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Epidemiology of subclinical ketosis in early lactation dairy cattle

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ABSTRACT

The purpose of this study was to describe the epidemiology of subclinical ketosis (SCK) in dairy cows in early lactation and determine the association of (1) days in milk (DIM) at onset of SCK, and (2) blood β-hydroxybutyrate (BHBA) concentration at onset of SCK with development of displaced abomasum (DA) and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and early lactation milk yield. Cows from 4 freestall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 DIM using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L. Mixed-effects multivariable Poisson regression was used to assess DA, removal from herd, and conception to first service. Semiparametric proportional hazards models were used to evaluate days to conception, and repeated-measures ANOVA was used to evaluate milk yield in the first 30 DIM. A total of 741 of 1,717 (43.2%) eligible cows had a least one BHBA test of 1.2 to 2.9 mmol/L. Peak incidence of SCK occurred at 5 DIM, when 22.3% of cows had their first SCK-positive test. Peak prevalence of SCK occurred at 5 DIM, when 28.9% of cows had a SCK-positive test. Median time from first positive SCK test until BHBA test <1.2 mmol/L was 5 d. Cows first testing SCK positive from 3 to 5 DIM were 6.1 times more likely [95% confidence interval (CI) = 2.3 to 16.0] to develop a DA than cows first testing SCK positive at 6 DIM or later. Cows first testing SCK positive from 3 to 7 DIM were 4.5 times more likely (95% CI = 1.7 to 11.7) to be removed from the herd, were 0.7 times as likely (95% CI = 0.6 to 0.8) to conceive to first service, and produced 2.2 kg less milk per day for the first 30 DIM than cows first testing positive at 8 DIM or later. Each 0.1 mmol/L increase in BHBA at first SCK-positive test increased the risk of developing a DA by a factor of 1.1 (95% CI = 1.0 to 1.2), increased the risk of removal from herd by a factor of 1.4 (95% CI = 1.1 to 1.8), and was associated with a decrease in milk production by 0.5 kg/d for the first 30 DIM. These results show that time of onset and BHBA concentration of first SCK-positive test are important indicators of individual cow performance.
Key words: dairy cow, ketosis, incidence, prevalence

INTRODUCTION

The ability of dairy cattle to adapt to the natural change of energy balance in early lactation is an important aspect of the transition period, as the demands for milk production cannot be met by feed intake alone (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). Cattle unable to adequately transition through this period are at a higher risk for metabolic disorders and decreased milk production (Cameron et al., 1998; Drackley, 1999; Herdt, 2000). One of these metabolic disorders, hyperketonemia, develops as a sequela to a poor adaptive response to negative energy balance and occurs when the liver is overwhelmed with NEFA. Hyperketonemia can manifest clinically as a decrease in appetite, weight loss, and a decrease in milk production, but cows are more likely to suffer from subclinical ketosis (**SCK**), defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988). Early lactational incidence of SCK was found to affect 40 to 60% of cows in herds undergoing repeated testing (Emery et al., 1964; Simensen et al., 1990; Duffield et al., 1998) and is much higher than the 2 to 15% incidence found with clinical ketosis (Duffield, 2000). Cows with SCK are at an increased risk of postpartum diseases such as displaced abomasum (**DA**) and metritis (Duffield et al., 2009; Ospina et al., 2010a), which may increase their risk of removal from the herd during early lactation. In addition to the effects on disease events, SCK has been found to decrease milk yield in early lactation (Dohoo and Martin, 1984; Ospina et al., 2010b) and may adversely affect reproduction.

Although studies have reported the prevalence of SCK over time (Dohoo and Martin, 1984; Duffield et al., 1998, 2009), none has adequately described the epidemiology of SCK. Aside from McArt et al. (2011),

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an interventional trial conducted concurrently with this observational study, no large field trials have reported monitoring BHBA concentrations more frequently than once a week. Practical difficulties of securing adequate labor to repeatedly sample a large number of cows and the lack of a rapid, accurate, and relatively inexpensive cow-side test for SCK may explain why cows were not sampled more frequently in previous studies. In addition, sampling cows only once weekly does not accurately determine SCK incidence, as the median time to resolution of SCK is approximately 5 d (McArt et al., 2011). The recent identification and validation of the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL) by Iwersen et al. (2009) and Konkol et al. (2009) eases many of the previous difficulties associated with intensive ketosis monitoring programs.

The objectives of this study were to describe the epidemiology of SCK in cows diagnosed with SCK in early lactation through an intensive monitoring program from 3 to 16 DIM and to determine the association of (1) DIM at onset of SCK, and (2) BHBA concentration at onset of SCK with development of DA and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and milk yield in the first 30 DIM.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York State from May 18 until September 8, 2010, and from 2 dairy farms (farms C and D) in Wisconsin from June 11 until August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing protocol. In-depth information concerning farm management, nutrition, and reproductive and disease events was published previously (McArt et al., 2011).

Data Collection and Study Design

Enrollment into the study occurred at calving. Cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for ketosis using a Precision Xtra meter. The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 88 to 96% and 96 to 98%, respectively, when using a cut-off value of $\geq$1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L; clinical ketosis was defined as BHBA $\geq$3.0 mmol/L (Oetzel, 2004). All testing of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Detailed blood collection and testing information has been reported previously (McArt et al., 2011).

This observational study was part of a larger randomized field trial in which cows with BHBA concentrations of 1.2 to 2.9 mmol/L were sequentially randomized to treatment group [oral propylene glycol (**PG**) drench] or control group (no PG) after their first SCK-positive test. For the study, cows assigned to the PG treatment group were removed from analysis after their first positive BHBA test, at which point they began receiving PG. Remaining cows were excluded from the study if their previous gestation length was <260 d, if they died or were sold before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before their first BHBA test, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included parity, DA, metritis, sold and died events, conception to first service, and DIM at conception. Displaced abomasa, cows died, cows sold, and pregnancy outcomes were exported throughout the study period from each farm's Dairy Comp 305 program. In addition, milk weights were exported for the 3 herds that recorded milk production on per milking basis (farms A, B, and D).

A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (#2008-0099) and the University of Wisconsin-Madison School of Veterinary Medicine Animal Care and Use Committee (#V01479-0-05-10). All farmers were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions including clinical milk fever, retained placenta, metritis, displaced abomasum, and clinical ketosis.

Statistical Analysis

Descriptive statistics were generated with the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC). Incidence and prevalence histograms were prepared in Excel (Microsoft Corp., Redmond, WA). Daily incidence of SCK was calculated by dividing the number of cows that tested BHBA positive for the first time on each DIM by the total number of cows at risk for that DIM. A

cow was determined to be at risk at any given DIM if she had a recorded BHBA value for that DIM and had not previously had a BHBA test ≥1.2 mmol/L. Data from all eligible cows were used to develop the incidence histogram. Daily prevalence of SCK was calculated by dividing the number of cows with a positive BHBA test on each DIM by the total number of cows tested for that DIM. To calculate prevalence for 3 and 4 DIM, data from all eligible cows were used because PG-treated cows were removed from the analysis after their first positive BHBA test. Prevalence for 5 to 16 DIM was calculated using only data from SCK-positive control cows and half of the nonketotic cows. One-half of the nonketotic cows were randomly removed to adjust for the fact that half of the SCK-positive cows were removed because they were assigned to the PG treatment group. If the prevalence denominator had not been adjusted in this manner, the calculated prevalence would have been falsely decreased by a factor of 2. Daily incidence of clinical ketosis included only data from SCK-positive control cows and nonketotic cows. As SCK-positive PG-treated cows were less likely to develop clinical ketosis than control cows (McArt et al., 2011), their data were excluded when developing the histogram to prevent bias. Similar to the SCK prevalence histogram, because half of the SCK-positive cows were removed due to PG treatment, half of the nonketotic cows were removed to appropriately adjust the denominator to calculate the incidence.

Time to resolution of SCK (BHBA <1.2 mmol/L) was analyzed with a Kaplan-Meier (Kaplan and Meier, 1958) model using the LIFETEST procedure of SAS. The time-series variables were defined as time from first BHBA test of 1.2 to 2.9 mmol/L until either 1 or 2 continuous subsequent BHBA tests of <1.2 mmol/L. A censoring variable was used to differentiate cows whose blood BHBA decreased to <1.2 mmol/L from cows that were removed from the herd, removed from the study, or did not have a BHBA <1.2 mmol/L by 16 DIM. Kaplan-Meier graphs were produced using MedCalc (MedCalc Software, Mariakerke, Belgium).

Five outcomes (development of a DA within 30 DIM, removal from herd within 30 DIM, conception to first service, time to conception within 150 DIM, and milk yield in the first 30 DIM) were analyzed for each of 3 conditions: SCK status, DIM at first positive SCK test, and BHBA concentration at first positive SCK test. The associations of the conditions with DA development, removal from herd, and conception to first service were analyzed using mixed-effects multivariable Poisson regression with the GENMOD procedure of SAS (Frome and Checkoway, 1985; Spiegelman and Hertzmark, 2005; Ospina et al., 2012). For the model evaluating DA development, the 2 cows that developed a DA before their first positive BHBA test were removed from the analysis. The potential confounding variables parity (lactation 1, lactation 2, and lactation ≥3) and metritis were offered to the models (the variable DA was also offered to the model concerning removal from herd) as independent variables, in addition to the model-respective variables on SCK status (SCK), DIM at first positive SCK test (**DIMPOS**), and BHBA concentration at first positive SCK test (**BHBAPOS**). The variable SCK was entered into the SCK status models as a dichotomous variable.

For all outcomes except DA development, the variable DIMPOS was entered into the DIM at first positive SCK test models as a dichotomous variable comparing cows with their first positive SCK test at 3 to 7 DIM with those having a first positive SCK test at 8 to 16 DIM. Because all cows that developed a DA had their first positive test ≤6 DIM, the variable DIMPOS was entered as a dichotomous variable comparing cows with their first positive SCK test at 3 to 5 DIM with those having their first positive SCK test at 6 to 16 DIM. This change in grouping was performed because at least one event in each category is needed to compute a ratio statistic; grouping cows for this outcome into 3 to 7 DIM and 8 to 16 DIM as for the other outcomes would produce a statistically impossible calculation. The variable BHBAPOS was entered into the BHBA at first positive SCK test models as a continuous variable.

The variable herd was entered as a random effect in the DA and removal models. Because of large differences in voluntary waiting period (**VWP**), conception to first service, and breeding strategy (inseminating many cows twice) on farm D, the variable herd was tested as a fixed effect in the conception to first service model; an offset term was used to adjust for the difference in VWP for each herd.

For all models, independent variables were removed by manual backward stepwise elimination if their contrast estimate was considered statistically nonsignificant ($P > 0.10$) and biologically not important. As only one fixed effect variable remained in all models after stepwise elimination, no interaction terms were tested. Statistical significance of the variable herd in the conception to first service model led to further analysis of conception by herd. Due to biologically plausible explanations for a change in outcome direction compared with the other 3 farms, farm D was excluded from both conception to first service and time to conception analyses for all conditions. The variable herd was then re-entered into the conception to first service model as a random effect to account for the unmeasured variations between the remaining 3 herds.

Analyses of days to conception was completed by semiparametric proportional hazards model (Cox, 1972) using the PHREG procedure of SAS. The time-series variable for the model was the number of days from calving until conception within 150 DIM. Censoring variables were used to identify cows that conceived from cows that were removed from the herd or did not conceive by 150 DIM. Independent variables offered to the model included model respective variables (SCK, DIMPOS, and BHBAPOS), parity, and herd. Independent variables and their respective interaction terms were manually removed by backward stepwise elimination if considered statistically nonsignificant ($P > 0.10$) and biologically not important. Proportional hazards assumptions were verified by evaluating the time-dependent covariates (Allison, 1995); noninformative censoring was evaluated using sensitivity analysis. Kaplan-Meier analyses using only the respective model variables SCK, DIMPOS, and BHBAPOS were completed using the LIFETEST procedure of SAS to determine median days from calving until conception.

Differences between groups in milk yield for individual milk weights until 30 DIM was analyzed using repeated-measures ANOVA with first-order autoregressive covariance using the MIXED procedure of SAS (Littell et al., 1998, 2000). Results were analyzed using different covariance structures; a first-order autoregressive covariance structure was chosen as it produced the lowest Akaike information criterion, a measure of the relative goodness-of-fit. Variables offered to the models included model-respective variables (SCK, DIMPOS, and BHBAPOS), parity (lactations 1, 2, ≥3), DIM, and herd as a random effect. Independent variables and their respective interaction terms were considered statistically significant if $P \leq 0.05$. A scatterplot, best-fit line, and R^2 statistic were produced from the ANOVA model with the mean predicted milk yield output for each concentration of BHBA from 1.2 to 2.9 mmol/L using Excel (Microsoft Corp.).

RESULTS

Descriptive Statistics

Of the 1,717 eligible cows, 741 (43.2%) were diagnosed with SCK at least once and randomized, with 372 cows receiving PG treatment and 369 control cows. Cows in the PG treatment group were removed from further analysis after their first positive BHBA test, which left 369 cows that had at least one positive test for SCK and 976 cows that never tested positive. The SCK group was composed of 106 (28.7%), 97 (26.3%), and 166 (45.0%) cows in parity 1, 2, and ≥3, respectively (median = 2); the nonketotic group contained 398 cows (40.8%) in parity 1, 327 (33.5%) in parity 2, and 251 (25.7%) in parity ≥3 (median = 2). A chi-squared test showed a difference in parity between the 2 groups ($P < 0.001$).

Displaced Abomasum and Removal from Herd. In the first 30 DIM, 3 of the 976 nonketotic cows (0.3%) developed a DA and 24 of 367 cows (6.5%) developed a DA after testing positive for SCK (2 cows developed a DA before testing positive for SCK and were excluded from the analysis). Cows testing positive for SCK were 19.3 times more likely [risk ratio (**RR**) 95% CI = 13.8 to 27.0, $P < 0.001$] to develop a DA than nonketotic cows. In the first 30 DIM, 18 of 976 (1.8%) nonketotic cows and 20 of 369 cows (5.4%) that tested positive for SCK were removed from the herd. Cows with SCK were 3.0 times more likely (RR 95% CI = 2.2 to 4.2, $P < 0.001$) to die or be culled than nonketotic cows. The final regression models for both DA and removal from herd included only SCK as a statistically meaningful predictor variable with herd as a random effect; the final model estimates are in Table 1.

Conception to First Service. Of the 3 farms used in the reproductive analyses (farms A, B, and C), 751 cows had data concerning conception at first service, of which 241 of 603 (40.0%) nonketotic cows and 52 of 148 (35.1%) SCK-positive cows conceived. Conception

Table 1. Estimates for 3 final Poisson regression models showing risk ratios (RR) comparing cows diagnosed with subclinical ketosis to nonketotic cows for 1,345 Holstein cows following intensive testing for subclinical ketosis from 3 to 16 DIM[1]

Model[1]	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	2.96	0.17	<0.001	19.3	13.8 to 27.0
Removal	1.10	0.17	<0.001	3.0	2.2 to 4.2
Conception	−0.06	0.11	0.55	0.9	0.8 to 1.2

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service.

[2]SE = standard error for estimate.

[3]*P*-value reported for estimate.

[4]CI for risk or hazard ratio.

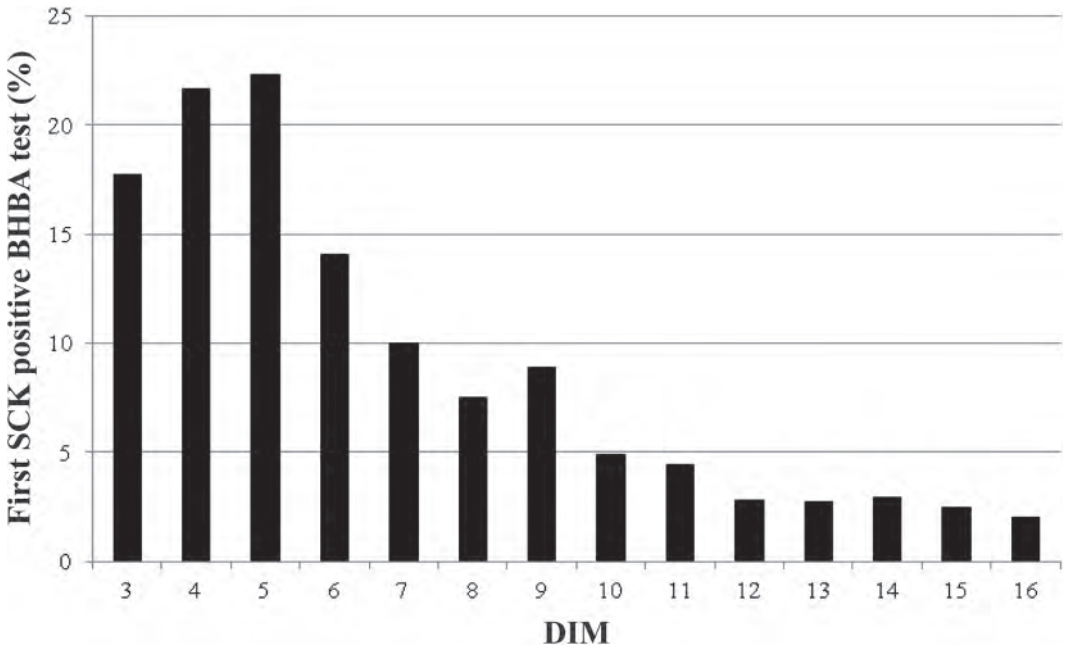

Figure 1. Histogram of incidence of subclinical ketosis (SCK) in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.

to first service did not differ between the 2 groups, with SCK cows equally likely (RR = 0.9, 95% CI = 0.8 to 1.2, $P = 0.55$) to conceive as nonketotic cows. The final regression model included only the SCK treatment variable with herd as a random effect; the estimate for the final conception to first service model is in Table 1.

Time to Conception. Of the 749 cows on farms A, B, and C with data on pregnancy status at 150 DIM, 496 of 601 (82.5%) nonketotic cows and 115 of 148 (77.7%) SCK-positive cows were pregnant. Days to conception within 150 DIM did not differ between the 2 groups [hazard ratio (**HR**) for SCK cows = 0.9, 95% CI = 0.7 to 1.1, $P = 0.40$], with a median time to conception of 96 d (95% CI = 92 to 101) and 104 d (95% CI = 95 to 114) for nonketotic and SCK cows, respectively. The final model concerning the association of SCK on time to pregnancy included the variables SCK, herd, and lactation group.

Milk Yield. In total, 1,115 cows from farms A, B, and D were used in the milk yield analysis: 804 nonketotic cows and 311 SCK-positive cows. The fixed-effect variables SCK, parity group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights with the variable herd as a random effect. Nonketotic cows produced 0.4 kg more milk per milking in the first 30 d of lactation than SCK-positive cows, at 11.7 and 11.3 kg, respectively ($P = 0.006$), for a total difference of 1.2 kg/cow per day.

Epidemiology of SCK

Histograms of SCK incidence and prevalence by DIM are given in Figures 1 and 2, respectively. Figure 3 is a histogram of incidence of clinical ketosis by DIM. A Kaplan-Meier curve describing time from first positive BHBA test to 1 negative BHBA test for the SCK group is shown in Figure 4. Median time until 1 negative test was 5 d (95% CI = 4 to 5). Of the 369 SCK-positive cows, 163 (44.2%) did not test <1.2 mmol/L by 16 DIM (n = 35) or died (n = 14), developed clinical ketosis (n = 52), or were treated by the farm (n = 62) before testing <1.2 mmol/L.

Association of DIM at First Positive BHBA Test

Displaced Abomasum and Removal from Herd. Of the 24 SCK-positive cows that developed a DA in the first 30 DIM, 22 (91.7%) had their first positive BHBA test from 3 to 5 DIM and 2 (8.3%) at 6 DIM. The median time from first positive BHBA test to DA was 5 d (range = 1 to 24 d). Cows diagnosed with SCK for the first time from 3 to 5 DIM were 6.1 times more likely (RR 95% CI = 2.3 to 16.0, $P < 0.001$) to develop a DA than cows first testing SCK positive at 6 DIM or later. Of the 20 SCK-positive cows that were removed from the herd in the first 30 DIM, 19 (95.0%) had their first positive BHBA test from 3 to 7 DIM and 1 (5.0%)

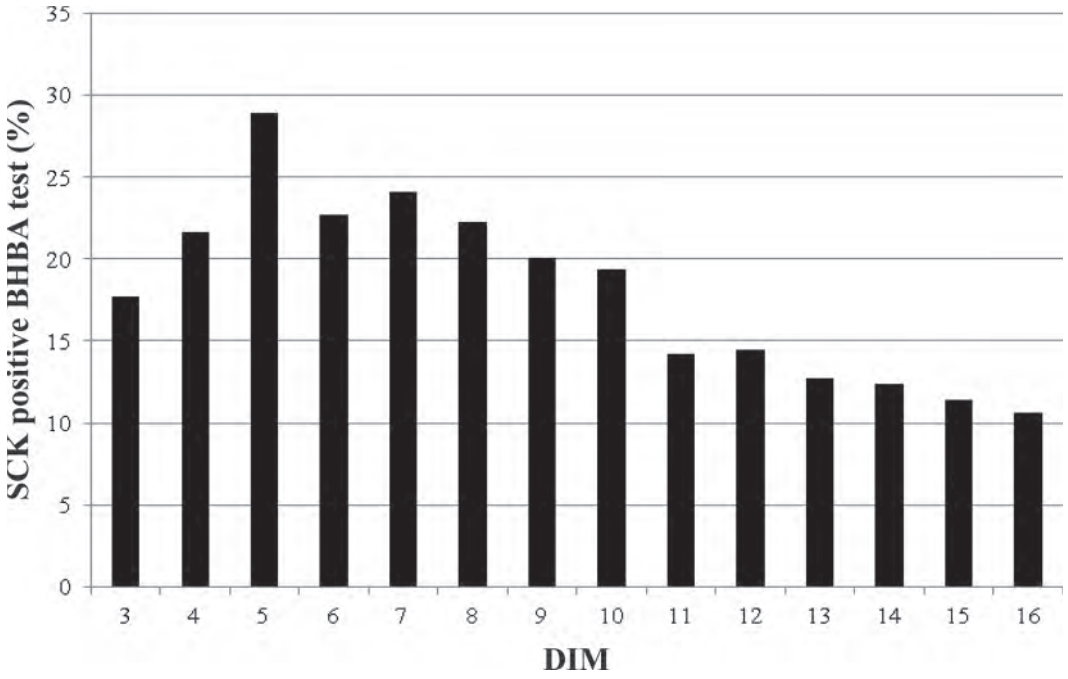

Figure 2. Histogram of prevalence of subclinical ketosis (SCK) in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.

at 12 DIM. The median time from first positive BHBA test to removal from herd was 9 d (range = 2 to 24 d). Cows diagnosed with SCK for the first time from 3 to 7 DIM were 4.5 times more likely (RR 95% CI = 1.7 to 11.7, $P = 0.002$) to be removed from the herd than cows first testing SCK positive at 8 DIM or later. The final regression models for both DA and removal from herd included only the variable DIMPOS with herd as

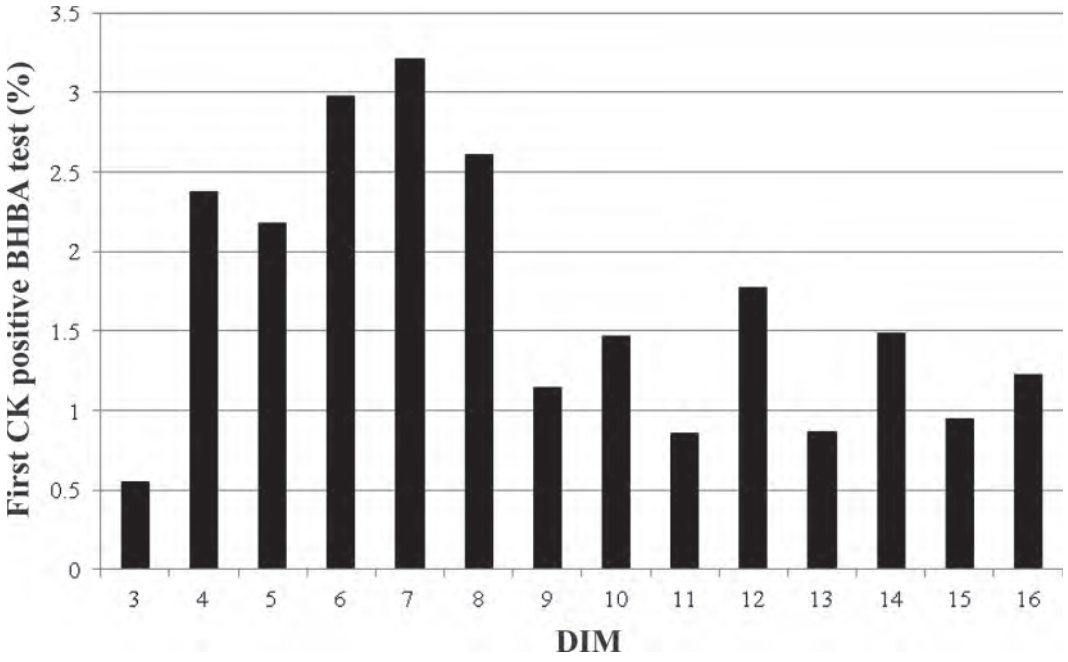

Figure 3. Histogram of incidence of clinical ketosis (CK) in 856 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of ≥3.0 mmol/L.

Table 2. Estimates for 3 final Poisson regression models showing risk ratios (RR) comparing cows diagnosed with subclinical ketosis (SCK) from 3 to 7 DIM with cows diagnosed with SCK from 8 to 16 DIM for 369 Holstein cows with at least one positive test for SCK from 3 to 16 DIM[1]

Model	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	1.81	0.49	<0.001	6.1	2.3 to 16.0
Removal	1.51	0.49	0.002	4.5	1.7 to 11.7
Conception	−0.35	0.08	<0.001	0.7	0.6 to 0.8

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service. For the DA outcome, cows were dichotomized into first positive test at 3 to 5 DIM or 6 to 16 DIM; for the remaining outcomes, cows were dichotomized into first positive test at 3 to 7 DIM or 8 to 16 DIM.

[2]SE = standard error for estimate.

[3]*P*-value reported for estimate.

[4]CI for risk or hazard ratio.

a random effect; the final model estimates are in Table 2.

Conception to First Service. In total, 148 SCK cows from farms A, B, and C had data concerning conception to first service, of which 52 (35.1%) conceived. Of these 52 cows, 35 (67.3%) had their first positive BHBA test from 3 to 7 DIM and 17 (32.7%) from 8 and 16 DIM. Cows diagnosed with SCK for the first time from 3 to 7 DIM were 0.7 times as likely (RR 95% CI = 0.6 to 0.8, $P < 0.001$) to conceive to first service than cows first testing SCK positive at 8 DIM or later. The final regression model included only the variable DIMPOS with herd as a random effect; the final model estimates are in Table 2.

Time to Conception. Of the 148 cows on farms A, B, and C with data concerning pregnancy status at 150 DIM, 115 (77.7%) were pregnant. Of these 115 cows, 82 (71.3%) had their first positive BHBA test between 3 to 7 DIM and 33 (28.7%) between 8 and 16 DIM. Cows diagnosed with SCK for the first time from 3 to 7 DIM were 0.7 times as likely (HR 95% CI = 0.5 to 1.1, $P = 0.13$) to conceive by 150 DIM than cows first testing SCK positive at 8 DIM or later, with a median time to conception of 107 d (95% CI = 95 to 119) and 98 d (95% CI = 78 to 117) for cows first testing SCK positive at 3 to 7 DIM and 8 to 16 DIM, respectively. The final model included the variables DIMPOS and herd.

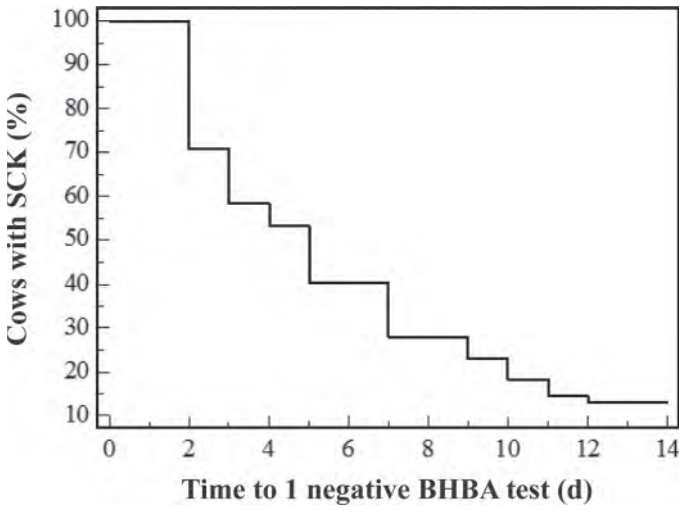

Figure 4. Kaplan-Meier curves of time from first positive test for subclinical ketosis (SCK; BHBA concentration of 1.2 to 2.9 mmol/L) to one blood BHBA concentration of <1.2 mmol/L in 369 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. Because of a Monday, Wednesday, Friday testing scheme, cows were not able to cure on d 0, 1, 6, or 8 after first positive BHBA test.

Milk Yield. In total, 311 cows from farms A, B, and D were used in the milk yield analysis. The fixed effect variables DIMPOS, lactation group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights with the variable herd as a random effect. Cows diagnosed with SCK for the first time from 3 to 7 DIM produced 0.7 kg less milk per milking in the first 30 d of lactation than cows diagnosed with SCK for the first time between 8 and 16 DIM, at 11.2 kg and 11.9 kg, respectively ($P = 0.04$). Given a 3-times-daily milking routine, the total difference in milk between the 2 groups was 2.1 kg per cow per day.

Association of Concentration at First Positive BHBA Test

Displaced Abomasum and Removal from Herd. Each 0.1 mmol/L increase in BHBA increased the risk of developing a DA by 30 DIM by a factor of 1.1 (95% CI = 1.0 to 1.2, $P = 0.002$). For example, cows whose first positive BHBA concentration was 1.3 mmol/L were 1.1 times more likely to develop a DA than cows whose first positive BHBA concentration was 1.2 mmol/L; cows whose first positive BHBA concentration was 2.4 mmol/L were 3.1 (1.1^{12}) times more likely to develop a DA than cows whose first positive BHBA concentration was 1.2 mmol/L. Each 0.1 mmol/L increase in BHBA

Table 3. Estimates for 3 final Poisson regression models showing risk ratios (RR) as BHBA concentration at first positive test increased for 369 Holstein cows with at least one positive test for subclinical ketosis from 3 to 16 DIM[1]

Model[1]	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	0.09	0.03	0.002	1.1	1.0 to 1.2
Removal	0.35	0.13	0.01	1.4	1.1 to 1.8
Conception	0.08	0.38	0.84	1.1	0.5 to 2.3

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service.

[2]SE = standard error for estimate.

[3]*P*-value reported for estimate.

[4]CI for risk or hazard ratio.

increased the risk of removal from the herd in the first 30 DIM by a factor of 1.4 (95% CI = 1.1 to 1.8, $P = 0.01$). For example, cows whose first positive BHBA concentration was 1.3 mmol/L were 1.4 times more likely to be removed from the herd than cows whose first positive BHBA concentration was 1.2 mmol/L; cows whose first positive BHBA concentration was 2.4 mmol/L were 56.7 (1.4^{12}) times more likely be removed from the herd than cows whose first positive BHBA concentration was 1.2 mmol/L. The final regression models for both DA and removal from herd included only the continuous variable BHBAPOS with herd as a random effect; the final model estimates are in Table 3.

Conception to First Service. We observed no difference in BHBA concentration at first positive test and conception to first service (0.1 mmol/L increase RR = 1.1, 95% CI = 0.5 to 2.3, $P = 0.84$). Thus, cows with a BHBA concentration of 1.2 mmol/L at first positive test had the same risk of conception as cows with a BHBA concentration at first positive test of 1.3 or 2.4 mmol/L. The final regression model included only the continuous variable BHBAPOS with herd as a random effect; the final model estimates are in Table 3.

Time to Conception. We observed no difference in BHBA concentration at first positive test and time to conception (0.1 mmol/L increase HR = 1.2, 95% CI = 0.8 to 1.9, $P = 0.38$). Thus, cows with a BHBA concentration of 1.2 mmol/L at first positive test had the same hazard of conception as cows with a BHBA concentration at first positive test of 1.3 or 2.4 mmol/L. The final model included the variables BHBAPOS and herd.

Milk Yield. The fixed-effect variables BHBA concentration at first positive test, parity group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights with the variable herd as a random effect. The milk yield per milking decreased as BHBA concentration at first positive test increased ($P < 0.001$). Predicted mean milk yield per milking at each subclinical BHBA concentration is shown in Figure 5 and can be estimated from the following formula:

$$\text{Predicted milk per day (kg)} = 42.6 - 5.1 \text{ (BHBA concentration at first positive test).}$$

Thus, for each 0.1 mmol/L increase in BHBA concentration at first positive test, the predicted milk produced for the first 30 d of lactation is estimated to decrease by 0.5 kg/d. The coefficient of determination for the model (R^2) was 0.4.

DISCUSSION

This observational study was conducted to describe the epidemiology of SCK in cows in early lactation and to determine the association of (1) DIM at onset of SCK and (2) blood BHBA concentration at onset of SCK with development of DA and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and early lactation milk yield. Our results indicated that cows develop SCK very early in lactation and that cows developing SCK within the first week postpartum were more likely to have adverse health events and produce less milk than cows developing SCK after the first week of lactation. In addition, as the concentration of BHBA at first positive SCK test increased, the risk of adverse events increased and milk production decreased.

The average incidence of SCK from 3 to 16 DIM in the reported study herds was 43% and ranged from 26 to 56% (McArt et al., 2011), with peak SCK incidence occurring at 5 DIM. Although cows were sampled beginning at 3 DIM, the shape of the incidence curve suggests that the incidence of SCK at 1 and 2 DIM is less than that at 5 DIM; however, the design of this study did not allow confirmation of this. No other studies have reported the incidence and prevalence of SCK in dairy cows undergoing BHBA testing more fre-

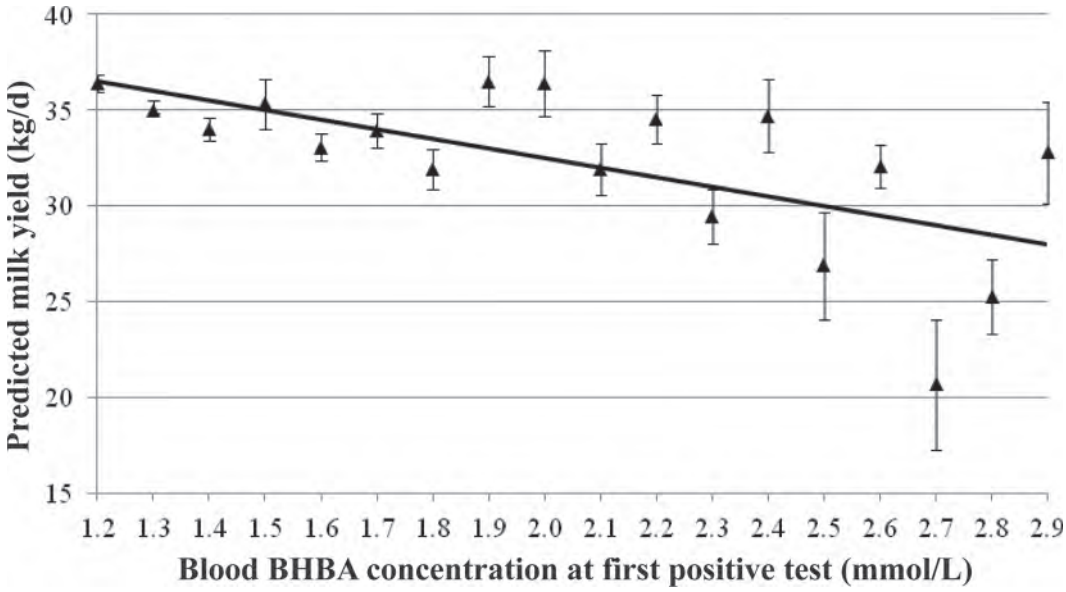

Figure 5. Regression plot of mean predicted milk yield per milking for the first 30 DIM by blood BHBA concentration of first positive BHBA test (1.2 to 2.9 mmol/L) for 369 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. The solid line represents the best linear fit; 95% CI are shown for each predicted milk yield by BHBA concentration.

quently than once a week in early lactation. Although previous studies from Emery et al. (1964), Simensen et al. (1990), and Duffield et al. (1998) had similar findings and reported cumulative SCK incidences of 49, 46, and 59%, respectively, these numbers most likely underestimate the true incidence as cows were tested once weekly. As shown in the current study, the median time from first SCK-positive BHBA test to first test <1.2 mmol/L was 5 d, which would allow cows to develop and resolve SCK between testing sessions if testing occurred only once weekly. In addition to underestimation due to the frequency of testing, previously reported SCK incidences may be falsely decreased due to timing of weekly testing. For example, Duffield et al. (2009) found a 24 and 25% incidence of SCK (BHBA concentration ≥1.2 mmol/L) for cows tested in the first and second weeks of lactation, respectively. Depending on what DIM cows were tested within the first week of lactation; for example, 4 versus 7 DIM, the true incidence could be falsely decreased by a factor of 2.

Both incidence (number of new cases divided by the number of cows at risk) and prevalence (number of existing cases divided by the number of cows sampled) of SCK are important measurements in herd settings. Incidence describes how quickly new cases develop, and it can be higher than the prevalence depending on the frequency of testing and the duration of disease. As a measure of SCK, incidence is a useful tool for individual animal treatment decisions. However, to get an accurate measure of incidence, frequent testing is necessary. The prevalence of SCK describes what proportion of the animals tested have SCK at the time of testing and can be used for herd monitoring over time as well as an outcome indicator for changes in dry or fresh cow management.

Results on the associations of SCK with adverse events are similar to those found in other studies concerning development of DA (Duffield et al., 2009; Ospina et al., 2010a) and early lactation milk yield (Dohoo and Martin, 1984; Ospina et al., 2010b), showing that the population of dairy cattle used in this study has good external validity with other published trials. Although no difference was found in conception to first service, similar to results found by Walsh et al. (2007) and Kessel et al. (2008), it is possible that the power of the reported study was too small to find a difference. The calculated power to find a difference in conception risk between SCK-positive and nonketotic cows was only 17%, most likely because of the smaller subset of SCK-positive cows used in the analysis. Thus, the findings concerning conception to first service should be interpreted with this in mind.

Whereas the studies mentioned above regarding the development of DA, early lactation milk yield, and reproduction have detailed the negative associations of SCK on health events, reproduction, and milk production in fresh cows, no studies have reported the effects of DIM or blood BHBA concentration at onset of SCK. Data from this study clearly show that cows developing SCK within the first week postpartum are at a much

higher risk for DA development, removal from herd, and poor milk production than cows developing SCK after 1 wk postpartum. It can be postulated that cows developing SCK within 1 wk postpartum have experienced extremely poor adaptation to negative energy balance through calving and into lactation, whereas cows developing SCK after the first week postpartum may have better adapted to the effects of decreased DMI during and immediately postcalving but are not able to sustain energy stores for the increase in milk production in early lactation.

Blood BHBA concentration at first SCK-positive test also has an effect on health events and milk production. Extrapolating from this study's findings, a cow with a BHBA concentration at the high end of the SCK range (2.4 mmol/L) is 3 times more likely to develop a DA, is >50 times more likely to be removed from the herd, and is expected to produce 180 kg less milk in the first 30 d of lactation than a cow with a BHBA concentration on the low end of the SCK range (1.2 mmol/L). Intuitively, cows with a more severe ketosis have less energy available to make milk; thus, cows with a poorer adaptation to negative energy balance (higher mobilization of NEFA leading to a more severe ketosis) may be at greater risk for adverse health events.

CONCLUSIONS

Cows that developed SCK within the first week postpartum were more likely to develop a DA or be removed from the herd within the first 30 DIM, were less likely to conceive to first service, and produced less milk in the first 30 DIM than cows developing SCK after the first week of lactation. In addition, as the concentration of BHBA at first positive SCK test increased, the risk of adverse events increased and milk production decreased. As 75% of all cows that developed SCK tested positive within 1 wk postpartum (with a peak at 5 DIM), it may be important to modify farm management protocols to maximize detection during this time. Cows that begin lactation with high BHBA concentrations may require special attention to decrease their risk of adverse events in early lactation.

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