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Effects of pathogen-specific clinical mastitis on probability of conception in Holstein dairy cows

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ABSTRACT

The objective of this study was to estimate the effects of pathogen-specific clinical mastitis (CM), occurring in different weekly intervals before or after artificial insemination (AI), on the probability of conception in Holstein cows. Clinical mastitis occurring in weekly intervals from 6 wk before until 6 wk after AI was modeled. The first 4 AI in a cow's lactation were included. The following categories of pathogens were studied: *Streptococcus* spp. (comprising *Streptococcus dysgalactiae*, *Streptococcus uberis*, and other *Streptococcus* spp.); *Staphylococcus aureus*; coagulase-negative staphylococci (CNS); *Escherichia coli*; *Klebsiella* spp.; cases with CM signs but no bacterial growth (above the level that can be detected from our microbiological procedures) observed in the culture sample and cases with contamination ($\geq$3 pathogens in the sample); and other pathogens [including *Citrobacter*, yeasts, *Trueperella pyogenes*, gram-negative bacilli (i.e., gram-negative organisms other than *E. coli*, *Klebsiella* spp., *Enterobacter*, and *Citrobacter*), *Corynebacterium bovis*, *Corynebacterium* spp., *Pasteurella*, *Enterococcus*, *Pseudomonas*, *Mycoplasma*, *Prototheca*, and others]. Other factors included in the model were parity (1, 2, 3, 4 and higher), season of AI (winter, spring, summer, autumn), day in lactation of first AI, farm, and other non-CM diseases (retained placenta, metritis, ketosis, displaced abomasum). Data from 90,271 AI in 39,361 lactations in 20,328 cows collected from 2003/2004 to 2011 from 5 New York State dairy farms were analyzed in a generalized linear mixed model with a Poisson distribution. The largest reductions in probability of conception were associated with CM occurring in the week before AI or in the 2 wk following AI. *Escherichia coli* and *Klebsiella* spp. had the greatest adverse effects on probability of conception. The probability of conception for a cow with any combination of characteristics may be calculated based on the parameter estimates. These findings may be helpful to farmers in assessing reproduction in their dairy cows for more effective cow management.

Key words: conception, bovine mastitis, pathogen, linear mixed model

INTRODUCTION

Successful reproduction of dairy cows is crucial to a dairy farm's livelihood. Both newborn calves and the maintenance of the cows' lactational cycles are important for a farm to thrive. When conception is delayed, or does not occur, it is a matter of great concern. Clinical mastitis (**CM**), a common problem in the dairy industry, can disrupt the fertility cycle (Santos et al., 2004; Hertl et al., 2010). Other factors interfering with conception include heat stress (Huang et al., 2008), high milk yield (Gustafsson and Emanuelson, 2002), and diseases other than mastitis (Fourichon et al., 2000). To minimize disruptions and irregularities in the fertility cycle, many farms use ovulation synchronization and planned breeding (AI) programs. The use of such interventions, however, is not a perfect solution. Cows still experience fertility problems due to the factors noted above.

The focus of the current study was the effect of CM due to different pathogens, occurring in different weekly time intervals before or after AI, on the probability of conception. We previously reported on the effects of CM caused by gram-negative and gram-positive bacteria and other organisms on probability of conception and found that all 3 types of CM reduced probability of conception, with gram-negative bacteria (comprising *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., and *Enterobacter* spp.) having the greatest effect (Hertl et al., 2010). Since completion of that report, we have continued to collect data on CM from 5 of the 7 farms in that study and have now amassed sufficient data to study the effects of CM at the pathogen-specific level. Although several studies have examined the effects of different CM pathogens on various aspects of reproduction in dairy cows (Moore et al., 1991; Hockett et al., 2005; Wilson et al., 2008), to our knowledge none have

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combined this information with timing of CM cases in specific intervals before or after AI.

The objective of the current study was to estimate the effects of different types of CM, due to different pathogens and occurring in different weekly intervals before or after AI, as well as other factors, on the probability of conception in New York State Holstein dairy cows.

MATERIALS AND METHODS

Herd Descriptions

Data on 90,271 AI in 39,361 lactations in 20,328 cows in 5 large, well-managed, high-producing Holstein herds in New York State were collected beginning in January 2003 (1 herd), February 2004 (2 herds), or June 2004 (2 herds) and continuing until July 2011, and analyzed. Three herds were in central New York State, 1 was in northern New York State, and 1 was in western New York State. The 305-d rolling herd average milk production ranged from 11,260 to 13,123 kg/cow per year, and monthly mean SCC ranged from 137,000 to 262,000 cells/mL. The farms recorded information on milk production and milk electrical conductivity, parity, reproduction, diseases, calving, drying-off, and herd exit using the DairyComp305 herd management software (Valley Agricultural Software, Tulare, CA). Cows were housed in freestalls in covered barns and managed in groups according to lactation month, production, and reproduction status. They were fed a balanced TMR and milked 3 times per day.

All cows in the study herds participated in ovulation synchronization and planned breeding programs. The study herds were selected for this reason, as such programs remove many potential biases that can arise when herd managers make decisions regarding estrus detection and breeding (Hertl et al., 2010). In the study herds, pregnancy checking and diagnosis was conducted at approximately 35 d post-AI, by either palpation or ultrasound, depending on the farm. Open cows were given GnRH, prostaglandin, or both, and resynchronized. Pregnancy rechecks were conducted between 50 and 95 d, depending on the farm. One farm did not perform rechecks, but instead watched cows for estrus and rebred any that may have previously been called pregnant.

Case Definition

All lactating cows that were inseminated for the first time between 40 and 90 DIM in the current lactation were eligible for inclusion in the study. Although a small proportion (5.5%) of cows in the study herds were first inseminated outside of this interval, they were not considered to have been inseminated normally. The voluntary waiting period in the study farms was 60 d (i.e., it was farm practice to plan the first insemination at approximately 60 DIM). Because these farms practiced synchronized breeding programs that are often on a weekly injection schedule, some cows may have started synchronization somewhat early. In some cows that were expressing strong heat symptoms, insemination would occur outside the synchronization schedule.

Milkers detected most CM cases, which presented as a warm, swollen udder or changes in milk consistency. Herdspersons also found cases in cows with elevated milk electrical conductivity (>115% of the average of the previous 10 d) and sudden concurrent milk loss (<70% of the average of the previous 10 d; Hertl et al., 2014). Such cows were then examined further for signs of CM. Sick cows were treated similarly on all 5 farms throughout the study, according to well-defined protocols (Cha et al., 2014). Microbiological diagnosis of milk samples from quarters with CM signs was performed at 3 of the Quality Milk Production Services laboratories (Ithaca, Canton, and Geneseo, NY); detailed information may be found in Gröhn et al. (2004).

If a second episode of CM occurred in the same quarter within 5 d after the first episode (with the same or a different pathogen isolated) or occurred within 14 d with the same pathogen isolated from both episodes, it was considered to be the same case of CM. Any episode occurring more than 14 d after the previous one, regardless of the pathogen isolated, was considered to be a new CM case (Barkema et al., 1998).

The pathogens included in the analysis were *Streptococcus* spp. [including *Strep. dysgalactiae*, *Strep. uberis*, other gram-positive catalase-negative cocci (such as *Strep. aerococcus*, *Strep. enterococcus*, *Strep. lactococcus*), and other *Streptococcus* spp.]; *Staphylococcus aureus*; staphylococci other than *Staph. aureus* (referred to as CNS throughout); *Escherichia coli*; *Klebsiella* spp.; cases with CM signs but no bacterial growth (above the level that can be detected using our microbiological procedures) observed in the culture sample, and cases with contamination (≥3 pathogens in the sample); and other pathogens (including *Citrobacter*, yeasts, *Trueperella pyogenes*, gram-negative bacilli (i.e., gram-negative organisms other than *E. coli*, *Klebsiella* spp., *Enterobacter*, and *Citrobacter*), *Corynebacterium bovis*, *Corynebacterium* spp., *Pasteurella*, *Enterococcus*, *Pseudomonas*, *Mycoplasma*, *Prototheca*, and others).

For the purposes of this study, where conception associated with the first 4 AI was of interest, conception status was based on either a repeated insemination (no conception as a result of the previous insemination) or the result of rectal pregnancy checks by the herd veterinarian.

Other Diseases

Besides CM, several other diseases were included as potential confounders. These were milk fever, retained placenta, metritis, ketosis, and displaced abomasum (**DA**). They were defined (Gröhn et al., 2004) as follows: (1) milk fever: a cow could not rise or had cool extremities and sluggish rumen motility near the time of calving, but was successfully treated with calcium; (2) retained placenta: retention of fetal membranes for ≥24 h after calving; (3) metritis: the cow had a fever and a purulent or fetid vaginal discharge, or an enlarged uterus detected by veterinary palpation; (4) ketosis: presence of ketones in milk or urine, and response to treatment; and (5) DA: an abomasum enlarged with gas, fluid, or both, and that was mechanically trapped in either the right or left side of the abdominal cavity. Written disease definitions and diagnostic criteria were provided to participating dairy producers and veterinarians to ensure uniformity.

Statistical Analysis

The outcome variable was whether or not a cow conceived following a particular AI. The independent variables included are described below. A set of 12 variables was created, indicating when CM occurred (if it occurred) with respect to time of an AI and the causative pathogen. The variables covered the period from 6 wk before an AI until 6 wk after an AI, in weekly intervals, for CM occurring 36 to 42 d, 29 to 35 d, 22 to 28 d, 15 to 21 d, 8 to 14 d, 1 to 7 d before, and 0 to 7 d, 8 to 14 d, 15 to 21 d, 22 to 28 d, 29 to 35 d, and 36 to 42 d after an AI. Artificial inseminations having 2 or more CM episodes in this 12-wk period around an AI were not included in the analysis (Hertl et al., 2010), as it would not be possible to determine which CM case was contributing to the probability of conception associated with a particular AI. When 2 pathogens occurred in one CM case (e.g., cow number 4 in Table 1), 1 pathogen was chosen as the leading cause according to their expected severity of effects in the cow (Hassan et al., 2009). Table 1 shows how the CM variables were coded for 4 example cows in the data set.

Other variables included in the analysis were parity (1, 2, 3, 4 and higher), season of AI [winter (January, February, March), spring (April, May, June), summer (July, August, September), autumn (October, November, December)], AI attempt (first, second, third, fourth), day of lactation of first AI, farm, and other diseases (milk fever, retained placenta, metritis, ketosis, DA).

Because the data set contained repeated measures (up to 4 AI per lactation), it was analyzed in SAS PROC GLIMMIX (version 9.2, 2009; SAS Institute Inc., Cary, NC). A model with a Poisson distribution for the response was considered appropriate as these were count data (number of AI). We fit the following generalized linear mixed model:

$$g(\mathbf{Y}) = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon}, \quad [1]$$

where g is a link function (here, the natural log of the odds of a cow conceiving after an AI), $\mathbf{Y}$ is the vector of observations (0 = did not conceive, 1 = conceived), $\boldsymbol{\beta}$ is an unknown vector of fixed-effect parameters with known design matrix $\mathbf{X}$, and $\boldsymbol{\varepsilon}$ is an unknown random error vector. A negative parameter estimate means that the cow with that characteristic is less likely to experience the event of interest; that is, to conceive. A positive parameter estimate means that the cow with that characteristic is more likely to experience the event of interest; that is, to conceive. Statistical significance was defined at $P < 0.05$.

Goodness of fit of the model was assessed by looking at the fit statistics −2(residual log pseudo-likelihood), generalized chi-square, and generalized chi-square/degrees of freedom. For the latter, a ratio of 1.0 is an indication of good model fit.

We calculated the probability, P, of an event (conception) occurring, due to the modeled factors, with the following formula:

$$P = [\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k)]/$$
$$[1 + \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k)], \quad [2]$$

where P is the probability of conception associated with a particular AI, β_0 is the regression parameter for the intercept, and β_1, β_2, …, β_k are the regression parameters for the effects x_1, x_2, …, x_k, respectively, in the model (Hertl et al., 2010).

Specification of a *residual* effect in the RANDOM statement of PROC GLIMMIX (SAS Institute, 2009) accounted for potential correlation of repeated AI within a lactation. The *subject* effect was a particular lactation of a specific cow in a specific herd. That is, events (AI) were correlated within each unique cow-lactation but were assumed independent among subjects (Hertl et al., 2010).

Least squares means; that is, means after controlling for other covariates in the model, were obtained for the levels of the fixed effects. Pairwise differences between levels or categories of each factor were assessed.

RESULTS

Descriptive Findings

A total of 97,869 first, second, third, and fourth AI events were recorded in the 5 herds during the study

Table 1. Data layout for variables relating to timing and type of clinical mastitis (CM) with respect to time of AI for 4 example cows

	CM occurring:											
	36–42 d before AI	29–35 d before	22–28 d before	15–21 d before	8–14 d before	1–7 d before	0–7 d after AI	8–14 d after	15–21 d after	22–28 d after	29–35 d after	36–42 d after
Cow 1[1]												
AI 1: 74 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
AI 2: 108 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
AI 3: 132 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
Cow 2[2]												
AI 1: 76 DIM	No CM	No CM	*Strep.* spp.	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
Cow 3[3]												
AI 1: 77 DIM	No CM	No CM	No CM	Other	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
AI 2: 110 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	*E. coli*	No CM
AI 3: 134 DIM	No CM	No CM	No CM	No CM	No CM	No CM	*E. coli*	No CM	No CM	No CM	No CM	No CM
AI 4: 157 DIM	No CM	No CM	No CM	*E. coli*	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
Cow 4[4]												
AI 1: 68 DIM	No CM	No CM	No CM	No CM	No CM	No CM	*Staph. aureus*	No CM	No CM	No CM	No CM	No CM
AI 2: 93 DIM	No CM	No CM	No CM	*Staph. aureus*	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
AI 3: 133 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM
AI 4: 157 DIM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM	No CM

[1]Cow 1 did not have CM, and conceived after the third AI.

[2]Cow 2 had *Streptococcus* spp. CM on d 52 of lactation, and conceived after the first AI.

[3]Cow 3 had CM due to yeast ("other") on d 60 of lactation and *Escherichia coli* CM on d 141 of lactation, and did not conceive.

[4]Cow 4 had CM due to *Staphylococcus aureus* and *Streptococcus* spp. on d 73 of lactation, and did not conceive. When 2 pathogens occurred in 1 CM case (as for cow 4), 1 was chosen as the leading cause (according to their effects in the cow; Hassan et al., 2009); here, *Staph. aureus*.

Table 2. Clinical mastitis (CM)-causing organisms (numbers[1]) in 39,361 lactations in 5 New York State Holstein herds, 2003–2011

Organism	All lactations	Lactation 1	Lactation 2	Lactation 3	Lactation 4+
Streptococcus spp.[2]	4,445	1,001	1,496	1,020	928
Staphylococcus aureus	1,194	341	412	252	189
CNS	1,238	338	398	259	243
Escherichia coli	4,200	933	1,404	998	865
Klebsiella spp.	1,612	243	578	433	358
Cases with CM signs but no bacterial growth					
No growth	4,443	1,003	1,445	1,085	910
Contamination	241	57	73	59	52
Other pathogens					
Citrobacter	7	1	—	1	5
Yeasts	259	79	81	53	46
Trueperella pyogenes	321	96	96	73	56
Gram-negative bacilli[3]	4	—	1	—	3
Corynebacterium bovis	61	22	15	14	10
Corynebacterium spp.	1	—	—	—	1
Pasteurella spp.	120	69	18	17	16
Enterococcus spp.	1	—	—	1	—
Pseudomonas spp.	29	8	9	4	8
Mycoplasma spp.	124	62	34	18	10
Prototheca	3	—	1	1	1
Others	2,214	497	706	532	479
Unknown[4]	1,178	339	371	274	194

[1]Total number of CM cases (comprising first, second, third, and fourth occurrences in a lactation) in which the organism was identified. A cow could have more than one organism in an episode.

[2]Includes *Strep. dysgalactiae*, *Strep. uberis*, *Streptococcus* spp.

[3]Gram-negative organisms other than *E. coli*, *Klebsiella* spp., *Enterobacter*, and *Citrobacter*.

[4]These cases were not included in the analysis.

period. Of these, 5,515 first AI that occurred before 40 DIM or after 90 DIM were excluded. A further 2,083 AI having 2 or more CM episodes occurring within 6 wk before to 6 wk after the AI were also excluded. Thus, the final data set analyzed consisted of 90,271 AI, in 39,361 lactations in 20,328 cows in 5 herds.

Forty percent of cows were in their first lactation, 30% were in their second lactation, 17% were in their third lactation, and 13% were in their fourth or higher lactation. Inseminations took place throughout the calendar year, with approximately equal proportions occurring in each season (spring, summer, autumn, winter). Although it was farm practice to wait until 60 DIM to inseminate for the first time, 16% of study cows were first inseminated between 41 and 59 DIM (most in the latter part of this interval).

In the data set, 30.8% lactations had at least one CM case. *Streptococcus* spp. and *E. coli* were the most commonly isolated pathogens. Cases with CM signs but no bacterial growth also frequently occurred (Table 2). Regarding non-CM diseases, 1% of lactations experienced milk fever, 10% had retained placenta, 4% had metritis, 9% had ketosis, and 3% had DA.

Regarding goodness of fit of the estimated model, the −2*residual log pseudo-likelihood was 336,408; generalized chi-square was 63,389; and the generalized chi-square/degrees of freedom was 0.7.

Effects of Variables (Other than CM) on Probability of Conception

Tables 3 and 4 present results from a model including parity, insemination attempt, season of AI, day in lactation of first AI, farm, non-CM diseases, and pathogen-specific CM occurring in different intervals with respect to time of AI. For ease of presentation, estimates for the non-CM variables are shown in Table 3 and estimates for the CM variables are shown in Table 4. All of the variables in both tables, however, appeared in the same model. Table 3 shows parameter estimates, standard errors, and 95% CI for variables other than CM. As a cow aged, her probability of conception at a given AI decreased. Parity 2 cows had a 14% lower [1 – exp(−0.15)] probability of conception ($P < 0.0001$) than did parity 1 cows. Parity 3 cows had a 15% lower ($P < 0.0001$) and parity 4+ cows a 20% lower ($P < 0.0001$) probability of conception than did parity 1 cows.

Of the 90,271 AI, 43% were the first in the studied lactation, 27% were the second, 18% were the third, and

Table 3. Probability of conception: Parameter estimates, standard errors (SE), and 95% CI for the non-CM factors in the generalized mixed model used to estimate the effects of clinical mastitis (CM) due to different pathogens, and other factors, in 39,361 lactations in 20,328 cows in 5 New York State herds[1,2]

Parameter	Level	Estimate	SE	95% CI
Intercept		−0.97***	0.05	−1.06, −0.88
Parity	1	Ref.[3]	—	—
	2	−0.15***	0.01	−0.17, −0.12
	3	−0.17***	0.02	−0.20, −0.14
	4	−0.22***	0.02	−0.26, −0.19
Insemination attempt (AI)	First	Ref.	—	—
	Second	−0.13***	0.01	−0.15, −0.10
	Third	−0.16***	0.01	−0.19, −0.13
	Fourth	−0.21***	0.02	−0.24, −0.17
Day in lactation of first AI	—	0.0025***	0.0006	0.001, 0.003
Season of AI	Winter	Ref.	—	—
	Spring	−0.01	0.01	−0.03, 0.02
	Summer	−0.20***	0.02	−0.22, −0.17
	Autumn	−0.04*	0.01	−0.06, −0.01
Farm	A	Ref.	—	—
	B	−0.06**	0.02	−0.10, −0.03
	C	−0.20***	0.01	−0.23, −0.18
	D	−0.15***	0.02	−0.18, −0.12
	E	−0.21***	0.02	−0.25, −0.18
Retained placenta	Absent	Ref.	—	—
	Present	−0.22***	0.02	−0.26, −0.19
Metritis	Absent	Ref.	—	—
	Present	−0.16**	0.03	−0.23, −0.09
Ketosis	Absent	Ref.	—	—
	Present	−0.09**	0.02	−0.14, −0.04
Displaced abomasum	Absent	Ref.	—	—
	Present	−0.12***	0.03	−0.19, −0.05

[1]The estimates in Tables 3 and 4 derive from the same model.

[2]Most values have been rounded to 2 decimal places, so results presented in the text may differ slightly due to rounding.

[3]Reference level of the factor (estimate = 0.00).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

12% were the fourth. Conception was marginally less likely with each successive AI (assuming that the first AI took place on d 60 of lactation): 31% {[exp(−0.97 + 60 × 0.0025)]/[1 + exp(−0.97 + 60 × 0.0025)]} for the first, 28% {[exp(−0.97 + 60 × 0.0025 − 0.13)]/[1 + exp(−0.97 + 60 × 0.0025 − 0.13)]} for the second, 27% for the third, and 26% for the fourth. A cow was more likely to conceive if the first AI occurred later rather than earlier (within the interval 40–90 DIM), in lactation.

The study farms varied in the success of their breeding programs, with the worst farm experiencing 19% fewer conceptions than the best farm. Conception was least likely to occur when AI took place in the summer. Retained placenta, metritis, ketosis, and DA were all associated with a decrease in probability of conception.

Effects of Pathogen-Specific CM on Probability of Conception

Clinical mastitis occurring before an AI had little effect on probability of conception until 1 to 7 d before insemination (Table 4). The greatest effect of *Streptococcus* spp. CM on probability of conception was when it occurred from 0 to 7 d {55% [1 – exp(−0.79)] less; $P < 0.0001$} after AI. *Staphylococcus aureus* CM was associated with a lower probability of conception only when occurring from 0 to 7 d. When CM due to CNS occurred 15 to 21 d after AI, it reduced the probability of conception, but when it occurred 29 to 35 d after AI, it increased the probability of conception.

Escherichia coli and *Klebsiella* spp. CM had their largest effects on probability of conception when occurring 0 to 7 d after AI. Clinical mastitis cases due to other pathogens and cases with signs but no growth also had their largest effects when occurring 0 to 7 d after AI.

As an example, Figure 1 shows the probability of conception associated with the first AI (assuming that it took place on d 60 of lactation), for a cow of parity 2 bred in autumn, with CM occurring in different intervals before or after AI. The horizontal line in each panel depicts the probability of conception for such a cow with no CM {probability = [exp(−0.97 + 60 × 0.0025 − 0.15 − 0.04)]/[1 + exp(−0.97 + 60 × 0.0025 − 0.15 − 0.04)] = 0.27; Table 3}. The probabilities for

Table 4. Probability of conception: Parameter estimates, standard errors (SE), and 95% CI for the clinical mastitis (CM) variables in the generalized mixed model used to estimate the effects of CM due to different pathogens, and other factors, in 39,361 lactations in 20,328 cows in 5 New York State herds[1,2]

Parameter	Level	Estimate	SE	95% CI
Intercept		−0.97***	0.05	−1.06, −0.88
CM occurring 36–42 d before AI	*Streptococcus* spp.	0.13	0.12	−0.10, 0.36
	Staphylococcus aureus	−0.17	0.30	−0.75, 0.42
	CNS	0.28	0.28	−0.27, 0.83
	Escherichia coli	0.01	0.11	−0.20, 0.22
	Klebsiella spp.	−0.53	0.30	−1.11, 0.05
	Other pathogens	0.42*	0.17	0.08, 0.77
	CM signs but no growth	−0.00	0.12	−0.23, 0.23
	None	Ref.[3]	—	—
CM occurring 29–35 d before AI	*Streptococcus* spp.	−0.14	0.14	−0.41, 0.13
	Staph. aureus	0.25	0.25	−0.25, 0.74
	CNS	0.54	0.30	−0.04, 1.13
	E. coli	0.07	0.10	−0.13, 0.26
	Klebsiella spp.	−0.01	0.24	−0.48, 0.47
	Other pathogens	0.21	0.19	−0.17, 0.58
	CM signs but no growth	−0.06	0.12	−0.30, 0.17
	None	Ref.	—	—
CM occurring 22–28 d before AI	*Streptococcus* spp.	−0.06	0.14	−0.33, 0.21
	Staph. aureus	0.07	0.25	−0.43, 0.57
	CNS	0.18	0.28	−0.37, 0.72
	E. coli	0.13	0.10	−0.05, 0.32
	Klebsiella spp.	0.05	0.21	−0.36, 0.46
	Other pathogens	0.03	0.22	−0.42, 0.47
	CM signs but no growth	0.08	0.10	−0.12, 0.27
	None	Ref.	—	—
CM occurring 15–21 d before AI	*Streptococcus* spp.	−0.18	0.13	−0.44, 0.08
	Staph. aureus	0.15	0.30	−0.43, 0.73
	CNS	−0.23	0.34	−0.91, 0.44
	E. coli	0.07	0.09	−0.11, 0.25
	Klebsiella spp.	−0.31	0.28	−0.86, 0.24
	Other pathogens	−0.20	0.23	−0.66, 0.25
	CM signs but no growth	0.27**	0.10	0.07, 0.47
	None	Ref.	—	—
CM occurring 8–14 d before AI	*Streptococcus* spp.	−0.28*	0.13	−0.54, −0.02
	Staph. aureus	−0.70	0.42	−1.52, 0.13
	CNS	0.05	0.30	−0.54, 0.63
	E. coli	−0.08	0.11	−0.29, 0.13
	Klebsiella spp.	0.04	0.24	−0.44, 0.52
	Other pathogens	0.37*	0.19	0.00, 0.74
	CM signs but no growth	−0.09	0.13	−0.34, 0.16
	None	Ref.	—	—
CM occurring 1–7 d before AI	*Streptococcus* spp.	−0.47*	0.18	−0.83, −0.11
	Staph. aureus	−0.46	0.34	−1.14, 0.21
	CNS	−0.13	0.34	−0.80, 0.54
	E. coli	−0.69***	0.19	−1.06, −0.32
	Klebsiella spp.	−1.32**	0.48	−2.27, −0.37
	Other pathogens	−1.31*	0.59	−2.48, −0.15
	CM signs but no growth	−0.28	0.15	−0.58, 0.02
	None	Ref.	—	—
CM occurring 0–7 d after AI	*Streptococcus* spp.	−0.79***	0.18	−1.15, −0.43
	Staph. aureus	−1.04*	0.42	−1.86, −0.22
	CNS	−0.54	0.38	−1.27, 0.20
	E. coli	−1.69***	0.22	−2.12, −1.27
	Klebsiella spp.	−1.33***	0.38	−2.06, −0.59
	Other pathogens	−1.05**	0.34	−1.72, −0.38
	CM signs but no growth	−0.53***	0.15	−0.82, −0.24
	None	Ref.	—	—
CM occurring 8–14 d after AI	*Streptococcus* spp.	−0.23	0.13	−0.49, 0.02
	Staph. aureus	−0.10	0.25	−0.60, 0.40
	CNS	−0.12	0.30	−0.71, 0.46
	E. coli	−0.77***	0.16	−1.08, −0.47
	Klebsiella spp.	−0.96**	0.32	−1.58, −0.34
	Other pathogens	−0.97**	0.32	−1.59, −0.35
	CM signs but no growth	−0.27*	0.13	−0.51, −0.02
	None	Ref.	—	—

Continued

Table 4 (Continued). Probability of conception: Parameter estimates, standard errors (SE), and 95% CI for the clinical mastitis (CM) variables in the generalized mixed model used to estimate the effects of CM due to different pathogens, and other factors, in 39,361 lactations in 20,328 cows in 5 New York State herds[1,2]

Parameter	Level	Estimate	SE	95% CI
CM occurring 15–21 d after AI	*Streptococcus* spp.	−0.02	0.12	−0.25, 0.22
	Staph. aureus	−0.23	0.27	−0.75, 0.29
	CNS	−1.10*	0.48	−2.05, −0.15
	E. coli	−0.78***	0.15	−1.07, −0.49
	Klebsiella spp.	−0.75**	0.28	−1.30, −0.20
	Other pathogens	−0.33	0.25	−0.83, 0.17
	CM signs but no growth	−0.20	0.13	−0.45, 0.04
	None	Ref.	—	—
CM occurring 22–28 d after AI	*Streptococcus* spp.	−0.42**	0.15	−0.72, −0.13
	Staph. aureus	0.15	0.22	−0.28, 0.57
	CNS	−0.18	0.30	−0.76, 0.40
	E. coli	−0.49***	0.13	−0.74, −0.24
	Klebsiella spp.	−0.57*	0.28	−1.12, −0.02
	Other pathogens	−0.27	0.25	−0.77, 0.22
	CM signs but no growth	−0.18	0.12	−0.41, 0.05
	None	Ref.	—	—
CM occurring 29–35 d after AI	*Streptococcus* spp.	−0.29*	0.13	−0.55, −0.03
	Staph. aureus	−0.40	0.27	−0.92, 0.12
	CNS	0.44*	0.22	0.02, 0.87
	E. coli	−0.16	0.11	−0.38, 0.07
	Klebsiella spp.	−0.29	0.23	−0.74, 0.17
	Other pathogens	−0.32	0.25	−0.82, 0.17
	CM signs but no growth	−0.09	0.12	−0.32, 0.14
	None	Ref.	—	—
CM occurring 36–42 d after AI	*Streptococcus* spp.	−0.16	0.13	−0.42, 0.10
	Staph. aureus	0.05	0.22	−0.38, 0.48
	CNS	−0.17	0.27	−0.69, 0.35
	E. coli	0.04	0.11	−0.17, 0.25
	Klebsiella spp.	−0.16	0.21	−0.58, 0.25
	Other pathogens	−0.04	0.22	−0.48, 0.40
	CM signs but no growth	0.00	0.11	−0.22, 0.22
	None	Ref.	—	—

[1]The estimates in Tables 3 and 4 derive from the same model.

[2]Values have been rounded to 2 decimal places, so results presented in the text may differ slightly due to rounding.

[3]Reference level of the factor (estimate = 0.00).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

each type of CM (*E. coli* and *Klebsiella* spp. in Figure 1A; *Streptococcus* spp. and *Staph. aureus* in Figure 1B; CNS, "other pathogens," and CM with signs but no growth in Figure 1C) were calculated from the estimates in Tables 3 and 4. Several pathogens had a large adverse effect when occurring immediately before AI (e.g., probability of conception for *Klebsiella* spp. CM occurring 1 to 7 d before AI decreased to only 0.09 $\{[\exp(-0.97 + 60 \times 0.0025 - 0.15 - 0.04 - 1.32)]/[1 + \exp(-0.97 + 60 \times 0.0025 - 0.15 - 0.04 - 1.32)]\}$ for the example cow (Figure 1A). Clinical mastitis occurring shortly after AI was most likely to reduce the probability of conception. For example, if the cow contracted *E. coli* CM within a week after AI, her probability of conception decreased to only 0.06 (Figure 1A). In a few instances, CM was associated with an increased probability of conception (Figure 1C).

Probability of conception also differed among some pathogens (Table 5), as assessed by differences in their least squares means. In general, *E. coli* and *Klebsiella* spp. had larger negative effects on the probability of conception than did the other study pathogens.

DISCUSSION

These results indicate a strong relationship between CM and fertility. Clinical mastitis due to virtually all pathogens was associated with a lower fertility. Not unexpectedly, some significant differences between cases of mastitis due to different pathogens were observed. In the current study, *E. coli* and *Klebsiella* spp., both of which are gram-negative organisms, had greater detrimental effects on probability of conception than did the other study pathogens. Previous studies, including Herath et al. (2009) and Hertl et al. (2010), have documented the adverse effects of gram-negative organisms on reproduction. In the current study, both *E. coli* and *Klebsiella* spp. CM reduced probability of conception

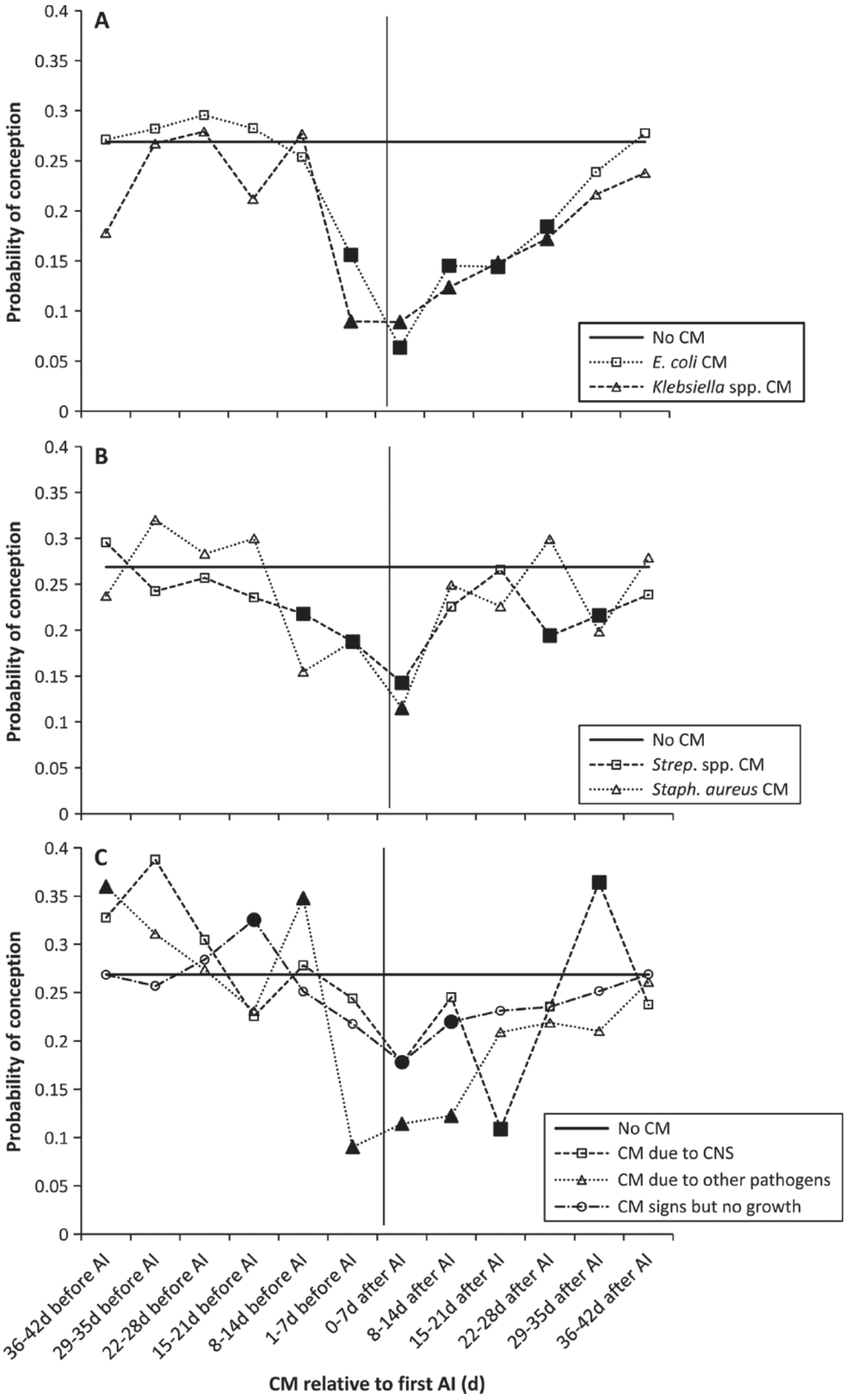

Figure 1. Probability of conception on first AI, for a parity 2 cow bred in autumn, without clinical mastitis (CM) or with CM caused by different organisms or organism groups. Larger, filled symbols indicate probabilities that were significantly different ($P < 0.05$) compared with "No CM" in the interval. The vertical dotted line indicates CM occurring before vs. after AI.

Table 5. Pathogens that differed significantly (at $\alpha = 0.05$) from each other, and their parameter estimates (see Table 4) in terms of their effect on probability of conception, as estimated by least squares means for the model, in each time interval of when clinical mastitis (CM) occurred in relation to an AI[1]

Group/pathogen	Parameter estimate	Group/pathogen	Parameter estimate	Difference	*P*-value
CM occurring 36–42 d before AI					
Escherichia coli	0.0117	Other pathogens	0.4243	−0.4126	0.04
Klebsiella spp.	−0.5294	*Streptococcus* spp.	0.1319	−0.6613	0.04
Klebsiella spp.	−0.5294	CNS	0.2816	−0.8110	0.05
Klebsiella spp.	−0.5294	Other pathogens	0.4243	−0.9537	0.01
No growth[2]	−0.0019	Other pathogens	0.4243	−0.4262	0.04
CM occurring 29–35 d before AI					
Streptococcus spp.	−0.1390	CNS	0.5439	−0.6829	0.04
CM occurring 15–21 d before AI					
Streptococcus spp.	−0.1773	No growth	0.2716	−0.4489	0.01
Klebsiella spp.	−0.3135	No growth	0.2716	−0.5851	0.05
CM occurring 8–14 d before AI					
Streptococcus spp.	−0.2778	Other pathogens	0.3722	−0.6500	0.005
Staph. aureus	−0.6976	Other pathogens	0.3722	−1.0698	0.02
E. coli	−0.0785	Other pathogens	0.3722	−0.4507	0.04
No growth	−0.0922	Other pathogens	0.3722	−0.4644	0.04
CM occurring 1–7 d before AI					
Klebsiella spp.	−1.3174	CNS	−0.1313	−1.1861	0.05
Klebsiella spp.	−1.3174	No growth	−0.2794	−1.0380	0.04
CM occurring 0–7 d after AI					
E. coli	−1.6911	*Streptococcus* spp.	−0.7921	−0.8990	0.002
E. coli	−1.6911	CNS	−0.5351	−1.1560	0.01
E. coli	−1.6911	No growth	−0.5298	−1.1613	<0.0001
Klebsiella spp.	−1.3260	No growth	−0.5298	−0.7962	0.05
CM occurring 8–14 d after AI					
E. coli	−0.7715	*Streptococcus* spp.	−0.2331	−0.5384	0.01
E. coli	−0.7715	*Staph. aureus*	−0.1014	−0.6701	0.02
E. coli	−0.7715	No growth	−0.2659	−0.5056	0.01
Klebsiella spp.	−0.9581	*Streptococcus* spp.	−0.2331	−0.7250	0.03
Klebsiella spp.	−0.9581	*Staph. aureus*	−0.1014	−0.8567	0.04
Klebsiella spp.	−0.9581	No growth	−0.2659	−0.6922	0.04
Other pathogens	−0.9685	*Streptococcus* spp.	−0.2331	−0.7354	0.03
Other pathogens	−0.9685	*Staph. aureus*	−0.1014	−0.8671	0.03
Other pathogens	−0.9685	No growth	−0.2659	−0.7026	0.04
CM occurring 15–21 d after AI					
CNS	−1.1025	*Streptococcus* spp.	−0.0153	−1.0872	0.03
E. coli	−0.7802	*Streptococcus* spp.	−0.0153	−0.7649	<0.0001
E. coli	−0.7802	No growth	−0.2012	−0.5790	0.003
Klebsiella spp.	−0.7486	*Streptococcus* spp.	−0.0153	−0.7333	0.02
CM occurring 22–28 d after AI					
E. coli	−0.4866	*Staph. aureus*	0.1492	−0.6358	0.01
Klebsiella spp.	−0.5712	*Staph. aureus*	0.1492	−0.7204	0.04
Streptococcus spp.	−0.4244	*Staph. aureus*	0.1492	−0.5736	0.03
CM occurring 29–35 d after AI					
Streptococcus spp.	−0.2889	CNS	0.4435	−0.7324	0.004
Staph. aureus	−0.3960	CNS	0.4435	−0.8395	0.01
E. coli	−0.1591	CNS	0.4435	−0.6026	0.01
Klebsiella spp.	−0.2863	CNS	0.4435	−0.7298	0.02
Other pathogens	−0.3239	CNS	0.4435	−0.7674	0.02
No growth	−0.0899	CNS	0.4435	−0.5334	0.03

[1]No significant differences were found among pathogens in the intervals "CM occurring 22–28 d before AI" and "CM occurring 36–42 d after AI."
[2]CM signs but no growth on culture.

when it occurred in the week before AI and at any time up to 4 wk after AI. Similarly, Wilson et al. (2008) found that cows with *E. coli* or *Klebsiella* CM needed more services per conception than did their herdmates without these types of CM.

In this study, *Streptococcus* spp. (*Strep. dysgalactiae*, *Strep. uberis*, and other *Streptococcus* spp.) CM reduced probability of conception both before and after AI (Table 4), with the greatest effect when it occurred 0 to 7 d after AI. This was in close agreement with a study by Wilson et al. (2008). Similarly, Hockett et al. (2005) found that cows experimentally infected with *Strep. uberis* were more likely to exhibit signs of impaired reproductive performance, including lack of

estrous behavior, LH surge, and ovulation, among other signs.

Staphylococcus aureus CM only affected probability of conception when it occurred immediately after AI. In a California herd in which *Staph. aureus* was the most commonly occurring pathogen, cows with CM were slightly less likely than their nonmastitic herdmates to have an estrus interval outside the normal range (18–24 d), although the difference was not statistically significant (Moore et al., 1991). In contrast, in another California herd in the same study, where gram-negative organisms, particularly *E. coli*, were most prevalent, cows with CM were nearly twice as likely to have an estrus interval outside this range. Those authors speculate that subclinical *Staph. aureus* may be protective against more severe coliform infections. Other differences between the herds, including heat detection efficiency and management practices, may also play a role (Moore et al., 1991).

Clinical mastitis due to CNS had no effect on probability of conception except when it occurred 15 to 21 d (reduction of 67%) or 29 to 35 d (increase of 55%) after AI. Coagulase-negative staphylococci are often considered "minor" pathogens, as they generally have few detrimental effects (in contrast to a major pathogen such as *E. coli*; Reyher et al., 2012). Lam et al. (1997) reported that quarters infected with minor pathogens were less likely than uninfected quarters to become infected with major pathogens. Thus, it may not be too surprising that a cow infected with a minor pathogen such as CNS may not have any important consequences for reproduction. Three-quarters of IMI (both clinical and subclinical) reported in heifers in 9 herds in a multi-state study were due to CNS (Borm et al., 2006). These pathogens, however, appeared to have no effect on reproduction, as measured by services per conception or days open, because no differences were observed between heifers treated and not treated with intramammary antibiotics prepartum (Borm et al., 2006).

Clinical mastitis due to other pathogens increased the probability of conception in 3 intervals before AI, but decreased it in the week immediately preceding AI and in the 2 wk immediately after AI. Two possible factors explain the increased conception observed in our study. First, it may be an artifact of statistical testing: at an α-level of 0.05, one would expect 5% of results to be statistically significant, whether they truly are or not. Second, many different pathogens are included in the "other" category, and we do not know which specific pathogen(s) was involved. Also, some infections are mild initially and resolve spontaneously (e.g., those due to *Enterobacter* spp.; Schukken et al., 2012). In our recent study on the effects of pathogen-specific CM on milk yield (Hertl et al., 2014), we found that CNS, unlike the other pathogens studied, were not associated with milk loss. In that study, we speculated that "minor" pathogens (such as CNS) have a protective effect against "major" pathogens (such as *E. coli*), as Lam et al. (1997) reported. A similar effect on reproduction may be occurring (if the other pathogen is a minor one) or if these cows for one or more unknown reasons are more likely to conceive.

Cases of CM with signs but no bacterial growth were common findings in the study herds. These cases, however, did not generally have a large effect on the probability of conception. In a previous study, a higher rate of premature luteolysis and longer follicular phase occurred in Hungarian Holstein cows with mastitis caused by either gram-negative or no detected pathogens compared with cows with gram-positive or no mastitis. Release of endotoxin from gram-negative and "no detected pathogen" (many of which were actually probably gram-negative) cases is likely involved (Huszenicza et al., 2005).

Table 5 presents a comparison of some of the pathogens, where they differed in their effects on probability of conception within the same time interval. The largest difference was between *Klebsiella* spp. and CNS occurring 1 to 7 d before AI, followed by *E. coli* and CNS occurring 0 to 7 d after AI. Similarly, Wilson et al. (2008) found *E. coli* and *Klebsiella* to have adverse effects on reproductive performance but CNS to have no effect. The reason for these differences, as mentioned above, may be that if a cow is already infected with a minor pathogen, such as CNS, a subsequent infection with a major pathogen such as *E. coli* may be prevented (Lam et al., 1997). Thus, only the lesser effects of the minor pathogen will manifest themselves. Without the presence of the minor pathogen, the greater effects of the major pathogen would be evident instead.

The effects of CM likely differ whether it occurs before or after AI, although the end result is the same: a lower probability of a confirmed pregnancy. When CM occurs before AI, the reproductive system may be too impaired to initiate a pregnancy, due to estrus cycle disruptions (Hockett et al., 2005; Williams et al., 2008; Herath et al., 2009). Alternatively, even if the embryo does successfully implant in the uterus, it may be lost before pregnancy is confirmed (Moore et al., 2005). Recently, several reports have been published on the interaction between pathogens and fertility, specifically on the proposed mechanisms of action in the case of LPS sensing by toll-like receptors (Bromfield and Sheldon, 2011; Cronin et al., 2012). It was shown that LPS perturbs oocyte meiotic progression through the toll-like receptor-4 pathway. As shown in our data, the strongest effect of CM before AI on conception was due

to gram-negative organisms, all of which have LPS in their cell walls. This relationship between LPS and meiotic progression is of increasing interest due not only to mastitis but also to presence of gram-negative organisms in the uterus immediately before AI (Bromfield and Sheldon, 2011).

When CM occurs after AI, the embryo may have already implanted in the uterus but be aborted before the pregnancy is confirmed (Risco et al., 1999; Hansen et al., 2004). In these cases, embryo loss may occur due in part to the actions of proinflammatory cytokines (Hansen et al., 2004) or the inflammatory pathway leading to prostaglandin production and subsequent luteotrophic effects of these prostaglandins (Herath et al., 2009). This early embryonic death is very common and frequently acknowledged in published papers (e.g., Bridges et al., 2013).

Although some studies have found milk yield to have an effect on reproduction [higher milk yield was associated with a higher risk of being a repeat breeder (Gustafsson and Emanuelson, 2002) and with decreased probability of conception (Hudson et al., 2012)], others have not found these associations (López-Gatius et al., 2002; Chebel et al., 2004). Contemporaneous milk yield was not modeled in the current study. Milk yield at the time of AI is an intervening variable in the relationship between CM and conception, so it would be difficult to determine how much of the CM effect on probability of conception is due to CM alone and how much is acting through milk yield. Milk production potential as measured through early-lactation milk production before both CM and AI was previously shown not to be related to conception risk (Hertl et al., 2010). Furthermore, the main interest of the current study was the effect of CM, not milk yield, on probability of conception.

The results of this study are generalizable to other herds with similar characteristics (i.e., large, well-managed, high-producing herds). From a biological perspective, the results can also probably be generalized to other herds that differ to some extent, as the pathogens are infecting individual cows within a herd. The findings, however, would not necessarily be as generalizable to farms using either no or a substantially different ovulation synchronization program. Conception is probably less likely to occur naturally (i.e., with no program in place) than when some type of planned breeding program is in place. Furthermore, farms performing pregnancy checks at different time intervals post-AI would exhibit varying conception probabilities. Pregnancy loss is substantial between 30 and 45 d post-AI but much less common after 45 d.

A valuable feature of this study is that the probability of conception for a cow with any combination of characteristics can be calculated, based on the estimates in Tables 3 and 4. Such calculations indicate the extent of the effect of different types of CM, and when they occur in relation to insemination, on probability of conception. They may be helpful, therefore, in determining the optimal allocation of resources (e.g., semen, labor, treatment costs) and management of reproduction. For example, it might be better to delay insemination in cows with certain types of CM. For cows that contract CM shortly after AI (e.g., the cow with *E. coli* in the above example), this is a moot point given that AI has occurred, but the farmer should keep in mind that such cows will very likely not conceive and will need to be reinseminated, and should account for this in management plans. In future, interventions that counteract the effect of pathogen-associated molecular patterns, such as LPS, may become available and would be of specific value to reduce the effect of IMI on subsequent reproductive outcomes.

CONCLUSIONS

This study estimated the effects of CM, due to different pathogens and occurring in different weekly intervals before or after AI, and other factors on the probability of conception in Holstein cows in 5 New York State dairy farms. Cases of CM, whether occurring immediately before or up until 4 wk after AI, had a strong negative effect on the probability of conception. The greatest reductions in probability of conception were observed when CM occurred in the week before AI or at some time in the first 2 wk after AI. *Escherichia coli* and *Klebsiella* spp. were associated with the largest reductions in probability of conception. Clinical mastitis cases due to the other pathogens studied here were also associated with lower probabilities of conception, albeit to a lesser extent. The probability of conception for a cow with any combination of characteristics may be calculated from the estimates provided. Such information may be beneficial to farmers in their attempts to optimize reproduction and management of their dairy cows.

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