Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows

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ABSTRACT

The objectives of this study were to obtain prevalence estimates for subclinical endometritis (SCE), determine cow- and herd-level risk factors, and evaluate the reproductive consequences of SCE. A cross-sectional study was used to determine prevalence and risk factors with cows followed in a prospective study to determine reproductive outcomes. Lactating Holstein cows were sampled between 40 and 60 d in milk using low-volume uterine lavage, and cytology was evaluated to determine SCE status. In total, 779 cows from 38 herds were used in the analysis. The cow-level prevalence of SCE was 25.9%. Within-herd level prevalence ranged from 4.8 to 52.6% (median 26.3%, interquartile range 15.6 to 33.3%). Cow-level risk factors identified were ketosis [odds ratio (OR) 3.83; 95% confidence interval (CI) 1.82–8.07], acute metritis (OR 1.86; 95% CI 1.05–3.30), and the interaction between milk production and parity. Primiparous cows that produced more milk had increased odds of having SCE, whereas multiparous cows that produced more milk had decreased odds of having SCE. Herd-level risk factors identified were housing early postpartum cows on bedded packs (herd-level SCE = 36.1%), which increased herd prevalence of SCE by 16.7% (SE 5.58) compared with early postpartum cows housed in freestalls (herd-level SCE = 19.4%), and straw bedding in the calving pen, which decreased herd prevalence of SCE by 10.7% (SE 3.59) compared with herds that used other bedding material. In this study, primiparous cows with and without SCE had similar reproductive performance; however, multiparous cows with SCE had median days open 44 d longer (159 d; 95% CI 126–186 d) compared with unaffected multiparous cows (115 d; 95% CI 106–132 d).

Key words: subclinical endometritis, risk factor, prevalence, dairy cow

INTRODUCTION

Subclinical endometritis (SCE) is a postpartum uterine disease characterized by uterine inflammation in the absence of clinical signs (Sheldon et al., 2006). Gilbert et al. (2005) found the proportion of cows with uterine inflammation diagnosed by cytology to decrease with time, from 100% at 2 wk postpartum to 89, 58, and 41% at 4, 6, and 8 wk postpartum, respectively. Most studies thus far have concentrated on developing diagnostic methods, establishing disease definitions, and describing consequences (Kasimanickam et al., 2006; Barlund et al., 2008). Risk factors for SCE have not been extensively studied at the cow or herd levels. In 5 herds, the prevalence of SCE varied from 37 to 74% at 40 to 60 DIM (Gilbert et al., 2005). This wide range suggests that herd-level risk factors exist that affect the prevalence of this disease. Herd-level risk factors for SCE have not been studied, possibly because of the obstacles of obtaining large numbers of samples for endometrial cytology and the difficulty in obtaining information from enough herds to reach valid conclusions and have precise estimates. Subclinical endometritis impairs reproductive performance by reducing pregnancy to first insemination and increasing median days open (Kasimanickam et al., 2004; Gilbert et al., 2005; Galvão et al., 2009). The economic losses could be substantial in herds with high prevalence of SCE. Identifying risk factors for SCE may allow for dairy management interventions to aid in controlling this costly disease. Our objectives were to 1) provide cow and within-herd level estimates of SCE prevalence, 2) determine cow- and herd-level risk factors for SCE, and 3) evaluate the effects of SCE on reproductive performance.

MATERIALS AND METHODS

Study Population and Sampling

The animal procedures performed for this study were approved by the Cornell University Institutional Animal Care and Use Committee. For identification of risk factors and prevalence, a cross-sectional study

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design was employed and a prospective cohort study was used to determine the effects of SCE on reproductive performance with the experimental unit cows clustered within herds. A convenience sample of 39 commercial dairy herds in New York State with freestall housing and TMR feeding was used based on the farmers' willingness to participate. Inclusion criteria for herds were >400 milking cows and use of DairyComp 305 record-keeping software (Valley Agricultural Software, Tulare, CA). The minimum herd size was used to ensure that adequate eligible cows (approximately 20 cows at 40 to 60 DIM) would be available at the time of sampling. Cows within these herds were eligible to be enrolled if they were between 40 and 60 DIM and before the end of the voluntary waiting period of the farm. Cows were excluded if they 1) were within 2 d of the end of the voluntary waiting period or 2) appeared sick or had abnormal vaginal discharge. Approximately 20 cows were sampled from each herd so that the within-herd prevalence could be estimated with 90% confidence (Win EpiScope 2.0; Thrusfield et al., 2001) assuming a prevalence of 25% based on experience and data from these herds and approximately 30 animals at risk in our sampling window. To represent normal herd demographics, a conscious effort was made to collect approximately one-third of the samples from first-lactation heifers and two-thirds from multiparous cows. Herdsmen were not informed of which cows were sampled or their SCE status until the end of the follow-up period, which was 6 mo after sampling. To limit differences in inter-herd data collection, all farms received a standardized consent form, survey, and case definitions for diseases of interest. Herd information was obtained through a survey completed by the herdsman or owner at the time of sampling. Herd-level risk factors evaluated were herd size, housing of dry cows, housing of cows within the first 30 DIM, calving pen bedding material, number of pen moves during the dry period, number of pen moves during the first 60 DIM, and average projected 305-d mature-equivalent milk yield. Cow-level risk factors were obtained from the dairy herd records collected on the day of sampling. Risk factor categories evaluated were calving related (calving ease scores, calf sex, twins, and stillbirths), postpartum disease (retained placenta, milk fever, acute metritis, ketosis, displaced abomasum), and milking related (first test-day SCC, 305-d mature-equivalent milk yield). At the time of sampling, cows were body condition scored (Ferguson et al., 1994). Data quality was evaluated for each herd. Herds with few recorded disease occurrences were excluded from the cow-level risk factor analysis. Risk factors with data on <50% of sampled animals were excluded from all analysis. Reproductive information (inseminations, pregnancy diagnosis) and censor-

ing (culling and management decision to not breed) were collected at 4 and 6 mo after sampling.

Presence of SCE was determined by cytological evaluation of low-volume uterine lavage as described previously (Gilbert et al., 2005). Briefly, the perineum of the cow was cleansed and a 25-inch Flex Tip sterile plastic infusion pipette (Exodus Breeders Corp., York, PA) was manipulated through the cervix into the uterus. Sterile saline solution (20 mL) was injected into the uterus and agitated gently per rectum; then a sample of the fluid was aspirated. The recovered fluid was centrifuged using a cytocentrifuge directly onto a glass slide. After drying, the slides were fixed and stained using a rapid Romanowsky-type staining procedure and examined under 400 $\times$ magnification. Cells were identified as PMNL (mostly neutrophils), large mononuclear leukocytes (macrophages), small mononuclear leukocytes (lymphocytes), and uterine epithelial cells. Two hundred cells were counted from each slide, and results expressed as a percentage of total cells (excluding erythrocytes). All the slides were read by the same investigator (SHC). Cows were designated as positive for SCE if >10% PMNL were identified, based on the recommendations of Sheldon et al. (2006).

Data Management and Statistical Analysis

Prevalence. Cow-level prevalence of SCE, as well as within-herd prevalence, was reported as the percentage of cows with >10% PMNL in uterine cytology. Within-herd prevalence was used as the dependent variable for herd-level risk factor analysis.

Cow-Level Risk Factors for SCE. The response variable for the cow-level risk factors was binomial, with cows classified as having SCE or being normal. Because the sampling of study unit was clustered within herds, herd was included as a random effect in all cow-level analyses. Analysis of putative risk factors was performed using PROC GLIMMIX of SAS, version 9.2 (SAS Institute Inc., Cary, NC). Risk factors examined were signalment, which included parity (primiparous or multiparous), days dry, DIM at sampling, and BCS (≥ 3.5 or < 3.5); calving-related risk factors, which included calving ease score (≥ 3 or < 3), twins (twins or singletons), and stillbirths (stillborn or alive); postpartum diseases, which included acute metritis, retained placenta, milk fever, ketosis, and displaced abomasum (diseased or nondiseased); and milking-related risk factors, which included log first-test-day SCC (**SCC1**) and 305-d mature equivalent milk yield at 30 DIM (**ME30**). Despite circulation of standard disease definitions to herdsmen, disease definition was heterogeneous between herds and for this study was defined as disease occurrence as recorded by farm personnel. Continuous

variables were examined for collinearity, outliers, and distribution, and SCC1 was log-transformed to fulfill the normal distribution assumption. Risk factors and interactions with P -values <0.20 were offered to the final model. The final multivariable model was built using manual backward stepwise variable selection with variables retained if $P < 0.05$.

Herd-Level Risk Factors. For the herd-level risk factor model, the response variable was the proportion of cows with SCE within each herd. Analysis was conducted using PROC GLM of SAS, version 9.2. Herd-level risk factors examined were herd size, average projected 305-d mature-equivalent milk yield, number of moves during the dry period (1 or 2 moves), number of pen moves during the first 60 DIM (1, 2, or 3 moves), bedding material (straw, sand, sawdust, paper, or combination of any of these) in the calving pen, type of housing for early postpartum cows (freestall or bedded pack), and interactions. Herd size was log-transformed. Calving pen bedding material comprised polytomous nominal categories and their combinations. For the risk factors with $P < 0.20$, new categories were created as either using that particular bedding material or not. The final model was built using manual backward stepwise variable selection with variables retained if $P < 0.05$.

Calving to Conception Interval. Kaplan-Meier survival analysis (Kaplan and Meier, 1958) was performed to produce survival curves of SCE effects on calving to conception interval alone and after stratification by potential confounders using PROC LIFETEST of SAS, version 9.2. Effect of SCE on calving to conception interval was also evaluated with a multivariable frailty model using STATA version 10 (Stata Corp., College Station, TX) to account for within-herd clustering. Potential confounders evaluated were parity (primiparous or multiparous), BCS (≥ 3.5 or <3.5), acute metritis, retained placenta, milk fever, displaced abomasum, ketosis, twins, stillbirth, calving ease score (≥ 3 or <3), log(SCC1), ME30, DIM at first insemination, and interactions. Covariates were evaluated with SCE forced into the model. Cows were censored when culled or as soon as designated do-not-breed by the herdsman. In addition, cows were censored if not pregnant by 210 DIM. The final model was built using manual backward stepwise variable selection with variables retained if $P < 0.05$.

Pregnancy to First Insemination. The effect of SCE on pregnancy to first insemination was examined using PROC GLIMMIX of SAS, version 9.2. Covariates were examined individually in a model with "herd" included as a random variable and SCE forced in as a fixed effect. Covariates evaluated were parity, BCS, acute metritis, retained placenta, milk fever, displaced

abomasum, ketosis, log(SCC1), ME30, calf sex, stillbirth, twins, DIM at first insemination, and interactions. To build the final model, variables with $P < 0.20$ were offered and retained if $P < 0.05$ using manual backward stepwise variable selection.

Evaluation of SCE Definition. Receiver operator characteristic curve analysis was performed using JMP8 (SAS Institute Inc.) with pregnancy by 210 DIM as the classifier and percentage PMNL in uterine cytology as the predictor. Optimal cutoff was evaluated overall and by parity as the value giving the greatest sum of sensitivity and specificity.

RESULTS

Descriptive Statistics

In total, 812 cows from 39 herds were sampled. One herd was excluded from all analysis as they had recently switched to DairyComp305 for herd recordkeeping, and the quality of data recording was generally poor. In addition, 15 cows were excluded from all analysis for poor quality cytology slides (either too few cells to evaluate or the morphology of the cells was unsatisfactory). Thus, 779 cows from 38 herds had satisfactory cytology and follow-up data. Average DIM at sampling was 46 (SD 6.6); 295 (37.9%) cows sampled were primiparous and 484 were multiparous.

Herd size ranged from 500 to 3,000 milking cows with a median of 880. Examination of herd records revealed unsatisfactory disease records in 4 herds, and cows from those herds had all disease recorded as missing values and were excluded from all cow-level risk factor analyses. The recorded incidences of diseases in the sampled cows were ketosis 5.0%, acute metritis 10.7%, retained placenta 6.6%, displaced abomasum 3.1%, and milk fever 0.1%. Calving-related risk factors analyzed were twinning (3%), calf sex (49.7% heifers), and stillbirths (6.0%). Milk production information (ME30) was available for all cows and the mean was 10,873 kg (23,921 lbs) (SD = 67.4 kg; 148.2 lbs). First test-day SCC was not tested in all herds; 212 cows had missing observations. The mean DIM at first insemination was later for primiparous cows (69.8 DIM) than for multiparous cows (68 DIM; $P = 0.05$) but was not different by SCE status (68.7 DIM for SCE and 68.7 for unaffected; $P = 0.95$). Calving pen bedding varied with 4 basic bedding materials and 4 combinations. During the follow-up period, 32 cows were designated "do not breed" and were censored at the day of designation, and 74 cows were culled, of which 9 cows were previously classified as "do not breed." In addition, 142 cows were not pregnant by 210 DIM and were censored.

Figure 1. Proportion of cows affected with subclinical endometritis (SCE) by herd arranged in order of sampling. *Herds with SCE prevalence different from the mean, 25.9% ($P < 0.05$).

Prevalence

The overall prevalence of SCE was 202 cows affected (25.9% exact binomial, 95% CI 22.3–28.4%) and 577 unaffected. Within-herd prevalence ranged from 4.8 to 52.6% (median 26.3%, interquartile range 15.6 to 33.3%). The distribution of within-herd prevalence is summarized in Figure 1. The prevalence of SCE in primiparous cows (20.1%) was similar ($P = 0.67$) to that in multiparous cows (26.4%).

Cow-Level Risk Factors

Individual risk factor analysis results are summarized in Table 1. Individual risk factors and interactions with $P < 0.20$ examined in the multivariable analysis were DIM at sampling, acute metritis, retained placenta,

ketosis, calf sex, stillbirth, twins, ME30, and the 2 interactions (ME30 $\times$ parity and acute metritis $\times$ parity). Parity was included to fulfill the hierarchical rule. Manual backward stepwise variable selection resulted in the final model, which included acute metritis, ketosis, and the interaction between ME30 and parity. Parity and ME30 were included to fulfill the hierarchical rule.

To further examine the interaction with parity, the risk factor analysis was repeated stratifying by parity; the results are summarized in Table 2. Primiparous cows that produced more milk were at increased odds of having SCE, and multiparous cows that produced more milk were at decreased odds of having SCE. In the stratified analysis, metritis was not significant as a risk factor for primiparous cows but primiparous cows with ketosis tended to be at greater odds of having SCE.

Table 1. Cow-level risk factor estimates for subclinical endometritis (SCE) with herd included as a random effect

Cow-level risk factor ¹	Individual risk factor			Final model		
	Odds ratio (estimate)	95% CI (SE)	P-value	Odds ratio (estimate)	95% CI (SE)	P-value
DIM at sampling ^{2,3}	(0.019)	(0.14)	0.16	—	—	—
Days dry ³	(−0.0043)	(0.0074)	0.56	—	—	—
Parity (multiparous)	1.10	0.78–1.54	0.60	(4.90)	(1.27)	<0.001
BCS (≥ 3.5)	0.98	0.68–1.40	0.89	—	—	—
Acute metritis ²	2.15	1.29–3.60	0.003	1.86	1.05–3.30	0.03
Retained placenta	1.56	0.82–2.97	0.18	—	—	—
Displaced abomasum	1.39	0.55–3.52	0.48	—	—	—
Milk fever	1.80	0.29–11.19	0.53	—	—	—
Ketosis ²	4.16	2.05–8.48	<0.001	3.83	1.82–8.07	<0.001
Log(SCC1) ³	(−0.024)	(0.056)	0.67	—	—	—
ME30 ^{2,3}	(0.000049)	(0.000022)	0.03	(−0.0008)	(0.00044)	0.06
Calf sex(bull) ²	0.80	0.58–1.11	0.18	—	—	—
Stillbirth ²	1.82	0.98–3.38	0.06	—	—	—
Twins ²	2.73	1.20–6.18	0.02	—	—	—
ME30 $\times$ parity ^{2,3}	(0.00016)	(0.000049)	<0.001	(0.00019)	(0.000052)	<0.001
Parity $\times$ acute metritis ³	(0.33)	(0.85)	0.08	—	—	—

¹Log(SCC1) = log-transformed first test-day SCC; ME30 = 305-d mature-equivalent milk yield at 30 DIM.

²Risk factors with $P < 0.20$ were offered to build the final model.

³Continuous variables reported as regression coefficient and standard deviation.

Table 2. Cow-level risk factor estimates for subclinical endometritis stratified by parity with herd included as a random effect

Risk factor	Primiparous ¹ (n = 231)			Multiparous (n = 424)		
	Odds ratio (coefficient)	95% CI (SE)	P-value	Odds ratio (coefficient)	95% CI (SE)	P-value
Ketosis	2.87	0.85–9.76	0.09	5.62	2.12–14.90	<0.001
Metritis	1.14	0.47–2.78	0.78	2.60	1.19–5.65	0.02
ME30 ^{2,3}	(0.0008)	(0.000045)	0.09	(–0.00011)	(0.000029)	<0.001

¹No variables were retained after model building for primiparous cows.

²ME30 = 305-d mature-equivalent milk yield at 30 DIM.

³Continuous variables reported as regression coefficient and standard error.

Milk yield tended to be significant in primiparous cows, but cows with higher production in this group were at increased risk of SCE compared with multiparous cows. In multiparous cows, ketosis and acute metritis were significant risk factors for SCE, and multiparous cows that produced less milk were at increased risk of SCE.

Herd-Level Risk Factors

Herd-level risk factors were examined individually and the univariable analysis is summarized in Table 3. Calving pen bedding material was identified as a risk factor in the univariable analysis ($P = 0.19$) and was split to 4 dichotomous categories, namely using or not using straw, sand, sawdust, and paper. No significant interactions were found between the main risk factors. Variables offered for building the multivariable model were early postpartum housing, straw bedding in calving pens, and paper bedding in calving pens. In the final model, only early postpartum housing and straw bedding in calving pens was retained. Herds housing early postpartum cows in freestall barns had 16.71% lower herd SCE prevalence (SE = 5.58; $P = 0.005$) compared with herds that housed early postpartum cows in bedded packs. Herds that used straw for calving pen bedding had 10.71% lower herd SCE (SE =

3.59; $P = 0.005$) compared with herds that did not use straw bedding.

Calving to Conception Interval

Kaplan-Meier survival curves showed that cows with SCE had median days open 26 d longer (142 d, 95% CI 119–159) than that of normal cows (116 d, 95% CI 110–124; $P = 0.02$). Kaplan-Meier survival curves for calving to conception interval were repeated stratifying by SCE status and parity and are shown in Figure 2. No difference in median days open was observed between primiparous cows with different SCE statuses, but multiparous cows with SCE had 159 d (95% CI 126–186 d) median days open, which was 44 d longer than in multiparous cows without SCE (115 d, 95% CI 106–132 d; $P = 0.006$). A similar interaction was found between parity and BCS on calving to conception interval. Multiparous cows with BCS <3.5 had 41 d longer median days open (148 d) compared with multiparous cows with BCS ≥ 3.5 (107 d). Body condition score did not affect median days open in primiparous cows ($\geq 3.5 = 118$ d, <3.5 = 119 d).

To account for clustering of cows within herds, a frailty model was used to screen covariates for the effect of SCE on calving to conception interval. The inter-

Table 3. Generalized linear model estimates of herd-level risk factors for subclinical endometritis

Herd-level risk factor	Individual risk factor			Final model		
	Estimate	SE	P-value	Estimate	SE	P-value
Log(herd size)	0.67	4.92	0.89	—	—	—
Average 305-d mature-equivalent milk yield	0.00067	0.0014	0.63	—	—	—
Early postpartum housing (freestall) ¹	–14.49	6.06	0.02	–16.71	5.58	0.005
Pen moves, dry (1)	–3.56	4.62	0.42	—	—	—
Pen moves, <60 DIM			0.68	—	—	—
(1 vs. 3)	2.55	9.50	0.79	—	—	—
(2 vs. 3)	–1.46	9.71	0.88	—	—	—
Calving pen bedding ¹			0.19	—	—	—
(straw)	–8.87	3.90	0.03	–10.71	3.59	0.005
(sand)	–4.71	6.52	0.47	—	—	—
(sawdust)	1.85	5.21	0.73	—	—	—
(paper) ¹	6.30	4.84	0.19	—	—	—

¹Risk factors with $P < 0.20$ were offered to build the final model.

Table 4. Final frailty model combined and stratified by parity for the hazard of pregnancy with subclinical endometritis (SCE) forced into the model and herd included as a random effect

Variable	Combined			Primiparous			Multiparous		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
SCE	1.16	0.77–1.76	0.47	1.18	0.76–1.83	0.45	0.68	0.49–0.94	0.021
BCS (≥ 3.5)	1.29	1.03–1.63	0.03	0.92	0.62–1.35	0.66	1.29	1.03–1.63	0.029
Log(SCC1) ¹	0.74	0.61–0.91	0.004	0.71	0.50–1.01	0.053	0.79	0.61–1.01	0.065
DIM at first service	0.99	0.98–1.00	0.01	0.99	0.9–1.00	0.13	0.98	0.97–1.00	0.018
Parity ²	1.11	0.86–1.45	0.42	—	—	—	—	—	—
Parity $\times$ SCE ²	0.58	0.34–0.99	0.05	—	—	—	—	—	—

¹Log-transformed first test-day SCC.

²Parity and the interaction between parity and SCE were dropped in the stratified model due to collinearity.

Figure 2. Kaplan-Meier survival analysis for cows with subclinical endometritis (SCE; thick line) compared with normal (thin line) cows: A) SCE did not increase calving to conception interval in primiparous cows ($P = 0.94$); B) multiparous cows with SCE had longer calving to conception interval ($P = 0.005$).

action between parity and BCS was collinear to the interaction between parity and SCE as described by the Kaplan-Meier survival analysis, and parity $\times$ SCE was used to build the final model. The interaction between parity and DIM at first service was not significant ($P = 0.23$) and was dropped from the final model. The final model included parity, BCS, log(SCC1), DIM at first service, and the interaction between parity and SCE. Estimates for the final model and the model stratified by parity are summarized in Table 4.

Pregnancy to First Insemination

A summary of individual covariates in a model with herd included as a random effect and SCE forced in as a fixed effect is shown in Table 5. No significant interactions were found and only log(SCC1) and BCS were evaluated to build the final model, in which only BCS was retained. Cows with SCE had lower odds (odds ratio = 0.61, 95% CI 0.40–0.93; $P = 0.02$) for pregnancy at first insemination compared with cows without SCE. Cows with BCS ≥ 3.5 had higher odds of pregnancy to first insemination (odds ratio = 1.78, 95% CI 1.24–2.56; $P = 0.002$) compared with cows with BCS < 3.5 .

PMNL Cutoff for SCE Definition

The overall sensitivity and specificity for the 10% PMNL cutoff for predicting pregnancy by 210 DIM were 0.79 and 0.43, respectively. The cutoff with the highest combined sensitivity and specificity was 9.5% PMNL, which is very close to the published recommendation of 10% PMNL. When stratified by parity, PMNL was not very predictive of pregnancy by 210 DIM ($P = 0.66$) for primiparous cows, with the optimal cutoff 30% PMNL (sensitivity = 0.93, specificity = 0.13). In multiparous cows, however, the optimal cutoff point was 9.5% PMNL (sensitivity = 0.80, specificity = 0.36) and was predictive of pregnancy by 210 DIM ($P = 0.005$).

Table 5. Coefficients for subclinical endometritis (SCE) effect on pregnancy to first insemination, with herd included as a random effect

Variable ¹	Individual factors			Final model		
	Odds ratio (coefficient)	95% CI (SE)	<i>P</i> -value	Odds ratio	95% CI	<i>P</i> -value
SCE ²	0.52	0.34–0.81	0.004	0.61	0.40–0.93	0.02
Parity (primiparous)	1.18	0.85–1.64	0.32	—	—	—
BCS (≥ 3.5) ²	1.78	1.24–2.56	0.002	1.78	1.24–2.56	0.002
Acute metritis	0.83	0.47–1.49	0.54	—	—	—
Retained placenta	0.78	0.38–1.60	0.50	—	—	—
Milk fever	1.83	0.29–11.45	0.52	—	—	—
Displaced abomasum	0.76	0.267–2.11	0.59	—	—	—
Ketosis	0.58	0.23–1.46	0.25	—	—	—
Log(SCC1) ^{2,3}	(0.11)	(0.05)	0.03	—	—	—
ME30 ³	(0.00003)	(0.000021)	0.23	—	—	—
Calf sex (bull)	1.189	0.86–1.64	0.29	—	—	—
Stillborn	1.059	0.54–2.07	0.87	—	—	—
Twins	0.983	0.40–2.42	0.97	—	—	—
DIM at first service ³	(0.0054)	(0.0073)	0.46	—	—	—

¹Log(SCC1) = log-transformed first test-day SCC; ME30 = 305-d mature-equivalent milk yield at 30 DIM.

²Risk factors with $P < 0.20$ were offered to build the final model.

³Continuous variables reported as regression coefficient and standard error.

DISCUSSION

The 779 cows from 38 herds sampled in this study make this the largest observational investigation of SCE in dairy cows to date. Herd-level risk factors were suspected to be substantial based on previous work by Gilbert et al. (2005), which showed large variability in the proportion of cows with SCE between herds, and part of our aim was to identify herd-level risk factors. This required a large number of herds, which, in turn, needed to be accounted for in the analysis.

The most significant cow-level risk factors for SCE were ketosis and milk production interaction with parity. Clinical ketosis is an indicator of severe negative energy balance (Duffield et al., 2009). Negative energy balance has been implicated in reduced reproductive performance primarily through impairment of the gonadotropin and somatotropin axes (Chagas et al., 2007) and more recently in uterine immune function (Hammon et al., 2006; Wathes et al., 2009). Ospina et al. (2010) described a profound effect of even subclinical ketosis on reproductive success. Subclinical endometritis is likely the result of impaired uterine immune function due to negative energy balance and a mechanism of energy balance affecting reproduction. The interaction between milk production and parity was strongly associated with the risk of SCE. We are not able to explain why primiparous and multiparous cows behaved differently with regard to milk yield and disease, but our current results, in which primiparous cows that produced more milk were at higher risk of SCE and multiparous cows that produced more milk had lower risk of SCE, reflect those of Galvão et al. (2010).

The only other significant risk of SCE was acute metritis. The odds ratio of 1.86 found in our study is slightly lower than the >2-fold increased risk reported by Rutigliano et al. (2008). Other postpartum diseases have been reported to increase the risk of developing SCE. Kasimanickam et al. (2004) reported retained placenta and assisted calving as important risk factors for SCE, and Rutigliano et al. (2008) found a tendency for cows with retained placenta to develop SCE but retained placenta was not significant in this study. Milk fever has previously been reported to be a risk factor for clinical endometritis (Whiteford and Sheldon, 2005). However, we did not find a correlation between clinical milk fever and SCE. This discrepancy could be due to unrecorded prophylactic calcium administration by herd managers, or failing to record clinical disease as discussed earlier. No diagnostic tests were performed; therefore, milk fever, and particularly subclinical hypocalcemia, cannot be ruled out as an important risk factor. The prevalence of SCE has been reported to be similar in primiparous and multiparous cows (Gilbert et al., 2005; Rutigliano et al., 2008; Galvão et al., 2009), and this finding was repeated in the current study. Days postpartum was not significant in our study perhaps because of the relatively small variation in the timing of sampling, with all samples being obtained between 40 and 60 DIM.

Herd-level risk factors have not been described previously. The prepartum period appears to be a critical period for future risk of metritis. Huzzey et al. (2007) found that DMI is reduced in cows 2 wk before metritis; aggressive behavior among cows in the dry period is also reported as a risk factor. Changing groups may

lead to aggressive behavior and decreased feed intake, which lead to the inclusion of pen moves in the dry and early postpartum period as a potential risk factor. However, the number of moves may not provide a reflection of the stress suffered by cows because several other management factors involved in the manner of making pen moves play a major role. Because the number of pen moves is decided on a herd basis, it was included as a potential herd-level risk factor. Based on our data, pen moves did not affect herd prevalence of SCE. Bedding material in the calving pens and early postpartum housing was the only significant risk factor at the herd level. Only 4 herds housed early postpartum cows on bedded packs and those herds had higher herd prevalence of SCE. Because of the small numbers, interpretations should be made with caution. Straw was used as bedding in calving pens in 21 of 38 herds, and those herds had lower herd prevalence for SCE. Although further studies need to be done to determine if changing to straw bedding and freestall housing for early postpartum cows will result in lowering of herd SCE prevalence, these represent potential interventions that were not previously identified.

Subclinical endometritis caused reproductive impairment with a longer calving to conception interval. Perhaps the most surprising finding was the difference between the effects of SCE on hazard of pregnancy between primiparous and multiparous cows, in which primiparous cows were not affected and all the observed difference was in multiparous cows. This finding provides a potential for targeted treatments and prophylaxis. In addition, thin multiparous cows were affected more severely by the negative reproductive effects of SCE (increase of 45 median days open) even though multiparous cows with good body condition were also affected (increase of 25 median days open). This may be a further manifestation of the effects of negative energy balance on uterine health. A large number of cows had missing values for log(SCC1) and BCS, resulting in the low number of observations in the final frailty model. A reduced frailty model was repeated containing only parity, SCE, and parity $\times$ SCE using all observations, and found the interaction parity $\times$ SCE to have a hazard ratio for pregnancy of 0.68 (95% CI 0.45–1.02; $P = 0.059$). Log(SCC1) was identified as the most significant covariate that increased calving to conception interval ($P = 0.002$). High SCC has been reported to decrease reproductive performance (Pinedo et al., 2009).

First-service conception rate was found to be significantly lower for cows with SCE, in agreement with previous reports (Gilbert et al., 2005; Barlund et al., 2008). The effects of SCE were also evaluated for subsequent inseminations, and the associations were similar

but smaller than for the first insemination (data not shown). Surprisingly, parity was not significant in this model even though the first-service conception rate difference for primiparous cows with SCE (28.4%) and unaffected primiparous cows (30.3%) was smaller, numerically, than the difference between multiparous cows with SCE (18.0%) and unaffected multiparous cows (29.5%). First test-day SCC was not retained in the final model for first-service conception, even though it had a greater effect than SCE on calving to conception interval. First test-day SCC and its interactions were not risk factors for SCE. These results suggest that the causes of inflammation in the udder and uterus are different and, although both affect reproductive performance, SCE appears to have greater effects on the first insemination.

The possibility of information bias arose from the nonuniformity of risk factor diagnosis and recording. Milk production and reproductive information are usually reliable and uniform across different herds, but disease occurrence and calving-related information are less reliable and occasionally not recorded at all. We attempted to reduce this bias first by examining the herd records for unusual patterns of each risk factor occurrence in each herd. Four herds had unusually low disease occurrence and were excluded for all cow-level risk factor analyses. The exclusion of these 4 herds did not change the odds ratio estimates or the final cow-level risk factor model although the P -values were decreased slightly. Several herds did not have good records of certain disease occurrence, particularly milk fever and, to a lesser extent, retained placenta. The poor recordkeeping for milk fever and retained placenta may have contributed to the inability to detect any association between these diseases and our outcomes of interest. In addition, some calving-related risk factors, particularly calving ease score, were poorly recorded and were excluded. In addition to screening the data, inclusion of herd as a random variable helps account for some herd variability.

Diagnosis of SCE depends on the proportion of PMNL in uterine cytology, estimation of which is repeatable between evaluators with kappa values from 0.76 to 0.85 (Gilbert et al., 2005; Barlund et al., 2008). To prevent further variability, all the slides were read by the same investigator and most samples (>90%) were collected by the same investigator. The herds sampled were all located within New York State but the average travel time was about 3 h from the herd to the college, with the furthest herd located over 6 h away. Some samples were unacceptably degraded when they were not processed promptly, usually when 2 herds were sampled on the same day. The presence of mucus in some of the samples resulted in poor adhesion of cells to the

slides, resulting in some slides not having sufficient cells to make a definitive diagnosis. Only one slide was produced per sample and making duplicates could have rescued some samples. However, only 15 samples were lost in this manner, which is <2% of the samples collected. Kasimanickam et al. (2004) reported difficulty in retrieving fluid from the low-volume uterine lavage but that was not experienced in this study. The optimal cutoff point for PMNL to define SCE using receiver operator characteristic curve analysis with pregnancy at 210 DIM agreed with the recommendations of Sheldon et al. (2006).

CONCLUSIONS

Cow-level risk factors identified for SCE were ketosis, acute metritis, and interaction between parity and milk production, in which primiparous cows with higher milk production were at higher risk and multiparous cows with higher production were at lower risk for SCE. Herd-level risk factors were early postpartum housing in bedded packs and calving pen bedding not using straw, although only 4 herds used bedded pack and thus this finding should be interpreted carefully. In our study, multiparous cows with SCE had increased calving to conception interval but primiparous cows with SCE were not observed to have the same impairment.

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REFERENCES

Barlund, C. S., T. D. Carruthers, C. L. Waldner, and C. W. Palmer. 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* 69:714–723.

- Chagas, L. M., J. J. Bass, D. Blache, C. R. Burke, J. K. Kay, D. R. Lindsay, M. C. Lucy, G. B. Martin, S. Meier, F. M. Rhodes, J. R. Roche, W. W. Thatcher, and R. Webb. 2007. New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. *J. Dairy Sci.* 90:4022–4032.
- Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.* 92:571–580.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal description of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Galvão, K. N., M. B. J. F. Flaminio, S. B. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C. L. Guard, W. R. Butler, and R. O. Gilbert. 2010. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. *J. Dairy Sci.* 93:2926–2937.
- Galvão, K. N., M. Frajblat, S. B. Brittin, W. R. Butler, C. L. Guard, and R. O. Gilbert. 2009. Effect of prostaglandin F2alpha on subclinical endometritis and fertility in dairy cows. *J. Dairy Sci.* 92:4906–4913.
- Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance in dairy cows. *Theriogenology* 64:1879–1888.
- Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunopathol.* 113:21–29.
- Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. *J. Dairy Sci.* 90:3220–3233.
- Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53:457–481.
- Kasimanickam, R., J. M. Cornwell, and R. L. Nebel. 2006. Effects of presence of clinical and subclinical endometritis at the initiation of the presynch ovsynch program on first service conception in dairy cows. *Anim. Reprod. Sci.* 95:214–223.
- Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* 62:9–23.
- Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Associations of elevated nonesterified fatty acids and beta hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *J. Dairy Sci.* 93:1596–1603.
- Pinedo, P. J., P. Melendez, J. A. Villagomez-Cortes, and C. A. Risco. 2009. Effect of high somatic cell counts on reproductive performance of Chilean dairy cattle. *J. Dairy Sci.* 92:1575–1580.
- Rutigliano, H. M., F. S. Lima, R. L. A. Cerri, L. F. Greco, J. M. Vilela, V. Magalhães, F. T. Silvestre, W. W. Thatcher, and J. E. P. Santos. 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. *J. Dairy Sci.* 91:3323–3336.
- Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining post-partum uterine disease in cattle. *Theriogenology* 65:1516–1530.
- Thrusfield, M., C. Ortega, I. de Blas, J. P. Noordhuizen, and K. Frankena. 2001. Win EPISCOPE 2.0: Improved epidemiological software for veterinary medicine. *Vet. Rec.* 148:567–572.
- Wathes, D. C., Z. Cheng, W. Chowdhury, M. A. Fenwick, R. Fitzpatrick, D. G. Morris, J. Patton, and J. J. Murphy. 2009. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. *Physiol. Genomics* 39:1–13.
- Whiteford, L. C., and I. M. Sheldon. 2005. Association between clinical hypocalcemia and postpartum endometritis. *Vet. Rec.* 157:202–203.