Results

Based on three concentrations of BHB in serum (> 0.8 mmol/L; > 1.0 mmol/L, and > 1.2 mmol/L) three ROC analyses were conducted. The highest area under the curve was at the value of 1.2 mmol/L; however, the highest agreement between milk and serum determinations (kappa estimator = 0.37) occurred at the value of 1.0 mmol/L. Therefore, the value of 1.0 mmol/L was used as the gold standard to classify an animal as positive or negative for subclinical ketosis. The value of milk BHB of 200 µmol/L gave the best combination of sensitivity and specificity to classify an animal as positive or negative (Se=71.4; Sp=83.5). This is in accordance with the recommendations of the commercial test strip used in the present study.

Twenty cows were culled between assignment and 14 d postpartum. Therefore, only 280 cows were tested for BHB in milk. Overall, the incidence of subclinical ketosis based on the milk test was 20.35% (57/280). Based on milk BHB test, 26.6% of control cows (n=139) and 14.5% of treated cows (n=141) were positive for subclinical ketosis. The adjusted effect of treatment was that cows receiving monensin were 0.68 times less likely to give a result of 200µmol/L (OR 95% CI =0.53-0.80) than control cows.

Concentrations of BHB in serum did not differ between groups (0.65 ± 0.07 mmol/L and 0.70 ± 0.07 mmol/L for controls and treated, respectively; P > 0.05). However cows with serum BHB concentration < 1.0 mmol/L were 0.063 times (CI 95% 0.013-0.302) as likely as controls to have a positive result for milk BHB ≥ 200 µmol/L.

Significance

If monensin is approved for use in lactating dairy cattle in the United States, a slow- release capsule in cows fed typical Florida diets will be useful to decrease the incidence of subclinical ketosis in the early postpartum period. In general, there is a valuable association between milk and serum BHB determinations. Consequently, Ketolac® test has the sensitivity to identify cows with subclinical ketosis. Therefore, it is a practical tool for use in a routine monitoring program to detect subclinical ketosis in early postpartum dairy cows.

Evaluation of Three Cow-side Tests for Detection of Subclinical Ketosis in Fresh Cows

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Introduction

Subclinical ketosis in dairy cattle can lead to economic losses through decreased milk production, decreased reproductive performance, increased risk of displaced abomasum and increased risk of clinical ketosis. For early detection of the disease, there is a need for rapid and accurate diagnostic tests.

The objective of the study was to evaluate the performance of three cow-side diagnostic tests for the detection of subclinical ketosis in fresh dairy cows, compared to the gold standard serum ß-hydroxybutyrate (BHBA). The cow-side tests were: (1) a commonly used test strip detecting acetoacetate in urine (Ketostix, Bayer Corporation, Elkhart, Indiana, USA), (2) a commonly used powder test used on milk, also detecting acetoacetate (KetoCheck, Great States Animal Health, St. Joseph, Missouri, USA), and (3) a milk test strip detecting BHBA (KetoTest, Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan, distributed by Elanco Animal Health/Provel, Guelph, Ontario, Canada).

Materials and Methods

The study was performed in a transition management facility housing dry and just-fresh cows for two large dairies in Wisconsin (total of 2500 milking cows). Serum, milk and urine samples were collected from fresh cows of all parities between two and 15 days in milk. Sensitivity and specificity of the cowside tests were cal-

culated over the range of possible cut-off points for each test, using a serum BHBA threshold of 1400 μmol/L to distinguish between normal and abnormal cows.

It is known that disease prevalence directly affects the number and the type of diagnostic errors that will arise from using imperfect tests for screening. This effect of the prevalence on the tests results thus complicates the comparison of diagnostic tests that have various combinations of sensitivity and specificity, as some tests may be more suitable in some situations, and less in others. Consequently, to evaluate and compare the three cowside tests in different screening situations, the number of true and false diagnostics was calculated over a range of hypothetical prevalence of subclinical ketosis, from a low prevalence (5%) to a high prevalence (30%).

Results

Groups of fresh cows were sampled on 16 different occasions from September 2002 to January 2003. The sample proportion of subclinical ketosis was 7.6%, ranging by sampling day from 0 to 20.8% (n = 859 samples from 545 cows).

The KetoCheck powder was very specific (99%) but poorly sensitive (41%) using milk samples, even at its most sensitive cut-point ("trace"). The sensitivity and specificity of the Ketostix strip using urine samples were 90 and 86%, respectively, with a cut-off point of 5 mg/dL ("trace"); 78 and 96% with a cut-off point of 15 mg/dL ("small"); 49 and 99% with a cut-off of 40 mg/dL ("moderate"); or 12 and 100% at the cut-off point of 80 mg/dL ("large"). The KetoTest strip using milk samples had a sensitivity and specificity of 88 and 90%, respectively, with a cut-off point of 50 μmol/L; 73 and 96% with a cut-off of 100 μmol/L; 27 and 99% with a cut-off of 200 μmol/L; or 3 and 100% with a cut-off of 500 μmol/L.

On average, the use of the Ketostix at 15 mg/dL ("small") or the KetoTest at 100 μmol/L would result in no more than three or four false positives per day of sampling in the screening of 100 cows with prevalence levels ranging from 5 to 30%, whereas the number of false negatives would range from 1 false negative at 5% prevalence to 7 or 8 false negatives at 30% prevalence. Over this prevalence range, the KetoCheck powder test would have a limited application as a screening test. Despite a low number of false positives (no more than 1 per 100 animals screened), the number of false negatives resulting from screening with the KetoCheck test would be too large, even at its most sensitive cut-off (from three false negatives at 5% prevalence to 18 at 30% prevalence in a population of 100 tested cows).

Significance

These results differ from earlier reports on the lack of specificity of the urine tests. However, it is important to mention that the Ketostix nitroprusside strips must be read promptly after contact with urine, as the color will tend to darken with time. Finally, the results of this study indicate that either the Ketostix urine strip or the KetoTest milk strip can provide acceptable results for the screening of individual cows on commercial dairies to detect subclinical ketosis.