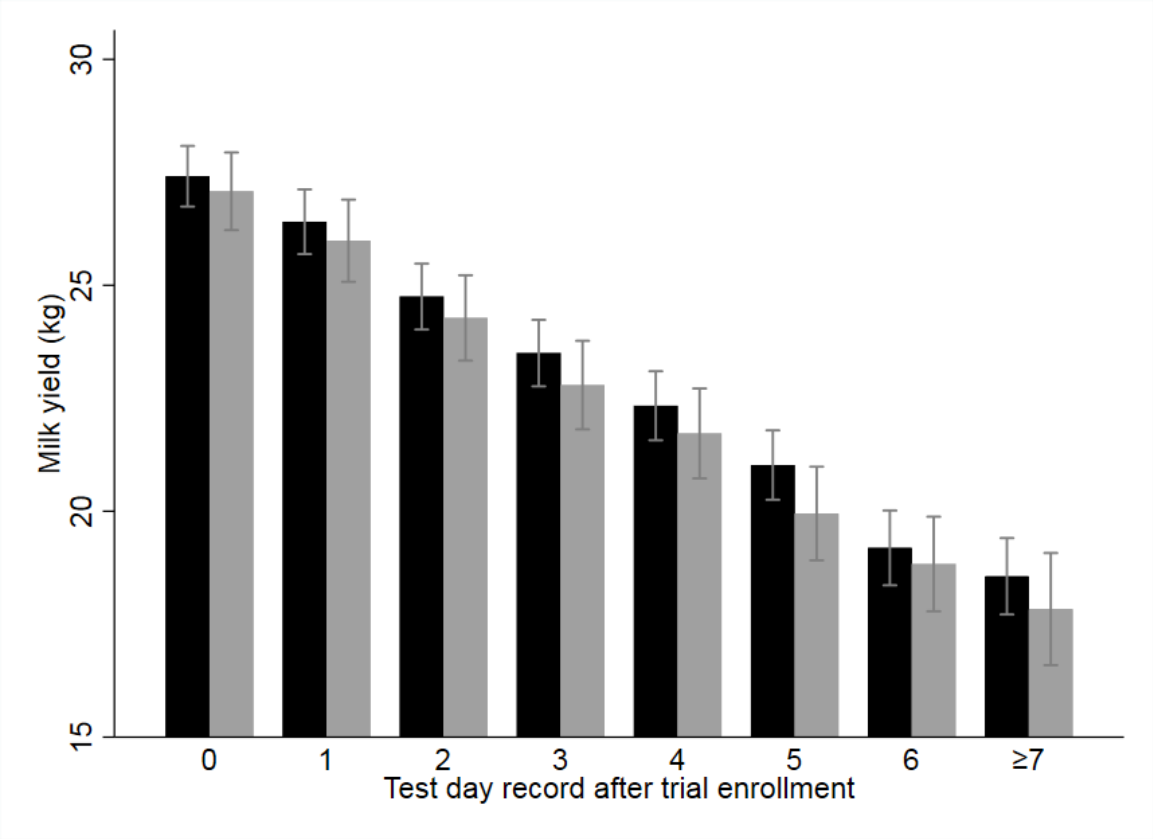
551 van den Borne et al. Figure 2.



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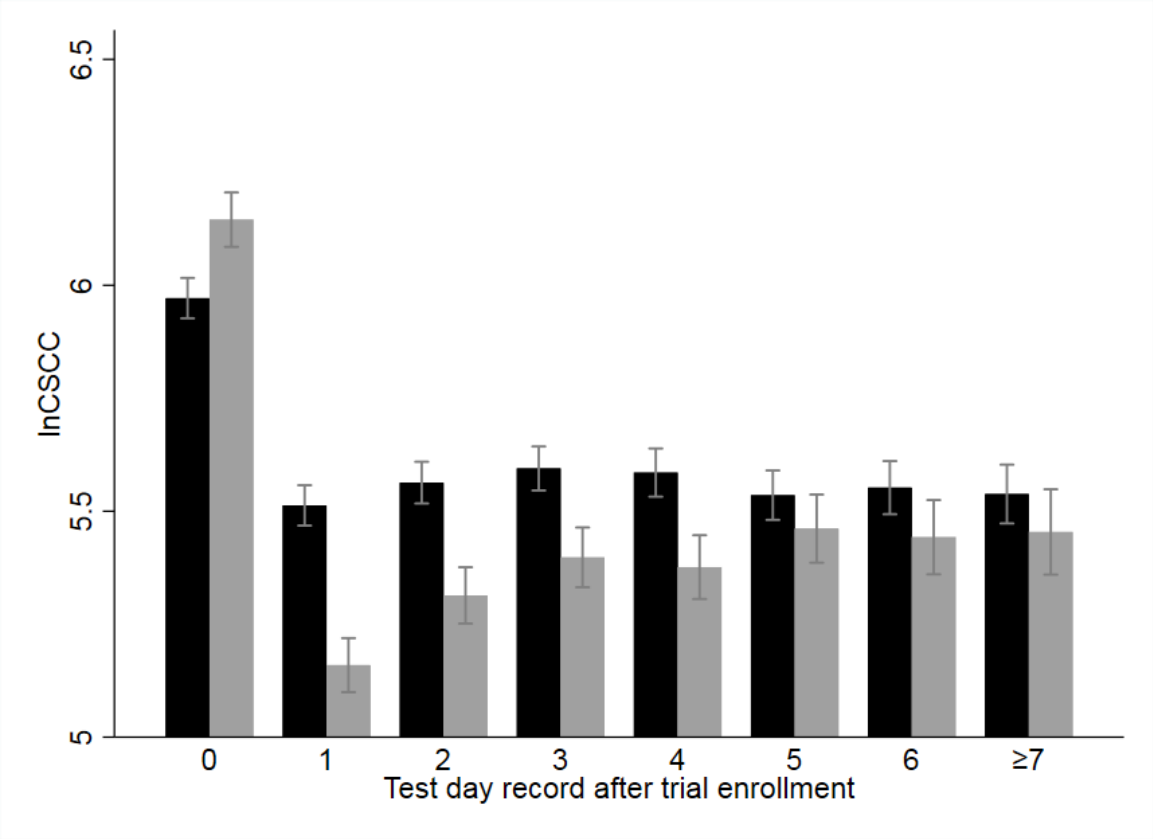
554 van den Borne et al. Figure 3.



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