

Validation of a Chemical Analysis Technique to Quantify β -hydroxybutyric Acid Concentration in Milk of Holstein Dairy Cows

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Introduction

Hyperketonemia is characterized by an increased concentration of circulating ketone bodies during the postpartum period of cows. A high prevalence of hyperketonemia in herds is associated with decreased milk production, decreased probability of pregnancy at first service, and increased risk of peripartum diseases. The gold standard diagnostic test for hyperketonemia is β -hydroxybutyric acid (BHBA) measurement in serum or plasma by a laboratory process. This technique requires money and time for sampling animals, which can be inconvenient for producers and veterinarians. Different cow-side tests have been developed to detect BHBA on blood or milk with variable accuracies. Skalar (Breda, Netherlands) designed a continuous flow analyzer (San++) that can be used to quantify BHBA in milk samples. The use of San++ may be a convenient way to determine the prevalence of hyperketonemia in a herd because the Dairy Herd Improvement Association (DHIA) samples milk from most herds every month. Unfortunately, little data is available regarding the accuracy of San++. Thus, the objective of this field study was to determine the accuracy of San++ for diagnosing hyperketonemia.

Materials and Methods

From July to September 2010, Holstein cows from 35 herds of the bovine ambulatory clinic of the Université de Montréal (Québec, Canada) were enrolled in this study. Herds were visited once during the study period within four hours of DHIA (Valacta, Ste-Anne-de-Bellevue, Québec, Canada) monthly milk sampling. During farm visits, five fresh cows (most recently calved) were selected for sampling by a research technician. Blood was drawn from coccygeal vessels of selected cows and was immediately analyzed to determine its BHBA concentration using a BHBA hand-held meter (Precision Xtra, Abbott, Mississauga, Ontario, Canada). An individual composite milk sample was collected from all selected cows during on-farm DHIA sampling. Bronopol (usual conservative agent) was added to all samples and they were refrigerated until analysis in the DHIA laboratory. A chemical analysis was performed on all milk samples

to determine BHBA and acetone concentrations using the SAN++ device. Statistical analyses were performed using Epi Info (version 3.5.5, Centers for Disease Control and Prevention, Atlanta, GA, USA) and Microsoft Excel (version 2003, Microsoft, Redmond, Washington, USA). Blood BHBA concentration (Precision Xtra) was considered as reference test, and hyperketonemia was defined as a BHBA concentration ≥ 1400 $\mu\text{mol/L}$. A Pearson correlation coefficient was calculated between blood and milk BHBA concentrations. Milk BHBA concentration values were dichotomized; all thresholds were compared to blood BHBA results in order to calculate likelihood ratio, sensitivity, specificity, and positive and negative predictive values.

Results

Samples from 175 cows were collected during this study. The average days-in-milk at sampling was 25.6 (SD = 16.9) and true prevalence of hyperketonemia was 19.2%. Pearson correlation coefficient between milk and blood BHBA concentrations was 0.90. Using a threshold of ≥ 0.15 mmol/L (San++) provided an apparent prevalence of hyperketonemia of 28.8%, which overestimated the true prevalence of this condition. The higher apparent prevalence was due to the presence of a great proportion of false-positive cases (1 – specificity = 13.3%). Sensitivity, specificity, and positive and negative predictive values were 94.1, 86.7, 62.7, and 98.4 %, respectively. The likelihood ratio for a positive result (≥ 0.15 mmol/L) was 7.1. Using a threshold of ≥ 0.18 mmol/L (San++) provided an apparent prevalence of hyperketonemia of 19.2%, which was equivalent to the true prevalence of this condition in our study population. Sensitivity, specificity, and positive and negative predictive values were 85.3, 96.5, 85.3, and 96.5 %, respectively. The likelihood ratio for a positive result (≥ 0.18 mmol/L) was 24.4.

Significance

These data showed that San++ had good accuracy compared to blood BHBA value. Correlation between the two tests was very good. These results indicate that the threshold level of ≥ 0.18 mmol/L was the best one

to use at the individual cow level, because a positive test was 24% more likely to come from cows affected by hyperketonemia than normal cows. On the other hand, if one considers using San++ results in a herd health monitoring program, a threshold value of ≥ 0.15 mmol/L would be more appropriate, given its higher sensitivity.

When using this threshold, the herd apparent prevalence of hyperketonemia is generally higher than true prevalence, but if the apparent prevalence is monitored over time using a control chart, it can be tracked to determine if the process becomes out of control.

Preliminary Evaluation of Two Methods for Estimating Lameness Prevalence on Western US Dairies

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Introduction

Lameness is an important problem in dairy herds because it decreases production and reproductive performance, increases culling, and has a negative impact on animal welfare and longevity. The Dairy F.A.R.M. (Farmers Assuring Responsible Management) program includes animal welfare assessment and requires a lameness prevalence of less than 10%, with lameness defined as a score of 3 or above in a 5-point locomotion scoring system. Previous studies of lameness in the United States estimate prevalence greater than 20%, suggesting a need for reduction of lameness in dairy cattle. Monitoring farm lameness prevalence and detecting lameness in individual cows will be important for dairy producers and veterinarians in their efforts to reduce lameness. If monitoring is to be increased, an efficient strategy for assessing lameness that is compatible with current dairy management is necessary. Furthermore, this method must be validated as an accurate estimate of the prevalence of lameness in a herd. Two strategies have recently been suggested to estimate lameness prevalence, one by strategic sampling of cows as they exit the milking parlor and one by observing back position as cows stand in lockups.

However, these studies were based on farms with less than 275 cows, most housed in one group, whereas half of the US dairy herd is on farms with greater than 500 cows that are housed in multiple, heterogeneous pens. The purpose of this study was to test these methods on dairies with multiple pens of cows.

Materials and Methods

All cows were locomotion-scored on a 5-point scale in which a score of 1 was not lame and a score of 5 was non-weight-bearing on one leg. Cows scoring ≥ 3 were defined as lame. On farm 1, locomotion score and parlor exit order were recorded for 886 cows in six pens (62-144 cows per pen) as they exited the milking parlor single file. Herd and pen-level lameness prevalence was calculated for all cows scored. In addition, lameness prevalence was calculated from a sample of cows in the middle of the parlor exit order using a published calculation to determine sample size. On farm 2, back position (arched or not arched, where arched was considered lame) was recorded for 200 cows in three pens locked at the feed bunk. Cows were individually released from headlocks and locomotion-scored. Lameness prevalence was calculated by locomotion score and by presence of arched back for the herd and for each pen. Sensitivity and specificity were calculated for the presence of arched back as a test for locomotion score ≥ 3 in individual cows, and kappa statistic was calculated for agreement between locomotion score and presence of arched back. All data were analyzed using Microsoft Excel.

Results

On farm 1, the lameness prevalence of all cows scored was 33%, and ranged by pen from 8.5% to 44%. Estimated lameness prevalence based on a sub-sample from the middle of each pen was 34% for all pen samples