Use of on-farm data to guide treatment and control mastitis caused by *Streptococcus uberis*

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ABSTRACT

Treatment of mastitis is the most common reason for use of antimicrobial agents 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, first nonsevere, repeat, or subclinical. Additional data were collected at the cow level [somatic cell count (SCC), parity, lactation stage, milk yield, fat and protein contents, treatment] and at the herd level (housing, bedding, premilking teat disinfection, postmilking teat disinfection). Severe cases were overrepresented among heifers and animals in early lactation, and repeat cases were overrepresented in cows with 3 or more lactations. The probability of cure was higher among first- and second-parity animals than among older cows, and was 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

INTRODUCTION

Antimicrobial resistance is increasingly perceived as a threat to human and animal health, and a range of national and international bodies (UK Department of Health, 2013; World Health Organization, 2015) have emphasized the need for the responsible use of antimicrobials. Key elements of the approach proposed by the World Health Organization include reduction of the incidence of infection and optimized use of antimicrobial medicines (World Health Organization, 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 reduce antibiotic usage, such as minimizing the risk of disease or developing 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

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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 been described in detail only 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 *Strep. uberis* can be transient, recurrent, or chronic, and a wide range of cure rates has been reported in response to treatment (Zadoks et al., 2003; Zadoks, 2007). Despite the importance of *Strep. uberis* as a mastitis pathogen, little is known about risk factors for the clinical manifestation or treatment outcome of *Strep. uberis* IMI. The aim of this study was to generate data that could inform low-cost guidelines for the improved management of *Strep. uberis* mastitis under field conditions. To that end, we conducted a farm-based study of herd- and cow-level risk factors associated with the clinical manifestation and likelihood of apparent cure of *Strep. uberis* IMI based on SCC. We used only tools and data that are routinely available to farmers and veterinary practices, including treatment and DHI records.

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. In total, 142 farmers submitted at least 1 milk sample, indicating an uptake of approximately 28%.

Milk samples were subjected to bacteriological culture in the Vetformance laboratory. Aliquots of milk

(10 μ L) were plated onto 3 media: (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) bromocresol purple (BCP) agar (bioMérieux; Ref. 43021). Plates were incubated at 37°C for 18 to 24 h. Cultures were considered pure if only 1 morphotype was present on the blood agar plate. For pure cultures, growth on both the CNA and BCP plates was considered evidence that the bacteria were gram-positive; growth on only the BCP plate was evidence that the bacteria were gram-negative. Among gram-negative bacteria, *Escherichia coli* was characterized by a positive lactose reaction (color change of the BCP plate from purple to yellow) and a negative urea reaction (bioMérieux; Ref. 55752), whereas *Klebsiella* was identified by positive lactose and urea reactions. Gram-positive bacteria were considered *Staph. 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 *Strep. uberis* was based on negative response in the catalase reaction and positive response in the esculin reaction (bioMérieux; Ref. 42086; National Mastitis Council, 1999). Susceptibility to penicillin was also 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 *Strep. uberis* at 0.5 McFarland (equivalent to approximately 10^8 cfu/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 μ g; 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: (1) private farm records on treatment, (2) monthly DHI data, and (3) questionnaires filled out by the farmer and the attending veterinarian. For each cow, the date of mastitis diagnosis (observation of CM or notification

of SCC data via DHI) and treatment were recorded, including intramammary administration of antimicrobials, parenteral administration of antimicrobials, and use of nonsteroidal antiinflammatory drugs (**NSAID**). As well, 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 (kg), fat content (g/kg), and protein content (g/kg). At the herd level, information was collected on the use of premilking teat disinfection (**PreMTD**) and postmilking 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 4 categories based on the 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 (temperature $>39^{\circ}\text{C}$ measured on clinical suspicion of fever by the farmers and results recorded on the data form accompanying the milk sample);
2. Nonsevere 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. Nonsevere repeat case (“repeat”): second 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); or
4. Subclinical: SCC $>200,000$ cells/mL at the cow level based on most recent DHI data, not accompanied by any clinical signs.

For 212 of the 251 animals that tested positive for *Strep. uberis*, SCC data were partially available (fewer than 3 records before or after diagnosis) or complete (3 records before and after diagnosis). We used SCC data to classify the duration of inflammation before diagnosis and the response to treatment after diagnosis. Inflammation was considered “short” if at least 2 monthly SCC records before diagnosis were $<200,000$ cells/mL and “long” if at least 2 monthly SCC records before diagnosis were $>200,000$ cells/mL. An animal

was considered cured if at least 2 monthly SCC after diagnosis were $<200,000$ cells/mL and not cured if at least 2 monthly SCC after diagnosis were $>200,000$ cells/mL. For other SCC combinations or missing data, duration and cure were not determined (**ND**), such as for animals in early lactation or for cows that were dried off or culled before completion of follow-up.

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 (e.g., for cow-level factors associated with clinical severity), data were analyzed using categorical methods (chi-square analyses). 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 based on SCC, we used logistic regression 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 was severe, first, repeat, or subclinical as defined above; duration was the inflammation history based on SCC (short, long, ND); parity was parity group (1, 2, 3+); DIM was categorized as early, mid, and late lactation (<100 , 100–200, 200+); and treatment was treatment for mastitis (intramammary antimicrobials, parenteral antimicrobials, NSAID, 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. We evaluated goodness of fit in the final model using 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-squared distribution with $10 - 2 = 8$ degrees of freedom (Hosmer and Lemeshow, 2013). A low value for the Hosmer-Lemeshow statistic indicates a good fit. A deviance value that is close to the remaining degrees of freedom implies no evidence of a poor fit.

RESULTS

Descriptive Analysis

Of 624 milk samples submitted for culture, 251 (40%) were positive for *Strep. uberis* in pure culture and 42 samples (7%) were culture-negative. The remaining samples tested positive for *E. coli* (n = 108; 17%), *Klebsiella* (n = 12; 2%), *Staph. aureus* (n = 76; 12%), *Staphylococcus* spp. (n = 103; 17%), or other species (n = 32; 5%). Samples positive for *Strep. uberis* originated from 142 farms. We obtained a single *Strep. uberis*-positive sample from 99 farms, and 2 or more *Strep. uberis*-positive samples from 20 and 23 farms, respectively. All isolates originated from cows with clinical or subclinical mastitis in 1 quarter with the exception of 3 cows, in which *Strep. uberis* was isolated from 2 quarters on the same sampling date. The clinical manifestation of *Strep. uberis*-positive mastitis cases was significantly different from the clinical manifestation of *Strep. uberis*-negative cases (chi-squared = 38.0, df = 3, $P < 0.005$; Figure 1): *Strep. uberis* was overrepresented among non-severe first cases and underrepresented among subclinical cases. Distribution across parities was not different between *Strep. uberis* and other diagnoses (chi-squared = 1.56, df = 2, $P = 0.46$). During the first 100 d of lactation, *Strep. uberis* was less common than other diagnoses, but it was more common between 100 and 200 DIM (chi-squared = 10.13, df = 2, $P < 0.05$). Milk yield, fat, and protein content were not different between *Strep. uberis* and non-*Strep. uberis* cases before infection (results not shown).

Cow-level data for *Strep. uberis* cases are summarized in Table 1. Severe and subclinical *Strep. uberis* cases were overrepresented in parity 1 compared to higher parities, but non-severe first cases were overrepresented in parity 2 and repeat cases were overrepresented in higher parities (chi-squared = 13.67, df = 6, $P < 0.05$). Severe cases were overrepresented in early lactation, but repeat cases were overrepresented in mid-lactation (chi-squared = 13.02, df = 6, $P < 0.05$; Table 1). Treatment records were available for approximately 80% of severe, first, and repeat cases, and for 42% of subclinical cases (chi-squared = 22, df = 3, $P = 0.0001$). When no treatment was recorded, we considered this to indicate that no treatment was administered. Intramammary antibiotics as the only treatment were used more commonly to treat non-severe first cases than severe, repeated, and subclinical cases; they were used more commonly in combination with parenteral treatment for repeat cases and subclinical cases (chi-squared = 31; df = 6, $P < 0.0001$; Table 1). The combination of intramammary and parenteral antimicrobials with antiinflammatory

Figure 1. Clinical manifestation of mastitis for quarters with *Strep. uberis*-negative (n = 373, white) and *Streptococcus uberis*-positive (n = 251, black) milk samples (chi-squared = 38.0, df = 3, $P < 0.005$). Severe = clinical mastitis (CM) with local and general symptoms (temperature $>39^{\circ}\text{C}$, checked upon clinical suspicion of fever); nonsevere first case = first occurrence of CM during the current lactation with local signs only (abnormalities of milk with or without abnormalities of the udder); nonsevere repeat case = repeat occurrence of CM during the current lactation with local signs only during the current episode; subclinical = elevated cow-level SCC ($>200,000$ cells/mL based on DHI data) not accompanied by any clinical signs.

treatment was used mostly 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 before diagnosis, but the difference was not significant. No differences were detected between severity classes with regard to fat or protein content of milk before or after diagnosis of mastitis (data not shown).

Herd-level data from farms with *Strep. uberis*-positive results are presented in Table 2. Most herds were housed, either full-time or part-time. Straw yards were the predominant housing system; only 22% of herds were housed in cubicles. Almost all herds used PostMTD, and more than half of herds used both PreMTD and PostMTD; use of PreMTD without PostMTD was not reported. Severe cases were overrepresented in herds without PostMTD (chi-squared = 10.23, df = 3, $P < 0.05$).

Factors Associated with Cure of *Strep. uberis* IMI

Cure was evaluated based on postdiagnosis 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 df; that is, the values were similar and showed no indication of a poor fit of the model to the data. 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 than in older animals. The probability of cure increased numerically with number of treatment types; that is, from no treatment to intramammary antimicrobials only to combined intramammary and parenteral antimicrobials, to both routes of antimicrobial administration combined with NSAID. However, we observed no statistically significant difference in cure between treatments. Finally, the probability of cure was higher in IMI with a short history of inflammation than in those with a long history of inflammation before treatment (Table 3). Clinical manifestation and herd-level variables were not associated with cure.

DISCUSSION

In this study, we aimed to use routinely available herd- and animal-level data to support control of *Strep. uberis* mastitis and the judicious use of antimicrobials. Risk factors for the incidence of *Strep. uberis* mastitis (CM or IMI) have been described at the herd level (Barkema et al., 1999; Ericsson Unnerstad et al., 2009) and animal level (Zadoks et al., 2001; Breen et al., 2009), and the effect of different treatment regimens on the outcome of treatment of *Strep. uberis* mastitis has been described for experimentally induced (Hillerton and Kliem, 2002; Oliver et al., 2003) and naturally occurring infections (Zadoks, 2007). To our knowledge,

Table 1. Cow-level data for *Streptococcus uberis*-positive mastitis cases, with breakdown by manifestation¹

Cow factor	All <i>Strep. uberis</i> cases, no. (%)	<i>Strep. uberis</i> cases by clinical manifestation, no. (%)			
		Severe	First	Repeat	Subclinical
DIM					
<100	123 (100)	26 (21)	56 (46)	27 (22)	14 (11)
100 to 200	63 (100)	7 (11)	25 (40)	27 (43)	4 (6)
>200	50 (100)	4 (8)	24 (48)	17 (34)	5 (10)
Subtotal	236 (100)	37 (16)	105 (44)	71 (30)	23 (10)
Parity					
First	61 (100)	12 (20)	23 (38)	16 (26)	10 (16)
Second	55 (100)	7 (13)	34 (62)	12 (22)	2 (4)
Higher	122 (100)	17 (14)	49 (40)	44 (36)	12 (10)
Subtotal	238 (100)	36 (15)	106 (45)	72 (30)	24 (10)
Treatment²					
None	49 (100)	7 (14)	17 (35)	12 (24)	13 (27)
IMM	67 (100)	6 (9)	42 (63)	17 (25)	2 (3)
IMM + PAR	103 (100)	14 (14)	41 (40)	40 (39)	8 (8)
IMM + PAR + NSAID	19 (100)	10 (53)	6 (32)	3 (16)	0 (0)
Subtotal	238 (100)	37 (16)	106 (45)	72 (30)	23 (10)
Duration³					
Short	78 (100)	8 (10)	35 (45)	30 (38)	5 (6)
Long	66 (100)	8 (12)	25 (38)	23 (35)	10 (15)
ND	96 (100)	22 (23)	46 (48)	19 (20)	9 (9)
Subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)
Cure⁴					
Yes	79 (100)	10 (13)	39 (49)	23 (29)	7 (9)
No	97 (100)	12 (12)	38 (39)	33 (34)	14 (14)
ND	64 (100)	16 (25)	29 (45)	16 (25)	3 (5)
Subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)

¹Severe = clinical mastitis (CM) with local and general symptoms (temperature >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 clinical signs.

²Treatment: IMM = intramammary antibiotic treatment, PAR = parenteral antibiotic treatment, NSAID = nonsteroidal antiinflammatory drug.

³Duration: 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.

⁴Cure: yes = at least 2 monthly cow-level SCC <200,000 cells/mL after diagnosis; no = 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 *Streptococcus uberis*-positive mastitis cases, with breakdown by manifestation¹

Herd factor	All <i>Strep. uberis</i> cases, no. (%)	<i>Strep. uberis</i> cases by clinical manifestation, no. (%)			
		Severe	First	Repeat	Subclinical
Housing					
Permanent	120 (100)	15 (13)	57 (48)	39 (33)	9 (8)
Partial	92 (100)	20 (22)	32 (35)	28 (30)	12 (13)
None	28 (100)	3 (11)	17 (61)	5 (18)	3 (11)
Subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)
Bedding					
Straw yard	187 (100)	27 (14)	84 (45)	58 (31)	18 (10)
Cubicles	53 (100)	11 (21)	22 (42)	14 (26)	6 (11)
Subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)
Predipping					
Yes	130 (100)	19 (15)	56 (43)	44 (34)	11 (8)
No	106 (100)	18 (17)	48 (45)	27 (25)	13 (12)
Subtotal	236 (100)	37 (16)	104 (44)	71 (30)	24 (10)
Postdipping					
Yes	214 (100)	29 (14)	94 (44)	67 (31)	24 (11)
No	22 (100)	8 (36)	10 (45)	4 (18)	0 (0)
Subtotal	236 (100)	37 (16)	104 (44)	71 (30)	24 (10)

¹Severe = clinical mastitis (CM) with local and general symptoms (temperature >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 clinical signs.

Table 3. Logistic regression of cow factors versus cure for 125 cases of *Streptococcus uberis* mastitis

Variable	Coefficient (SE)	Odds ratio	95% CI for odds ratio	Z-value	P-value
Constant	-4.8 (1.3)			-3.68	0.0002
Parity					
First	1.7 (0.6)	5.5	1.8 to 5.5	3.1	0.0023
Second	1.3 (0.6)	3.8	1.3 to 11.1	2.42	0.016
Third or higher	Base				
Clinical manifestation ¹					
Severe	1.6 (1.3)	4.8	0.3 to 68.7	1.16	0.24
First	2.0 (1.2)	7.1	0.7 to 77.0	1.65	0.10
Repeat	1.9 (1.2)	6.4	0.6 to 70.9	1.54	0.13
Subclinical	Base				
Duration ²					
Short	1.7 (0.5)	3.1	2.2 to 14.2	3.74	0.0002
Long	Base				
Treatment ³					
IMM + PAR + NSAID	2.2 (1.3)	9.4	0.7 to 9.4	1.68	0.09
IMM + PAR	1.1 (0.6)	3.1	0.9 to 3.1	1.79	0.074
IMM	0.7 (0.7)	2.1	0.6 to 8.2	1.09	0.28
None	Base				

¹Severe = clinical mastitis (CM) with local and general symptoms (temperature >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 clinical signs.

²Duration: 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.

³Treatment: IMM = intramammary antibiotic treatment, PAR = parenteral antibiotic treatment, NSAID = nonsteroidal antiinflammatory drug.

animal-level risk factors for severity of disease or treatment outcome of *Strep. 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 for *Strep. uberis* mastitis and to guide treatment decisions. Specifically, the probability of cure was higher among first- and second-parity animals compared with older cows, and in animals with, at most, a single elevated cow-level SCC before diagnosis compared with those with multiple high SCC records. Those findings are strikingly similar to results obtained for *Staph. aureus* IMI across a range of studies covering both clinical and subclinical mastitis (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 and use this information to provide a realistic prognosis. Pathogen-specific predictors for cure, as described here for *Strep. 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 *Strep. uberis* and *Staph. 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 finding was 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 the reduced probability of cure in older animals is unknown. Possible explanations were discussed by Barkema et al. (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 *Strep. uberis* treatment. Immunosenescence, the waning of the immune response with age, could be postulated to play a role in the deterioration of treatment outcome with age, but no specific evidence for this is available 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 cows age healthily, such as 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 *Strep. 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 the number of elevated monthly SCC before treatment, is predictive of cure is compatible with previous data on both *Strep. uberis* and *Staph. aureus*. A detailed longitudinal study of *Strep. uberis* IMI in 2 herds showed that some episodes of CM were due to recent IMI and other CM episodes were preceded by periods of elevated SCC (Zadoks et al., 2003). Clinical manifestation 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 before treatment was associated with a decreased probability of cure (Barkema et al., 2006). A poor response of chronic *Staph. aureus* IMI to treatment may be explained in part by micro-abscess formation and fibrosis (Erskine et al., 2003). Fibrosis also occurs during *Strep. uberis* mastitis, starting as early as 6 d after infection in experimental challenge studies. It is accompanied by presence of the pathogen in subepithelial and septal tissue, and in lymphatic vessels and lymph nodes (Thomas et al., 1994). This may explain why the response of *Strep. uberis* mastitis to treatment can be poor, even after extended therapy (Milne et al., 2005). In both experimentally induced and persistent *Strep. 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 the increased costs of antibiotics and milk discard, and the 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 likelihood of a particular treatment outcome, but a specific outcome in any individual animal cannot be predicted.

In the current study, a numerical but nonsignificant 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 antiinflammatory products; first clinical cases with intramammary treatment only; and repeated and subclinical cases with antimicrobial treatment by both the intramammary and parenteral routes. This implies that farmers are aware of the usefulness of cow-specific treatment, with consideration of both animal welfare and the 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 toward judicious use of antimicrobials, we envisage implementation of cow-specific protocols.

Animals with short-duration mastitis (no or single SCC elevation before diagnosis) were likely to cure (no or single SCC elevation after diagnosis), whereas animals with long-duration mastitis (multiple SCC elevations before diagnosis) were likely not to cure (multiple SCC elevations after diagnosis). Similarly, data availability after diagnosis mirrored data availability before diagnosis: animals with incomplete SCC data before diagnosis often had incomplete SCC data after diagnosis too (data not shown). This would apply mostly 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 were included in the analysis. Indeed, loss to follow-up as indicated by the 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 *Strep. uberis* mastitis compared with first cases of mastitis. The value of PostMTD in reducing the risk of *Strep. 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 CM of *Strep. 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: adequate care of nonlactating animals. Considering that *Strep. uberis* is

common in the feces 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 (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 posttreatment 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 CM, subclinical mastitis, and dry cow treatment (St. Rose et al., 2003; Lago et al., 2011; Persson et al., 2015). Somatic cell count 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). Somatic cell count 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 posttreatment. Thus, SCC is a convenient, affordable, and meaningful indicator of treatment outcome.

CONCLUSIONS

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 *Strep. 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 value than in older animals or animals with multiple high SCC values before 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 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 for informing decisions on treatment of *Strep. uberis* mastitis, the most common type of mastitis observed in this and many other studies.

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