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Interpretive summary. Use of on-farm data to guide treatment and control of mastitis caused by Streptococcus uberis, by Samson et al. To reduce the risk of antimicrobial resistance, judicious use of antimicrobials is advocated. We show that routinely available DHI and treatment data can be used in veterinary practice to predict cure of S. uberis mastitis. Probability of apparent cure is higher among 1st and 2nd parity animals compared to older cows, and in animals with short-duration elevated SCC compared to those with repeated SCC elevation before occurrence of mastitis. This knowledge enables farmers and veterinarians to tailor antimicrobial use for treatment of mastitis, and to put increased emphasis on prevention of cases with poor prognosis.
Use of on-farm data to guide treatment and control of mastitis caused by *Streptococcus uberis*

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ABSTRACT

Treatment of mastitis is the most common reason for use of antimicrobials in dairy cattle. The responsible use of antimicrobials could be strengthened by knowledge of predictors for cure, which would help to tailor treatment decisions. Ideally, to allow for widespread uptake, this would be achieved using data that are routinely available. To assess whether this is feasible in practice, farmers were invited to submit milk samples from mastitis cases to their veterinary practice for bacteriological culture. Among 624 culture-positive samples, 251 were positive for *Streptococcus uberis*. Using cow-level data, cases were classified as severe clinical mastitis (CM; “severe”), 1st non-severe CM (“first”), repeated non-severe CM (“repeat”), or subclinical mastitis (“subclinical”). Additional data were collected at cow-level (somatic cell count (SCC), parity, lactation stage, milk yield, fat and protein content, treatment) and at herd-level (housing, bedding, pre-milking teat disinfection, post-milking teat disinfection). Severe cases were overrepresented among heifers and animals in early lactation whereas repeat cases were overrepresented in cows with 3 or more lactations. The probability of cure was higher among 1st and 2nd parity animals than among older cows, and higher in animals with a single elevated cow-level SCC than in animals with multiple high SCC records. Results obtained in the current study are similar to those previously described for *Staphylococcus aureus* mastitis. Thus, routinely available cow-level information can help to predict the outcome of antimicrobial treatment of the most common causes of Gram-positive mastitis.

Key words: mastitis, *Streptococcus uberis*, prognosis, antimicrobial treatment, clinical manifestation
Antimicrobial resistance is increasingly perceived as a threat to human and animal health and the need for responsible use of antimicrobials is emphasized by a range of national and international bodies (UK Department of Health 2013; World Health Organisation 2015). Key elements of the approach proposed by the World Health Organisation (WHO) include reduction of the incidence of infection and optimized use of antimicrobial medicines (WHO, 2015). Farmers are increasingly aware of the need to use antimicrobials responsibly. In a recent survey in the UK over 70% of dairy farmers said that reducing antibiotic usage would be “a good thing to do” (Jones et al., 2015). Veterinarians can play an important role in this process by providing information on ways to achieve reductions in antibiotic usage, e.g. by minimizing the risk of disease or through development of treatment protocols (Raymond et al., 2006; Jones et al., 2015).

On dairy farms, treatment of mastitis is a major reason for use of antimicrobials. For example, Pol and Ruegg (2007) calculated the estimated overall exposure to antimicrobial drugs of cattle on conventional dairy farms as 5.43 defined daily doses (DDD) per cow per year. This included 3.58 DDD of intramammary applications (2.02 DDD during lactation and 1.56 DDD at dry off) and 1.85 DDD of parenteral use. Clinical mastitis (CM) was the most common reason for intramammary or parenteral antimicrobial usage. To reduce the use of antimicrobials protocols for selective treatment of dry cows and cattle with CM have been developed, including protocols based on culture and on cow-factors such as SCC (Lago et al., 2011; Cameron et al., 2014; Scherpenzeel et al., 2014). In culture-based protocols, treatment decisions are largely based on the distinction between gram-positive growth, gram-negative growth and no growth (Lago et al., 2011; Cameron et al., 2014). Further refinement of treatment decisions may be possible when pathogen factors, such as antimicrobial resistance, and host characteristics, including duration of infection and parity, are taken into account, but this has only been described in detail for Staphylococcus aureus (Barkema et al., 2006).
In many countries on different continents *Streptococcus uberis* is among the most common
gram-positive causes of CM (Olde Riekerink *et al*., 2008; Petrovski *et al*., 2011; Verbeke *et al*., 2014).

The organism is also responsible for a considerable proportion of subclinical mastitis cases (Bradley
*et al*., 2007; Sampimon *et al*., 2009). Intramammary infections and CM caused by *S. uberis* can be
temporary, recurrent or chronic and a wide range of cure rates has been reported in response to
treatment (Zadoks *et al*., 2003; Zadoks 2007). Despite its importance as a mastitis pathogen little is
known about risk factors for the clinical manifestation or treatment outcome of *S. uberis* IMI.

Therefore, the aim of this study was to generate data that could inform guidelines for improved
management of *S. uberis* mastitis under field conditions. To that end, we conducted a farm-based
study of herd- and cow-level risk factors that are associated with the clinical manifestation and
likelihood of apparent cure of *S. uberis* IMI as based on SCC. In doing so, we only used tools and data
that are routinely available to farmers and veterinary practices, including treatment and DHI records,
because our aim was to generate low-cost guidelines for improved management of *S. uberis* mastitis
under field conditions.
MATERIALS AND METHODS

Milk sampling and Bacteriological culture

From August 2012 until January 2014, quarter milk samples (n=624) were collected from French dairy cows with clinical or subclinical mastitis using standard aseptic sample collection methods (National Mastitis Council, 1999). Detection and sampling of mastitis cases was driven by participating farmers. To motivate farmers to participate in the study, all clients of our veterinary practice (Vetformance, Villaines la Juhel, France) with more than 50 lactating cows (approximately 500 farms), received an invitation to sample clinical and subclinical mastitis cases at their farms. The bacteriological analysis of the samples was free of charge for the farmers. In addition, upon return of completed data information sheets, a head collar for a cow was offered to the farmers. One hundred and forty two farmers submitted at least one milk sample, indicating an uptake of approximately 28%.

Milk samples were subjected to bacteriological culture in the laboratory of Vetformance. Aliquots of milk (10 µl) were plated onto three media, i.e. (1) Colombia blood agar containing 5% sheep blood (bioMérieux, Craponne, France; Ref. 43041); (2) Colistin Nalidixic Acid (CNA) agar (blood agar plate containing 5% sheep blood, Colistin (10 mg/L) and Nalidixic Acid (15 mg/L) (bioMérieux; Ref. 43071) and (3) Bromo Cresol Purple (BCP) agar (bioMérieux; Ref. 43021). Plates were incubated at 37°C for 18 to 24 h. Cultures were considered pure if only one morphotype was present on the blood agar plate. For pure cultures, growth on both the CNA and BCP plates was considered as evidence of the bacteria being gram-positive; growth on only the BCP plate as evidence of the bacteria being gram-negative. Among gram-negative bacteria, *E.coli* was characterized by a positive lactose reaction (colour change of the BCP plate from purple to yellow) and a negative urea reaction (bioMérieux; Ref. 55752), whereas *Klebsiella* was identified by both positive lactose and positive urea reactions. Gram-positive bacteria were considered to be *Staphylococcus aureus* based on positive catalase and coagulase reactions (bioMérieux; Ref. 73112) and Staphylococcus spp. in the
case of positive catalase and negative coagulase results. Identification of *S. uberis* was based on negative response in the catalase reaction and positive response in the esculin reaction (bioMérieux; Ref 42086) (National Mastitis Council, 1999). In addition, susceptibility to penicillin was evaluated. This procedure increases specificity by excluding enterococcal isolates, which are more likely to be penicillin-resistant than streptococci (Makovec and Ruegg, 2003; Nam et al., 2010). Susceptibility to penicillin was tested using the disc diffusion method in accordance with the recommendations of the Société Française de Microbiologie (Soussy, 2013). Using a swab, a Mueller Hinton agar plate containing 5% sheep blood (MH2, bioMérieux; Ref. 43321) was homogenously plated with a suspension of *S. uberis* at 0.5 McFarland (equivalent to approximately $10^8$ colony forming units/ml). Plates were incubated at 37°C for 18 to 24 h. Bacteria were considered sensitive to penicillin if they expressed a growth inhibition zone of more than 21 mm around an Oxacillin disc (5 µg; Soussy, 2013). Bacteria that could not be classified using the criteria described here were considered “other species”.

**Cow and Herd Data**

Three data sources were used to obtain information about individual cows and their herds of origin, i.e. (i) private farm records on treatment; (ii) monthly DHI data; and (iii) questionnaires that were filled out by the farmer and the attending veterinarian. For each cow the date of mastitis diagnosis (observation of clinical mastitis or notification of SCC data via DHI) and treatment were recorded, including use of intramammary administration of antimicrobials (IMM), parenteral administration of antimicrobials (PAR), and use of non-steroidal anti-inflammatory drugs (NSAIDs). DHI data were collected from the 3 milk recordings preceding the diagnosis of mastitis, the month of diagnosis and the 3 recordings after diagnosis, if available. This included cow-level SCC data, parity (1 = first lactation, 2 = second lactation, 3 = third or higher lactation), DIM, milk yield (MY, in kg), fat content (g/kg), and protein content (g/kg). At herd level, information was collected on the use of pre-milking...
teat disinfection (PreMTD) and post-milking teat disinfection (PostMTD), use of housing (yes or no) and, where applicable, on housing type (cubicles or straw yards).

Classification of Cases

Clinical manifestation was classified into four categories based on clinical severity of the current episode and information on previous episodes of CM in the same animal:

1. Severe: CM with both local and general symptoms (T > 39°C; temperature measured on clinical suspicion of fever by the farmers and results recorded on the data form accompanying the milk sample);

2. Non-severe first case (“first”): first episode of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder);

3. Non-severe repeat case (“repeat”): 2nd or subsequent episode of CM during the current lactation with local signs only in the current episode (abnormalities of milk with or without abnormalities of the udder);

4. Subclinical: SCC > 200,000 cells/ml at cow level based on most recent DHI data, not accompanied by any clinical signs.

For 212 of the 251 S. uberis positive animals SCC data were partially available (fewer than 3 records before or after diagnosis) or complete (3 records before and after diagnosis). SCC data were used to classify the duration of inflammation prior to diagnosis and the response to treatment after diagnosis. Inflammation was considered short if at least 2 monthly SCC records prior to diagnosis were below 200,000 cells/ml (SHORT) and long if at least 2 monthly SCC records exceeded 200,000 cells/ml prior to diagnosis (LONG). An animal was considered cured if at least 2 monthly SCC after diagnosis were below 200,000 cells/ml (CURE) and not cured if at least 2 monthly SCC exceeded 200,000 cells/ml after diagnosis (NO CURE). For other SCC combinations or missing data, duration
Statistical Analysis

Statistical analyses were performed using Statistix, version 10 (Analytical Software, Tallahassee, FL). Data were inspected for outliers and missing values and descriptive analyses were conducted using tabular and graphical formats. For outcomes of interest with 3 or more categories, data were analysed using categorical methods (Chi-Square analyses), e.g. for cow-level factors associated with clinical severity. The association between clinical severity and milk, fat or protein yield relative to occurrence of mastitis was evaluated using a t-test at each time point. To identify cow- and herd level risk factors for apparent cure as based on SCC, logistic regression was used with backward stepwise analysis. The final logistic regression equation was:

\[
\text{Logit (SCC cure)} = \text{intercept} + \text{Clinical manifestation} + \text{Duration} + \text{Parity} + \text{Treatment} + \text{error}
\]

where Clinical manifestation is severe, first, repeat or subclinical as defined above, Duration is the inflammation history based on SCC (short, long, ND), parity is parity group (1, 2, 3+), DIM is categorized into early, mid and late lactation (<100, 100-200, 200+) and treatment is treatment for mastitis (IMM, PAR, and NSAIDs, or no treatment). Two way interactions between the main variables were also evaluated for statistical significance. No correction was made for clustering of cases within herd, because the model would not converge when herd was included due to the large number of herds and the limited number of cases per herd. Goodness of fit of the final model was evaluated using the model deviance and the Hosmer-Lemeshow statistic. In the Hosmer-Lemeshow statistic, the data are divided into 10 approximately equal deciles of observed risk. In these deciles the observed and expected number of observation are compared using a Chi-square distribution with 10-2 = 8 degrees of freedom (Hosmer and Lemeshow, 2013). A low value of the Hosmer-Lemeshow
statistic indicates a good fit to the data. In addition, a deviance value that is close to the remaining degrees of freedom implies that there is no evidence of a poor fit of the model to the data.
RESULTS

Descriptive Analysis

Of 624 milk samples submitted for culture 251 (40%) were positive for *S. uberis* in pure culture whilst 42 samples (7%) were culture negative. The remaining samples tested positive for *Escherichia coli* \( n = 108 \); 17%), *Klebsiella* \( n = 12 \); 2 %), *Staphylococcus aureus* \( n = 76 \); 12%), *Staphylococcus* spp. \( n = 103 \); 17%) or other species \( n = 32 \); 5%). Samples positive for *S. uberis* originated from 142 farms.

From 99 farms, a single *S. uberis* positive sample was obtained whilst 20 and 23 farms provided 2 or more *S. uberis* positive samples, respectively. All isolates originated from cows with clinical or subclinical mastitis in one quarter with the exception of three cows where *S. uberis* was isolated from two quarters on the same sampling date. The clinical manifestation of *S. uberis* positive mastitis cases was significantly different from the clinical manifestation of *S. uberis* negative cases (Chi-square = 38.0, df = 3, \( P < 0.005 \); Figure 1), with *S. uberis* overrepresented among non-severe first cases and underrepresented among subclinical cases. Distribution across parities was not different between *S. uberis* and other diagnoses (Chi-square = 1.56, df = 2, \( P = 0.46 \)). During the first 100 days of lactation *S. uberis* was less common than other diagnoses, whereas it was more common between 100 and 200 DIM (Chi-square=10.13, df = 2, \( P < 0.05 \)). Milk yield, fat and protein content were not different between *S. uberis* and non *S. uberis* cases prior to infection (results not shown).

Cow level data for *S. uberis* cases are summarized in Table 1. Severe and subclinical *S. uberis* cases were overrepresented in parity 1 compared to higher parities, whereas non-severe first cases were overrepresented in parity 2 and repeat cases in higher parities, respectively (Chi-square = 13.67, df = 6, \( P < .05 \)). Severe cases were overrepresented in early lactation, whereas repeat cases were overrepresented in mid-lactation (Chi-square = 13,02, df = 6, \( P < 0.05 \); Table 1). Treatment records were available for ca. 80% of severe, first and repeat cases and for 42% of subclinical cases (Chi-square = 22, df = 3, \( P = 0.0001 \)). When no treatment was recorded, this was considered to
indicate that no treatment was administered. Intramammary antibiotics as the only treatment were more commonly used to treat non-severe first cases as compared to severe, repeated and subclinical cases, whereas they were more commonly used in combination with parenteral treatment for repeat cases and subclinical cases (Chi-square = 31; df = 6, P < 0.0001; Table 1). The combination of intramammary and parenteral antimicrobials with anti-inflammatory treatment was mostly used in severe cases and never for subclinical cases (Table 1). Milk production was numerically lower in severe cases than in non-severe cases, both before and after diagnosis of clinical or subclinical mastitis, with the exception of yield at 3 DHI recordings prior to diagnosis, but the difference was not significant. No differences were detected between severity classes with regard to fat and protein content of milk before or after diagnosis of mastitis (data not shown).

Herd-level data was collected on farms with *S. uberis* positive results and is presented in Table 2. Most herds were housed, either full time or part time. Straw yards were the predominant housing system, with only 22% of herds housed in cubicles. PostMTD was used in almost all herds and in more than half of all herds both PreMTD and PostMTD were used. Use of PreMTD without PostMTD was not reported. Severe cases were overrepresented in herds without PostMTD (Chi-square = 10.23, df = 3, P < 0.05).

**Factors Associated with Cure of S. uberis IMI**

Cure was evaluated based on post diagnosis SCC values and results from the regression model are shown in Table 3. A total of 125 cases had complete data and were included in this analysis. Model deviance was 127.4 on 115 degrees of freedom, i.e. the values were similar and there was no indication of a poor fit of the model to the data. In addition, the Hosmer-Lemeshow statistic was low (6.05), implying a good fit to the data. The probability of cure was significantly higher in animals in lactation 1 and 2 compared to older animals. The probability of cure increased numerically with increasing number of treatment types, i.e. from no treatment to intramammary antimicrobials only
to combined intramammary and parenteral antimicrobials, to both routes of antimicrobial administration combined with NSAID. However, there was no statistically significant difference in cure between treatments. Finally, the probability of cure was higher among IMI with a short history of inflammation than those with a long history of inflammation prior to treatment (Table 3). Clinical manifestation and herd level variables were not associated with cure.
In this study we aimed to use routinely available herd and animal-level data to support control of *S. uberis* mastitis and the judicious use of antimicrobials. Risk factors for the incidence of *S. uberis* mastitis (clinical mastitis or IMI) have been described at herd-level (Barkema *et al.*, 1999; Ericsson *et al.*, 2009) and animal-level (Zadoks *et al.*, 2001; Breen *et al.*, 2009), and the impact of different treatment regimens on the outcome of treatment of *S. uberis* mastitis has been described for experimentally induced (Hillerton and Kliem, 2002; Oliver *et al.*, 2003) and naturally occurring infections (reviewed in Zadoks, 2007). To our knowledge, animal-level risk factors for severity of disease or treatment outcome of *S. uberis* IMI have not been described. Here, we show for the first time that animal-level data can be used to predict the outcome of antimicrobial treatment of *S. uberis* mastitis and to guide treatment decisions. Specifically, the probability of cure was higher among 1st and 2nd parity animals compared to older cows, and in animals with at most a single elevated cow-level SCC before diagnosis compared to those with multiple high SCC records. Those findings are strikingly similar to results obtained for *Staphylococcus aureus* IMI across a range of studies covering both clinical and subclinical mastitis (reviewed in Barkema *et al.*, 2006) and can be used to inform decisions about treatment duration or the choice between treatment and culling. The individual making treatment decisions will be able to weigh these factors in the decision making and to use this information to provide a realistic prognosis. Pathogen-specific predictors for cure, as described here for *S. uberis*, are particularly useful when information on the causative agent is available. Several studies have demonstrated the feasibility of using on-farm diagnostics to inform case management (Lago *et al.*, 2011; Cameron *et al.*, 2014) and additional tests for rapid or on-farm screening of milk samples are under development, including culture and DNA-based tests (Viora *et al.*, 2014; Bosward *et al.*, 2016). Considering the similarities between results obtained for *S. uberis* and *S. aureus*, some of this information may also be of value in the absence of an etiological diagnosis, although further field evaluation will be needed to validate such a generic approach.
Increased parity was associated with a reduced likelihood of cure. This is not merely a reflection of the chronicity of infection, because parity was significant after correction for SCC, which is a proxy for duration (Barkema et al., 2006). The mechanism behind reduced probability of cure in older animals is unknown. Possible explanations were discussed by Barkema and co-workers (2006) for the response to treatment of Staph. aureus IMI. One potential explanation is the change in ratio between udder volume, which increases with age, and the administered dose of antimicrobials, which is independent of age, resulting in a lower dose per unit udder volume in older animals (Barkema et al., 2006). This reasoning would also apply to S. uberis treatment. Immunosenescence, the waning of the immune response with age, could be postulated to play a role in deterioration of treatment outcome with age, but there is no specific evidence for this in the context of bovine mastitis. Regardless of the underlying mechanism, the comparatively poor treatment response of older cows can be interpreted as an imperative to help our cows to age healthily, e.g. by selecting for cows with high genetic merit for udder health or immune responsiveness (Thompson-Crispi et al., 2014). In addition, animal-level risk factors should be minimized where possible. For example, severe teat end hyperkeratosis is an animal-level risk factor for S. uberis CM, and the risk of hyperkeratosis can be reduced by avoiding overmilking (Breen et al., 2009; Edwards et al., 2013).

The observation that duration of IMI, as measured by number of elevated monthly SCC prior to treatment, is predictive of cure is compatible with previous data on both S. uberis and Staph. aureus. A detailed longitudinal study of S. uberis IMI in 2 herds showed that some episodes of CM are due to recent IMI, whereas other CM episodes are preceded by periods of elevated (Zadoks et al., 2003). CM episodes without preceding SCC elevation were more likely to be followed by cure than CM episodes with preceding SCC elevation. Similarly, in several treatment trials of Staph. aureus IMI, higher or longer SCC elevation prior to treatment was associated with a decreased probability of cure (reviewed in Barkema et al., 2006). A poor response of chronic Staph. aureus IMI to treatment may be explained in part by micro-absscess formation and fibrosis (Erskine et al., 2003). Fibrosis also occurs during S. uberis mastitis, starting as early as 6 days after infection in
experimental challenge studies. It is accompanied by presence of the pathogen in subepithelial and
septal tissue and lymphatic vessels and lymph nodes (Thomas et al., 1994). This may explain why the
response of S. uberis mastitis to treatment can be poor, even after extended therapy (Milne et al.,
2005). Both in experimentally induced and in persistent S. uberis IMI, extended therapy increases
the probability of cure (Oliver et al., 2003; Swinkels et al., 2014). The benefits of extended therapy
must be weighed against its disadvantages, including increased costs of antibiotics and milk discard,
and increased risk of residue in milk and selection for antimicrobial resistance (Hillerton and Kliem,
2002; Barkema et al., 2006). As in any risk factor study, the risk factors identified in the current
study, including treatment modality, and their coefficients allow us to quantify the increase or
decrease in the likelihood of a particular treatment outcome, but the specific outcome in any
individual animal cannot be predicted.

In the current study a numerical but non-significant increase in cure was observed with an
increase in treatment modalities (intramammary and parenteral antimicrobials and NSAIDs). This
study was not, however, a randomized controlled clinical trial, nor was it meant to be. Farmers
tended to treat severe cases of mastitis with a combination of intramammary, parenteral and anti-
inflammatory products, first clinical cases with intra-mammary treatment only and repeated- and
subclinical cases with antimicrobial treatment by both the intra-mammary and parenteral route.
This implies farmers’ awareness of the usefulness of cow-specific treatment, with consideration of
both animal welfare and economic aspects of treatment. This information provides evidence that
farmers are willing to make cow-specific decisions and bodes well for the feasibility of including cow-
specific risk factors in future protocols. In our practice, farm specific treatment protocols are already
discussed with each farmer on an annual basis and the treatment choices reported by the farmers
are in line with those protocols. As a next step towards judicious use of antimicrobials, we envisage
implementation of cow-specific protocols.
Animals with short duration mastitis (no or single SCC elevation prior to diagnosis) were likely to cure (no or single SCC elevation after diagnosis), whereas animals with long duration mastitis (multiple SCC elevation prior to diagnosis) were likely not to cure (multiple SCC elevations after diagnosis). Similarly, data availability post-diagnosis mirrored data availability pre-diagnosis, i.e. animals with incomplete SCC data prior to diagnosis often had incomplete SCC data after diagnosis too (data not shown). This would mostly apply to animals in early lactation that were lost to follow-up due to culling. Thus, although our analysis shows no significant difference in cure between different severity classes, this result is affected by “healthy worker bias”, whereby only surviving cows are included in the analysis. Indeed, loss to follow-up as indicated by absence of data on cure was proportionally higher for severe cases than for non-severe cases (Table 1).

Of the herd-level factors considered in this study, use of PostMTD was associated with a reduced risk of severe, repeat and subclinical *S. uberis* mastitis compared to first cases of mastitis. The value of PostMTD in reducing the risk of *S. uberis* IMI has been documented repeatedly (Zadoks et al., 2003; Galton, 2004; Williamson and Lacy-Hulbert, 2013) but it has not been linked to clinical manifestation. Strain-specific transmission and virulence patterns have previously been suggested or documented (Zadoks et al., 2003; Tassi et al., 2013) and could theoretically contribute to an association between PostMTD and clinical manifestation. It has also been hypothesized that host immune status may contribute to clinical manifestation of *S. uberis* IMI (Tassi et al., 2013). Indeed, in the current study, severe cases of CM were overrepresented among heifers and animals in early lactation. This emphasizes the importance of another herd-level management factor, i.e. adequate care for non-lactating animals. Considering that *S. uberis* is common in the faeces and environment of cattle (Zadoks *et al.*, 2005), environmental hygiene is of particular importance. The risk of infection in heifers and dry cows can also be reduced through use of teat spray and internal teat sealants, respectively (Lopez-Benavides *et al.*, 2009; Compton *et al.*, 2014). With increasing pressure to reduce antimicrobial use, implementation of non-antimicrobial mastitis prevention measures becomes increasingly important.
In this field study, definitions of transient and persistent IMI and cure were based on SCC data. Although repeated post-treatment culture has been considered the “gold standard” for cure in clinical trials, additional or alternative metrics for cure are increasingly reported in field studies. SCC has been used as a primary criterion for cure in studies of clinical mastitis, subclinical mastitis, and dry cow treatment (St. Rose et al., 2003; Lago et al., 2011; Persson et al., 2015). SCC is routinely used as an indicator of infection status (Schukken et al., 2003), although the probability of bacteriological cure is higher than the probability of SCC-based cure in studies of chronic streptococcal mastitis (St. Rose et al., 2003). SCC is of immediate interest to farmers, unlike bacteriological cure which is primarily of academic interest. Moreover, SCC is routinely available at very low cost, which makes its large-scale use feasible in field studies, veterinary practice and farm management. Finally, SCC captures long-term outcomes of mastitis treatment, whereas culture results generally only reflect the first few weeks post-treatment. Thus, SCC is a convenient, affordable and meaningful indicator of treatment outcome.

In conclusion, we show that treatment recommendations can be informed by animal-level data that is routinely available to farmers and veterinarians, such as parity and SCC. To some extent, treatment recommendations can be animal-specific rather than pathogen-specific, as both S. uberis IMI and Staph. aureus IMI show a better response to treatment in animals in first or second lactation and in animals with a single high SCC than in older animals or animals with multiple high SCC values prior to treatment. In older animals or animals with multiple high SCC values the simultaneous use of multiple treatment modalities may enhance the probability of cure but this would result in increased use of antimicrobials. To limit the need for such treatment, continued or renewed emphasis on herd management and infection prevention is needed. Formal validation of the observations described here through a randomized controlled clinical trial may strengthen the evidence base underpinning the suggested treatment decisions. In the absence of such validation, the evidence presented here is the best available evidence to inform decisions on treatment of S. uberis mastitis, the most common type of mastitis observed in this and many other studies.
Bibliography


40. Zadoks, R. N. 2007. Sources and epidemiology of *Streptococcus uberis*, with special emphasis on
mastitis in dairy cattle. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition
and Natural Resources, 2, 030, 15 pp.

Schukken. 2001. Cow- and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus

Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy
herds. Epidemiol Infect. 130:335-49.

Figure 1. Clinical manifestation of mastitis for quarters with *S. uberis* negative (n = 373, white) and *S. uberis* positive (n = 251, black) milk samples (Chi-square = 38.0, df = 3, \( P < 0.005 \)). Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical suspicion of fever); Non-severe first case: first occurrence of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder). Non-severe repeat case: repeat occurrence of CM during the current lactation with local signs only during the current episode; Subclinical mastitis: elevated cow-level SCC (> 200,000 cells/ml based on DHI data) not accompanied by any visible abnormalities.
Classification of mastitis based on clinical expression

Samson et al., Figure 1.
Table 1. Cow-level data for *S. uberis* positive mastitis cases (number and (%)) with break-down by manifestation. Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical suspicion of fever); First: first occurrence of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat occurrence of CM during the current lactation with local signs only during current episode; Subclinical: cow-level SCC (> 200,000 cells/ml based on DHI data) not accompanied by any signs.

<table>
<thead>
<tr>
<th>Cow factor</th>
<th>All <em>S. uberis</em> cases, n (%)</th>
<th><em>S. uberis</em> cases by clinical manifestation, n (%)</th>
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<td>Severe</td>
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<tr>
<td><strong>subtotal</strong></td>
<td>236 (100)</td>
<td>37 (16)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>61 (100)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Second</td>
<td>55 (100)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Higher</td>
<td>122 (100)</td>
<td>17 (14)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td>238 (100)</td>
<td>36 (15)</td>
</tr>
<tr>
<td>Treatment¹</td>
<td></td>
<td></td>
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<tr>
<td>none</td>
<td>49 (100)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>IMM</td>
<td>67 (100)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>IMM + PAR</td>
<td>103 (100)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>IMM + PAR + NSAID</td>
<td>19 (100)</td>
<td>10 (53)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td>238 (100)</td>
<td>37 (16)</td>
</tr>
</tbody>
</table>
### Duration

<table>
<thead>
<tr>
<th></th>
<th>Short</th>
<th>Long</th>
<th>ND</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>78 (100)</td>
<td>66 (100)</td>
<td>96 (100)</td>
<td>240 (100)</td>
</tr>
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<td></td>
<td>8 (10)</td>
<td>8 (12)</td>
<td>22 (23)</td>
<td>38 (16)</td>
</tr>
<tr>
<td></td>
<td>35 (45)</td>
<td>25 (38)</td>
<td>46 (48)</td>
<td>106 (44)</td>
</tr>
<tr>
<td></td>
<td>30 (38)</td>
<td>23 (35)</td>
<td>19 (20)</td>
<td>72 (30)</td>
</tr>
<tr>
<td></td>
<td>5 (6)</td>
<td>10 (15)</td>
<td>9 (9)</td>
<td>24 (10)</td>
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</tbody>
</table>

### Cure

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>ND</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>79 (100)</td>
<td>97 (100)</td>
<td>64 (100)</td>
<td>240 (100)</td>
</tr>
<tr>
<td></td>
<td>10 (13)</td>
<td>12 (12)</td>
<td>16 (25)</td>
<td>38 (16)</td>
</tr>
<tr>
<td></td>
<td>39 (49)</td>
<td>38 (39)</td>
<td>29 (45)</td>
<td>106 (44)</td>
</tr>
<tr>
<td></td>
<td>23 (29)</td>
<td>33 (34)</td>
<td>16 (25)</td>
<td>72 (30)</td>
</tr>
<tr>
<td></td>
<td>7 (9)</td>
<td>14 (14)</td>
<td>3 (5)</td>
<td>24 (10)</td>
</tr>
</tbody>
</table>

2. Duration: Short = at least 2 monthly cow-level SCC < 200,000 cells/ml before diagnosis; Long = at least 2 monthly cow-level SCC > 200,000 cells/ml before diagnosis; ND = not determined due to insufficient SCC data before diagnosis.
3. Cure: Yes = at least 2 monthly cow-level SCC < 200,000 cells/ml after diagnosis; Long = at least 2 monthly cow-level SCC > 200,000 cells/ml after diagnosis; ND = not determined due to insufficient SCC data after diagnosis.
Table 2. Herd-level data for *S. uberis* positive mastitis cases (number and (%)) with breakdown by manifestation. Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical suspicion of fever); First: first occurrence of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat occurrence of CM during the current lactation with local signs only during current episode; Subclinical: cow-level SCC (> 200,000 cells/ml based on DHI data) not accompanied by any signs.

<table>
<thead>
<tr>
<th>Herd factor</th>
<th>All <em>S. uberis</em> cases, n (%)</th>
<th><em>S. uberis</em> cases by clinical manifestation, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Severe</td>
</tr>
<tr>
<td>Housing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent</td>
<td>120 (100)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Partial</td>
<td>92 (100)</td>
<td>20 (22)</td>
</tr>
<tr>
<td>None</td>
<td>28 (100)</td>
<td>3 (29)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td><strong>240 (100)</strong></td>
<td><strong>38 (16)</strong></td>
</tr>
<tr>
<td>Bedding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Straw yard</td>
<td>187 (100)</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Cubicles</td>
<td>53 (100)</td>
<td>11 (21)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td><strong>240 (100)</strong></td>
<td><strong>38 (16)</strong></td>
</tr>
<tr>
<td>Pre-dipping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>130 (100)</td>
<td>19 (15)</td>
</tr>
<tr>
<td>No</td>
<td>106 (100)</td>
<td>18 (17)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td><strong>236 (100)</strong></td>
<td><strong>37 (16)</strong></td>
</tr>
<tr>
<td>Post-dipping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>214 (100)</td>
<td>29 (14)</td>
</tr>
<tr>
<td>No</td>
<td>22 (100)</td>
<td>8 (36)</td>
</tr>
<tr>
<td><strong>subtotal</strong></td>
<td><strong>236 (100)</strong></td>
<td><strong>37 (16)</strong></td>
</tr>
</tbody>
</table>
Table 3. Logistic regression of cow-factors versus cure for 125 cases of *S. uberis* mastitis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (SE)</th>
<th>Odds Ratio</th>
<th>95% C.I. for Odds Ratio</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-4.8 (1.3)</td>
<td></td>
<td>-3.68</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>1.7 (0.6)</td>
<td>5.5</td>
<td>1.8 to 5.5</td>
<td>3.1</td>
<td>0.0023</td>
</tr>
<tr>
<td>Second</td>
<td>1.3 (0.6)</td>
<td>3.8</td>
<td>1.3 to 11.1</td>
<td>2.42</td>
<td>0.016</td>
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<tr>
<td>Third or higher</td>
<td>Base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical manifestation(^1)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Severe</td>
<td>1.6 (1.3)</td>
<td>4.8</td>
<td>0.3 to 68.7</td>
<td>1.16</td>
<td>0.24</td>
</tr>
<tr>
<td>First</td>
<td>2.0 (1.2)</td>
<td>7.1</td>
<td>0.7 to 77.0</td>
<td>1.65</td>
<td>0.10</td>
</tr>
<tr>
<td>Repeat</td>
<td>1.9 (1.2)</td>
<td>6.4</td>
<td>0.6 to 70.9</td>
<td>1.54</td>
<td>0.13</td>
</tr>
<tr>
<td>Subclinical</td>
<td>Base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>1.7 (0.5)</td>
<td>3.1</td>
<td>2.2 to 14.2</td>
<td>3.74</td>
<td>0.0002</td>
</tr>
<tr>
<td>Long</td>
<td>Base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (^3)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IMM + PAR + NSAID</td>
<td>2.2 (1.3)</td>
<td>9.4</td>
<td>0.7 to 9.4</td>
<td>1.68</td>
<td>0.09</td>
</tr>
<tr>
<td>IMM + PAR</td>
<td>1.1 (0.6)</td>
<td>3.1</td>
<td>0.9 to 3.1</td>
<td>1.79</td>
<td>0.074</td>
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<tr>
<td>IMM</td>
<td>0.7 (0.7)</td>
<td>2.1</td>
<td>0.6 to 8.2</td>
<td>1.09</td>
<td>.28</td>
</tr>
<tr>
<td>None</td>
<td>Base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical suspicion of fever); First: first occurrence of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat occurrence.
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