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# An estimation of the cow- and herd-level prevalence of post-partum subclinical ketosis in large Washington state dairy herds and evaluation of mean β-hydroxybutyrate concentration for herd-level assessment

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**Abstract**

The purpose of this study was to estimate the cow- and herd-level prevalence of subclinical ketosis (SCK) on dairies in the state of Washington and evaluate the arithmetic mean of individual samples as a proxy for pooled-sample testing to screen herds for SCK. The cow-level prevalence of SCK in 589 cows from 40 Washington dairies was 31, 25, and 19% using β-hydroxybutyrate (BHB) concentration cutpoints of ≥ 1.0, 1.2, and 1.4 mmol/L, respectively. For all BHB cutpoints, no difference was observed in the prevalence of SCK between cows of parity 1 and 2 ($P > 0.358$), but SCK was higher in cows of parity ≥ 3 versus those in lactations 1 and 2 ($P < 0.001$). The mean BHB concentration (mmol/L) in cows of parity ≥ 3 (1.16) was higher than that of parity 1 (0.72) and parity 2 (0.88) cows ($P < 0.001$). Using a BHB cutpoint of ≥ 1.2 mmol/L and herd alarm level of >15% cows exceeding that cutpoint, 23 herds (58%) demonstrated a herd-level problem with SCK. When a BHB cutpoint of ≥ 1.4 and herd alarm level of >25% was used, 12 herds (30%) had SCK. A herd mean BHB concentration of 0.77 mmol/L was correlated with a 15% herd-alarm level using a ≥ 1.2 mmol/L BHB cutpoint. Sensitivity and specificity of the herd mean BHB cutpoint of ≥0.8 mmol/L to identify herds with > 15% cows with BHB ≥ 1.2 mmol/L was 91 and 75%, respectively. The prevalence of SCK in Washington dairies was numerically higher than previous reports. Results of this study highlight the importance of obtaining representative samples from parity risk groups. Furthermore, results suggest parity 2 cows may be better grouped with parity 1 rather than ≥ 3 parity cows. Herd mean BHB concentration performed well as a test to identify herds with a potential SCK problem when a mean value specific cutpoint was used. Further research evaluating the relationship between pooled-sample BHB and important outcomes, such as disease, milk production, reproduction, and removal, are needed.

**Key words:** dairy, subclinical, ketosis, BHB

**Résumé**

Le but de cette étude était d'estimer la prévalence de la cétose subclinique (SCK) en nombre de vaches et de troupeaux dans les entreprises laitières de l'état de Washington et d'évaluer la moyenne arithmétique d'échantillons individuels comme approximation dans l'analyse d'échantillons groupés pour le dépistage de SCK dans les troupeaux. La prévalence de SCK chez 589 vaches provenant de 40 entreprises laitières dans l'état de Washington était de 31, 25, et 19% lorsque les valeurs admissibles de concentration en β-hydroxybutyrate (BHB) étaient ≥ 1.0, 1.2, et 1.4 mmol/L, respectivement. Pour toutes les valeurs admissibles en BHB, aucune différence n'a été observée dans la prévalence de SCK entre les vaches de 1ère et 2e parités ($P > 0.358$), mais la prévalence de SCK était plus élevée chez les vaches de parité ≥ 3 versus celles en 1re et 2e lactation ($P < 0.001$). La concentration moyenne en BHB (mmol/L) chez les vaches de parité ≥ 3 (1.16) était plus élevée que celle des vaches de première (0.72) et deuxième (0.88) parité ($P < 0.001$). Pour une valeur seuil en BHB ≥ 1.2 mmol/L et un niveau d'alarme de troupeau > à 15% de vaches dépassant cette valeur seuil, 23 troupeaux (58%) ont démontré un problème de SCK au niveau du troupeau. Lorsqu'une valeur seuil en BHB ≥ 1.4 et un niveau d'alarme de troupeau > à 25% étaient utilisés, 12 troupeaux (30%) étaient atteints de SCK. Une concentration moyenne de troupeau en BHB de 0.77 mmol/L corrélait avec un niveau d'alarme de troupeau de 15% lorsqu'une valeur admissible en BHB ≥ 1.2 mmol/L était utilisée. La sensibilité et la spécificité de la valeur moyenne seuil de troupeau en BHB ≥0.8 mmol/L pour identifier les troupeaux avec > 15% des vaches avec un BHB ≥ 1.2 mmol/L étaient de 91 et 75%, respectivement. La prévalence de SCK dans les entreprises laitières de l'état de Washington était numériquement plus

élevée que celle rapportée précédemment. Les résultats de la présente étude soulignent l'importance d'obtenir des échantillons représentatifs des groupes à risque selon la parité. De plus, les résultats suggèrent qu'il est préférable de grouper les vaches de deuxième parité avec celles de première parité plutôt que celles de parité ≥ 3. La concentration moyenne en BHB pour le troupeau a représenté un bon test pour identifier les troupeaux avec un problème potentiel de SCK lorsqu'une valeur seuil moyenne spécifique était utilisée. Plus de recherche est nécessaire dans l'évaluation du lien entre la valeur en BHB d'échantillons groupés et d'importants facteurs, tels que la maladie, la production de lait, la reproduction, et la réforme.

## Introduction

Dairy cattle experience negative energy balance (NEB) peripartum as they transition from the non-lactating to lactating period.[4] This is a normal condition associated with the abrupt, substantially increased energy demand of lactation and a brief period of anorexia around parturition. The adaptive response to NEB is mobilization of body fat and production of ketones as glucose-sparing, alternative energy sources for tissues, particularly skeletal muscle.[5] However, excessive NEB, usually due to reduced dry matter intake, predisposes cows to post-partum disease, reduced milk production, reduced first-service pregnancy rate, and increased risk of removal from the herd.[14]

Measuring beta-hydroxybutyrate (BHB) in plasma or serum has been well established as an efficient, cost-effective method of assessing the energy balance of post-partum dairy cows and identifying cows with subclinical ketosis (SCK).[6,8,11] However, the cutpoint of blood BHB concentration post-partum that defines SCK is unclear. Studies associating post-partum disease, milk production, and reproductive performance with BHB concentration have suggested a range of threshold BHB values from 0.9 mmol/L to 1.7 mmol/L to define SCK at the cow level.[2,9,16] The wide range of BHB cutpoints for SCK can be attributed to variation in the days-in-milk (DIM) at sampling, parity, and farm-specific management of cows during the transition period, as well as differences in the outcomes assessed across studies. McArt et al recently reviewed the literature evaluating testing of energy-related metabolites. Based on 8 studies, they suggested 1.2 mmol/L BHB as a cutoff with a "good combination" of sensitivity (range: 31 to 68%) and specificity (range: 75 to 82%) "useful in many situations".[8] However, the studies reporting these test characteristics only did so for the outcome of displaced abomasum. Furthermore, a recent metanalysis of the associations between SCK and post-partum outcomes reported 1.4 mmol/L BHB was an appropriate threshold for defining a cow with SCK.[14] It has been suggested that cows with clinical ketosis typically have a serum BHB ≥3.0 mmol/L.[12]

Evaluating potential herd-level problems with excessive NEB by assessing herd-level prevalence of SCK consists of selection of a cow-level cutpoint used to identify cows with SCK, and the proportion of cows sampled with SCK above which action should be considered (herd alarm-level). As with the cow-level cutpoint for BHB concentration, the herd alarm-level that most accurately identifies a "problem herd" is uncertain. Values from 10 to 25% of sampled cows with SCK have been reported[3,10,12] with variation likely explained by the number of cows sampled at a given time, the BHB cutpoint used, and the outcomes assessed.

The mean BHB concentration of pooled sera from cows sampled at a given time has been evaluated as a less expensive method of monitoring the herd prevalence of SCK. This strategy could reduce testing costs while potentially improving the accuracy of herd-level SCK monitoring by routinely sampling and pooling cows of different parity and DIM risk groups. Borchardt and Staufenbiel evaluated the use of pooled serum samples for herd-based detection of SCK by sampling 10 cows from each of 110 German dairy herds.[1] They concluded pooled samples could be successfully used to identify herds with SCK. By contrast, Ospina et al reported a low test sensitivity (30%) for pooled-sample testing compared with the proportion of individual sample tests for identifying herds above a specified herd alarm-level.[9] However, both studies indicated pooled samples were well correlated with the arithmetic means of individual samples.

The purpose of this study was to estimate the cow- and herd-level prevalence of SCK on large dairies in the state of Washington and to evaluate the arithmetic mean of individual samples as a proxy for pooled-sample screening to identify herds with a potential SCK problem or monitor SCK status of a herd.

## Materials and Methods

Washington state dairies with > 250 milking cows that fed a total mixed ration were eligible for enrollment in the study. In 2011 there were 460 licensed dairies in WA, 33% of which had more than 200 milking cows, resulting in a potential study population of about 152 dairy herds. A sample size of 44 herds was necessary to estimate the prevalence of SCK in this population with a precision of 25%, assuming 50% prevalence and 95% confidence.[15]

Forty-five herds that met the enrollment criteria were sampled for BHB determination from May to August 2011. The study herds chosen were a convenience sample of volunteer herds, and the study was observational and cross-sectional in design. In each herd, a convenience sample of 13 to 15 apparently healthy cows, 3 to 14 DIM, were sampled. Healthy cows were defined as not being in the hospital pen and not being treated for disease prior to or at the time of sampling. Enrolled cows showed no overt signs of being sick as assessed by the person performing sample collection; however, a complete physical exam was not performed. Whole blood was collected from the coccy-

geal vein and immediately analyzed using a handheld BHB meter[a] by trained study personnel or the herd veterinarian. Cow identification number, parity, and DIM were obtained from farm computer records.

Beta-hydroxybutyrate measurements were reported in mmol/L because readily available handheld meters, commonly used to measure BHB in the field, report results in these units. Individual-cow SCK was defined based on 3 different cutpoints, where whole-blood BHB was ≥ 1.0, 1.2, and 1.4 mmol/L. Herd-level prevalence of SCK was calculated as the number of cows with SCK divided by the total number of cows sampled for each BHB cutpoint. Cow-level prevalence was also evaluated by parity, where cows were grouped as parity 1, 2, and ≥3. Comparison of SCK prevalence by parity group within each BHB cutpoint was done by Fisher's Exact Test, and arithmetic mean BHB concentrations of lactation groups were compared by T test.[b] A Bonferroni adjustment for multiple comparisons was applied such that lactation groups were considered different if the *P*-value of the test used was < 0.017 (0.05/3 comparisons). Dairies with > 15% herd-level prevalence of SCK using the 1.2 mmol/L cutpoint (HA1.2>15%) were classified as SCK-positive herds.[10] Those with > 25% within-herd prevalence of SCK using the 1.4 mmol/L cutpoint (HA1.4>25%) were classified as SCK-positive herds.[3] Correlation between the herd arithmetic mean BHB concentration and the percent of cows with SCK in a herd for HA1.2>15% and HA1.4>25% was determined by linear regression and visualized by scattergraphs.[c] The sensitivity and specificity of mean BHB concentration to identify herds with SCK based on HA1.2>15% and HA1.4>25% was determined using mean BHB concentrations (mmol/L) rounded to the tenths place, since that is the precision of available handheld BHB meters commonly used in the field.

## Results

Five herds were excluded because too many cows were sampled outside of 3 to 14 DIM, resulting in <10 cows with appropriate data from each farm and insufficient resources to sample additional herds. Data of 589 Holstein cows from 40 herds were included in the analysis. Individual-cow BHB measurements were excluded for 17 cows because they were sampled outside of 3 to 14 DIM. As a result, the number of cows sampled/herd was 11 to 17 (mean = 15). There were 171 (29%), 178 (30%), and 218 (37%) parity 1, 2, and ≥3 cows, respectively. Parity data were missing from 22 (3.7%) cows. These cows were included in all analyses except those where parity was a variable of interest. Cows with parity data were sampled in lactation 1 to 11 with an average of 2.4 lactations. Within a herd, parity 1 cows represented 0 to 60%, parity 2 cows 7 to 69%, and cows of parity ≥ 3 represented 14 to 60% of those sampled. The average number of milking cows in the herds sampled was 2808 (SD = 2098, range: 312 to 7500 cows).

*Cow-level prevalence of SCK*

The cow-level prevalence of SCK observed in a sample of 589 cows from 40 Washington state dairy herds was 31, 25, and 19% using the ≥ 1.0, 1.2, and 1.4 mmol/L cutpoints, respectively. The prevalence of SCK varied by parity, and was greater in higher-parity groups (Figure 1). For all cutpoints evaluated, no difference was observed in the prevalence of SCK between cows of parity 1 and 2 ($P > 0.358$), but prevalence was higher in cows of parity ≥ 3 vs those of parity 1 and 2 ($P < 0.001$) (Figure 1). The average whole-blood concentration of BHB for all cows sampled was 0.93 mmol/L (range: 0.1 to 5.2 mmol/L; Table 1). The average BHB concentration (mmol/L) in cows of parity ≥ 3 (1.16) was higher than that of parity 1 (0.72) and parity 2 (0.88) cows ($P < 0.001$). The concentration of BHB was ≥ 3.0 mmol/L in 2.7% of all cows. No difference was observed between parity groups where 1.8%, 2.3%, and 4.1% of lactation 1, 2, and ≥ 3 cows sampled, respectively, had BHB ≥ 3.0 mmol/L ($P = 0.320$).

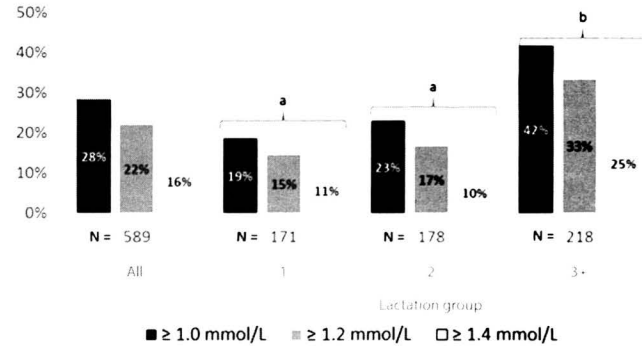


**Figure 1.** Prevalence of subclinical ketosis for all cows sampled (All) and by lactation group in 40 Washington state dairies based on 3 commonly used cutpoints for beta-hydroxybutyrate concentration in whole blood. Values above lactation groups in the axis label represent the number of cows sampled (N). Lactation number was missing for 22 cows. For a given cutpoint within a lactation group, differences were observed between lactation groups with different letters above the brackets ($P < 0.001$)

**Table 1.** Descriptive statistics of whole-blood BHB concentration of cows sampled 3 to 14 days-in-milk from 40 Washington state dairies by lactation group. Number sampled (N), standard deviation (SD), minimum (Min), maximum (Max), and parity not recorded (NR).

| | | | BHB (mmol/L) | | | |
|---|---|---|---|---|---|---|
| **Parity** | **N** | **Mean*** | **SD** | **Median** | **Min** | **Max** |
| 1 | 171 | 0.72[a] | 0.61 | 0.60 | 0.1 | 4.1 |
| 2 | 178 | 0.88[a] | 0.70 | 0.70 | 0.1 | 4.7 |
| ≥3 | 218 | 1.16[b] | 0.87 | 0.90 | 0.2 | 5.2 |
| NR | 22 | 0.71 | 0.55 | 0.60 | 0.1 | 2.6 |
| ALL | 589 | 0.93 | 0.76 | 0.70 | 0.1 | 5.2 |

*Parity mean BHB (mmol/L) with a different letter were different ($P < 0.001$).

*Herd-level prevalence of SCK*

Using a BHB cutpoint of ≥ 1.2 mmol/L and a herd alarm-level of > 15% of cows exceeding that cutpoint (HA1.2>15%), 23 herds (58%; 95% CI: 42 to 73%) were considered to have SCK at the time of sampling. When a BHB cutpoint of ≥ 1.4 and herd alarm-level of > 25% (HA1.4>25%) were used, 12 herds (30%; 95% CI: 16 to 44%) were considered to have SCK at the time of sampling. All 12 herds identified with SCK by HA1.4>25% were also identified using the HA1.2>15% criteria. All 17 herds considered not to have SCK by HA1.2>15% were designated the same by the HA1.4>25% criteria. Eleven (28%) of the herds sampled were considered SCK-positive by HA1.2>15%, but negative by HA1.4>25%.

*Herd mean BHB and prevalence of SCK*

A linear relationship was observed between the arithmetic mean BHB concentration of a herd and the percentage of cows sampled in the herd with ≥ 1.2 mmol/L BHB ($r = 0.95$, $r^2 = 0.90$ $P < 0.001$; Figure 2A) and with ≥ 1.4 mmol/L BHB ($r = 0.92$, $r^2 = 0.84$ $P < 0.001$; Figure 2B). A herd mean BHB concentration of 0.77 mmol/L and 1.04 mmol/L was correlated with HA1.2>15% and HA1.4>25%, respectively. Available handheld BHB meters used in the field only report to the tenth of a mmol/L, therefore sensitivity and specificity of these cutpoints to identify herds with SCK were calculated using a range of mean BHB concentrations from 0.6 to 1.0 mmol/L (Table 2). The best combination of sensitivity and specificity was observed at mean BHB cutpoints of > 0.8 and > 1.0 mmol/L to identify herds with SCK, based on HA1.2>15% and HA1.4>25%, respectively (Table 2).

## Discussion

An objective of this study was to estimate the cow- and herd-level prevalence of SCK on large Washington state dairies. The average herd size of licensed dairies in WA was about 565 cows, whereas the average herd size of the convenience sample of cows in this study was 2808, and 78% of herds sampled were milking 1000 or more cows. The bias toward larger herds was in part due to enrollment criteria where herds with more than 250 milking cows were selected so an adequate number of post-partum cows could be sampled within a month, assuming the number of cows calving in a month was about 10% of the milking herd. The larger herd size of the sample population was primarily influenced by the convenience of sampling, since sample collection could be completed on a single visit. Thus the results of this study are more representative of larger WA dairies.

To achieve a precision of 25% on the prevalence estimate of SCK, a sample size of 44 herds was needed; however, only 40 herds had adequate data for analysis. As a result, the precision of the prevalence estimate of SCK for herds with more than 250 milking cows is lower than if data from 44 herds were included in the analysis.

A herd alarm-level of 15% of cows in the herd with >1.2 mmol/L BHB was reported to be associated with a 1.8 percentage point increase in displaced abomasum (DA), and a 0.8 percentage point decrease in pregnancy rate 42 days after the voluntary wait period.[10] That study reported 40% of 60 herds from the northeastern US were above the alarm level. In the current study, 58% of herds sampled had >

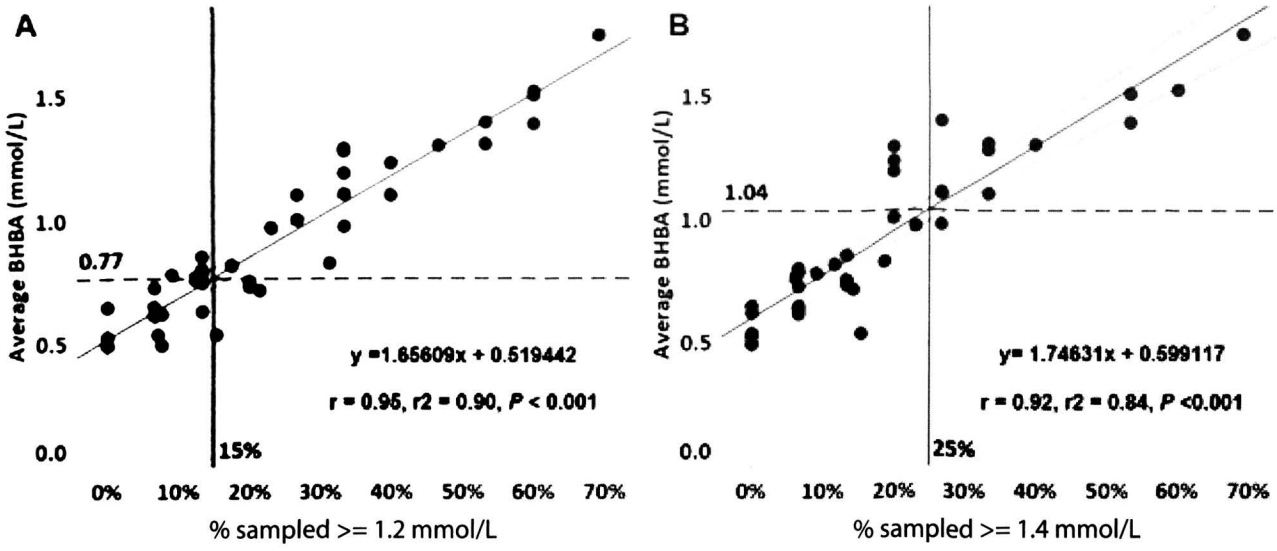


**Figure 2.** Scattergraphs showing the correlation between the % cows sampled in a herd with ≥ 1.2 mmol/L BHB (A), or ≥ 1.4 (B) and the herd average BHB (mmol/L) of cows sampled from 40 Washington state dairies. The linear trendline with 95% confidence band is shown. The vertical solid line marks a herd "alarm level" of 15% of cows sampled with BHB ≥ 1.2 mmol/L (A) and 25% of cows with BHB ≥ 1.4 mmol/L (B). Herds exceeding those levels were considered to have a potential problem with subclinical ketosis. The horizontal dashed lines mark the average BHBA of herds sampled that correlated with the specified herd alarm level.

15% of cows with a whole-blood BHB ≥ 1.2 mmol/L. A herd alarm-level of > 25% of cows in the herd with > 1.4 mmol/L BHB was associated with a 2-fold increased risk of DA.[3] That study reported 15% of 55 herds across the US and Canada were above the threshold of 25%. In the current study, 30% of herds had > 25% of cows with a BHB ≥ 1.4 mmol/L. To our knowledge there are no studies that have determined a herd alarm-level for the 1.0 mmol/L BHB cutpoint associated with an increased risk of detrimental post-partum outcomes at the herd level. Season of the year, geographical region, parity of cows sampled, and herd size could explain why the apparent prevalence of SCK observed in this study was numerically higher than previous reports. In the current study, cows were sampled during the summer, whereas the other 2 studies sampled across multiple seasons. Decreased dry matter intake during the summer months may have resulted in more cows sampled with higher BHB, resulting in a higher prevalence of SCK in the current study. Regional differences in rations, weather, and farm structure and management exist among dairies.[13] Cows in the current study were all from Washington (west), vs New York and Wisconsin (northeast and midwest) in previous studies.[3,10] Chapinal et al included herds from California (west); however, the majority of herds and cows were from the midwest, northeast, and southeast.[3] The prevalence of SCK in the California herds sampled was not specifically reported; however, DA incidence risk was 0.5% compared to 4.1 to 5.2% for the other regions studied. This is consistent with anecdotal reports of a lower DA incidence in Washington and western herds, compared with those in the midwest and northeast. Since DA was a major outcome used to establish BHB cutpoints and herd-alarm levels, it is possible those values are different in larger western dairy herds. However, Ospina et al also identified impaired production and reproduction using similar cutpoints.[10] Nonetheless, more research is warranted to determine if these commonly used cutpoints apply to larger western dairy herds.

Parity distribution of cows sampled could also explain the differences in SCK prevalence. In the current study, 29% of cows sampled were primiparous. This was similar to the 27% reported by Chapinal et al, but lower than the 41% in the study by Ospina et al.[3,10] Results of the current study are consistent with previous reports that found a lower prevalence of SCK in younger cows.[7,10,16] A recent review of the literature did not report parity group-specific BHB cutpoints or parity group-specific herd alarm levels.[8] The prevalence of SCK in the current study was about twice as high in cows parity ≥ 3 than both parity 1 and 2 groups. This suggests post-partum energy balance in parity 2 cows is more like primiparous cows than cows of parity ≥ 3, and should be grouped with primiparous cows. Previous studies have typically grouped cows as primiparous or multiparous and cows sampled from each herd were a mixed population, as was the case in the current study. Given that the prevalence of SCK in cows parity ≥ 3 may be about twice that of younger cows, obtaining an adequate sample size of each risk group (at least 15 cows) would improve the herd (or risk group) level sensitivity (HeSe) for a given individual test cutpoint and herd alarm-level (e.g. HA1.2>15%). This would be particularly important in the parity < 3 group because HeSe of a testing scheme is reduced more by smaller sample sizes at a lower disease prevalence. This is demonstrated in Table 3, which depicts the HeSe for 2 sample sizes and a range of disease prevalence for a given herd alarm-level, and individual test sensitivity and specificity generated using the online EpiTools resource.[15] When the prevalence of SCK was 15% (similar to the prevalence seen in the current study for parity < 3 cows), reducing sample size from 15 to 10 cows resulted in a 33% decrease in HeSe. By comparison, a 9.1% reduction in HeSe resulted when the prevalence was 30% (similar to the prevalence observed in this study for parity ≥ 3 cows). In larger herds like those in this study, 15 cows that are 3 to 14 DIM can typically be sampled from each parity risk group on a single day, and should improve the sensitivity of the monitoring program to identify excessive NEB among parity risk-groups in the herd.

Routine (e.g. weekly) testing of pooled samples of parity risk-groups would modestly reduce testing costs. If 15 cows were sampled from each parity risk-group at $2 (USD)/test, the cost would be $60/week. Pooled-sample testing of each parity risk-group would cost $4. Material costs for sample pooling (2 mL EDTA tube and transfer pipette) would cost approximately $0.30/sample or $9 for 30 cows sampled, for a total testing cost of $13/week. Based on our experience, the time (labor) to pool and test 2 samples

**Table 2.** Test characteristics of herd mean BHB concentration (mmol/L) to identify herds with SCK based on 2 individual-cow testing schemes.

| Testing scheme* | Mean BHB cutpoint (mmol/L) | Sensitivity | Specificity |
|---|---|---|---|
| HA1.2>15% | > 0.6 | 0.96 | 0.67 |
| | > 0.7 | 0.92 | 0.79 |
| | > 0.8 | 0.74 | 0.94 |
| **HA1.4>25%** | > 0.8 | 1 | 0.67 |
| | > 0.9 | 1 | 0.71 |
| | > 1.0 | 0.91 | 0.75 |

*HA1.2>15% - BHB cutpoint of ≥ 1.2 mmol/L and a herd alarm-level of >15% of cows exceeding that cutpoint
HA1.4>25% - BHB cutpoint of ≥ 1.4 mmol/L and a herd alarm-level of >25% of cows exceeding that cutpoint

**Table 3.** Herd-level sensitivity for 2 sample sizes and a range of SCK prevalence assuming 35 post-partum cows at risk, an individual BHB test sensitivity of 0.96 and specificity of 0.97, and a herd alarm-level of 2 positive individuals.

| | SCK prevalence | | | | |
|---|---|---|---|---|---|
| Sample size | 0.15 | 0.20 | 0.25 | 0.3 | 0.35 |
| 10 | 0.56 | 0.71 | 0.83 | 0.90 | 0.95 |
| 15 | 0.83 | 0.93 | 0.97 | 0.99 | 0.91 |

would be comparable if not lower than running 30 individual tests. Thus, pooled-sample testing would cost about 75% less than individual-sample testing. Admittedly, the cost of individual-sample testing is not prohibitive. However, use of a validated, pooled-sample BHB cutoff for each parity group has the potential to more accurately assess energy balance in a herd given the various individual BHB test cutoffs and herd alarm-levels that have been reported.

Borchardt and Staufenbiel reported that measuring BHB on pooled serum samples from 10 post-partum cows accurately identified herds with SCK based on proportion of cows sampled at or above a specified BHB cutpoint.[1] They sampled 10 cows from 110 German dairies and used 2 herd alarm-levels to classified herds as negative, borderline or positive if they had 0, 1 to 2 or > 2 cows, respectively, with BHB greater than a specified cutpoint. This testing scheme was based on that proposed by Oetzel.[12] They found a pooled sample of cows exceeding a cutpoint of 0.75 mmol/L distinguished between negative and borderline herds (sensitivity: 77%, specificity: 85%). A pooled-sample cutpoint of 1.1 mmol/L BHB distinguished between borderline and positive herds (sensitivity: 91%, specificity: 92%). This is in contrast to another report that evaluated use of a pooled-sample cutpoint of 1.2 mmol/L, the same cutpoint used on an individual cow test.[11] That study observed a sensitivity and specificity of 30 and 100% respectively, to identify herds where the proportion of 12 individual cows with BHB ≥1.2 mmol/L was >15%. The low sensitivity reported in that study was likely the result of using the individual cow cutpoint on pooled samples rather than determining a pooled sample cutpoint as was done by Borchardt and Staufenbiel.[1] However, both studies found individual sample arithmetic mean BHB values were highly correlated with pooled serum sample BHB concentrations ($r$ = 0.97). Furthermore, evaluation of agreement between pooled-sample and mean BHB values indicated no clinically relevant differences in results obtained by the 2 methods.[1]

In the current study pooled samples were not available; however, given the high correlation with the arithmetic mean, herd-average BHB concentrations were used to evaluate pooled-sample test characteristics compared to individual-cow testing schemes commonly reported. We determined that a mean BHB cutpoint of 0.77 and 1.04 mmol/L were highly correlated with HA1.2>15% ($r$ = 0.95, $P$ < 0.001) and HA1.4>25% ($r$ = 0.92, $P$ < 0.001), respectively (Figure 2). Given the precision of BHB meters commonly used in the field, mean BHB cutpoints evaluated were rounded to the tenth of a mmol/L. The test characteristics of the mean BHB concentration cutpoints in the current study were similar to those based on pooled-sample BHB testing reported by Borchardt and Staufenbiel.[1] A mean BHB cutpoint of > 0.8 mmol/L had the best combination of sensitivity (74%) and specificity (94%) for identifying herds SCK-positive based on HA1.2>15%. A mean BHB cutpoint > 1.0 mmol/L had a sensitivity and specificity of 91 and 75%, respectively, for identifying herds SCK-positive based on HA1.4>25%. These results were similar to that previously reported by Borchardt and Staufenbiel.[1] In the current study, using lower mean BHB cutpoints resulted in higher sensitivity at the expense of lower specificity, as expected. In the case of monitoring for negative energy balance, failure to identify herds or risk groups with a problem (lower herd-level sensitivity) may be more costly than further investigating a potential SCK problem when it doesn't exist (the cost of lower herd-level specificity). Regardless of whether samples are pooled or individually tested, targeted sampling of different risk groups (e.g. parity groups) would increase herd-level sensitivity. In the current and previous studies, evaluation of aggregate sample testing used established individual-cow testing schemes (e.g., HA1.2>15% and HA1.4>25%) as the "gold standard" to classify herd SCK status. Research to evaluate the ability of pooled-sample BHB testing to identify herds at greater risk of poor outcomes related to disease, milk production, reproduction, and removal are needed.

The impact of individual samples with extreme (high or low) BHB concentration on the pooled sample or mean BHB value is a concern. It has been shown that minimum and maximum serum BHB concentrations significantly influenced pooled-sample values.[1] Nonetheless, pooled-sample[1] and mean-sample BHB test characteristics suggested aggregate sample testing was useful for identifying herds classified as having SCK based on individual sample testing schemes.

## Conclusions

The prevalence of SCK in Washington dairies observed in this study was numerically higher than previous reports. This may have been due to regional and seasonal differences in the study populations. Given regional differences in rations, housing, and climate, further research is warranted to determine if the established BHB cutpoints to define SCK apply to larger western dairies. When measuring BHB to monitor post-partum energy balance, representative samples should be taken from parity risk-groups. Results of this study suggest parity 2 cows should be included in the parity 1 group rather than the parity ≥ 3 sample. The arithmetic mean BHB of a sample performed well as a test to identify herds with a potential SCK problem. This suggests pooled samples of risk groups could be used to assess SCK and monitor post-partum energy balance. However, further research evaluating the relationship between pooled-sample BHB and important outcomes (disease, milk production, reproduction, and removal) are needed.

## Endnotes

[a]Precision Xtra, Abbott Labs, Abbott Park, Illinois
[b]SAS for Windows v9.3, SAS Institute, Cary, NC
[c]Tableau Desktop v9.1, Tableau Software, Seattle, WA

## Acknowledgements

The authors would like to thank the veterinarians and dairy farmers who participated in the study, as well as Dr. Jill Stevenson and Dr. Sarah Giebel for technical support in sampling. Partial funding was provided by a Washington State University College of Veterinary Medicine Summer Research Fellowship grant. The authors declare no conflict of interest.

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